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ARTICLE

Fecal microbiota transplantation improves metabolism and gut microbiome composition in *db/db* mice

Pei-pei Zhang[1], Lin-lin Li[1,2], Xue Han[1], Qin-wei Li[3], Xu-hua Zhang[1], Johnson J. Liu[4] and Ye Wang[1]

Fecal microbiota transplantation (FMT) has become an effective strategy to treat metabolic diseases, including type 2 diabetes mellitus (T2DM). We previously reported that the intestinal microbiome had significant difference between individuals with normal glucose tolerance and T2DM in Chinese Kazak ethnic group. In this study, we investigated the effects of transplanted fecal bacteria from Kazaks with normal glucose tolerance (KNGT) in *db/db* mice. The mice were treated with 0.2 mL of fecal bacteria solution from KNGT daily for 10 weeks. We showed that the fecal bacteria from KNGT successfully colonized in the intestinal tract of *db/db* mice detected on day 14. In the FMT-treated *db/db* mice, the levels of fasting blood glucose, postprandial glucose, total cholesterol, triglyceride, and low-density lipoprotein–cholesterol were significantly downregulated, whereas high-density lipoprotein–cholesterol levels were upregulated. In the FMT-treated *db/db* mice, *Desulfovibrio* and *Clostridium coccoides* levels in gut were significantly decreased, but the fecal levels of *Akkermansia muciniphila* and colon histone deacetylase-3 (HDAC3) protein expression were increased. At 8 weeks, both intestinal target bacteria and HDAC3 were correlated with glycolipid levels; *Akkermansia muciniphila* level was positively correlated with HDAC3 protein expression ($r = +0.620$, $P = 0.037$). Our results suggest that fecal bacteria from KNGT could potentially be used to treat diabetic patients.

Keywords: fecal microbial transplantation; Chinese Kazak ethnic group; metabolic diseases; *db/db* mice; intestinal microbiome; *Desulfovibrio*; *Clostridium coccoides*; *Akkermansia muciniphila*; colon histone deacetylase-3

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INTRODUCTION

The prevalence of diabetes mellitus has increased dramatically worldwide over the past 2 decades. According to the International Diabetes Federation [1], there were 415 million diabetic patients between the ages of 20 and 79 years in 2015, and it is estimated that this number will increase to 642 million by 2040. China has the highest number of patients with diabetes, estimated at 109.6 million [1], and over 90% have type 2 diabetes mellitus (T2DM) [2, 3].

T2DM is characterized by glycolipid metabolism disorders and is caused by both genetic and environmental factors. In our previous study, we found a significant difference in the composition and proportion of the intestinal microbiome between individuals with T2DM and those with normal glucose tolerance (NGT) [4]. It has also been shown that fecal bacteria from T2DM patients can induce abnormal glucose and lipid metabolism in mice [5].

Intestinal microbial metagenomics analysis has shown that T2DM patients have more opportunistic pathogens in their intestinal tracts compared with those of healthy individuals [6]. Previous studies by our group showed that the fecal levels of *Clostridium coccoides* from diabetic mice were significantly higher than the levels in normal control mice. We also found a positive correlation with fasting blood glucose (FBG) levels [7]. Another study reported that an increase in *Desulfovibrio* in the intestinal tract can promote the development of diabetes, while *Akkermansia muciniphila* has antidiabetic effects [8].

The colon has the largest distribution of intestinal flora. Intestinal microbiota modulate metabolism and associate closely with epithelial cells in the intestine. Epithelial expression of histone deacetylase-3 (HDAC3) plays a critical role in integrating microbiota-derived signals to maintain intestinal homeostasis [9]. For example, *A. muciniphila* can epigenetically activate or silence genes involved in host lipid metabolism, leading to a significant decrease in the expression of G-protein-coupled receptor 43 (Gpr43) and peroxisome proliferator-activated receptor (Ppar) and increased expression of HDAC3 and histone deacetylase-5 (HDAC5) [10]. Local tissue depletion of HDAC3 can cause severe systemic insulin resistance in mice [11].

Fecal microbiota transplantation (FMT) is a procedure in which stool from a healthy donor is transferred into another patient's intestinal tract. This procedure can reverse intestinal microbiome dysbiosis in the recipient [12]. FMT has been used to treat intestinal infections and metabolic diseases because the transplanted microbiota can rebuild the recipient's intestinal microbial ecosystem [13]. Recently, FMT has been proven to be a safe and effective method for treating and preventing the recurrence of

[1]Department of Pharmacology, Xinjiang Medical University, Urumqi 830000, China; [2]State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia of Xinjiang Medical University, Urumqi 830000, China; [3]Department of Otorhinolaryngology, Heze Municipal Hospital, Heze 274031, China and [4]Department of Pharmacology, School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia
Correspondence: Ye Wang (wangye56@xjmu.edu.cn)
These authors contributed equally: Pei-pei Zhang, Lin-lin Li

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gastrointestinal disorders [14]. In addition, FMT from lean donors can increase intestinal microbial diversity and improve insulin sensitivity in patients suffering from metabolic syndrome [15]. Although FMT has been successful in human studies [15], the molecular mechanism underlying FMT's therapeutic benefits is not well understood. In this study, we used a combination of human fecal bacteria (as the donor) and an animal model (*db/db* mice as recipients) to investigate the mechanism of FMT. Specifically, we transplanted fecal bacteria from individuals in the Chinese Kazak ethnic group with NGT (KNGT) into *db/db* mice to investigate whether FMT confers protection. The *db/db* mouse is a common mouse model of diabetic dyslipidemia. Our goal was to determine the feasibility and potential mechanism of FMT for T2DM treatment.

MATERIALS AND METHODS

Ethics statement

The use of human subjects and the *db/db* mouse model were approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Urumqi, China; approval No: 20130216-141). All participants provided written consent prior to the study.

Criteria for NGT

NGT was determined according to the diagnostic criteria for diabetes established by the American Diabetes Association (2005) [16] and the recommendations of the Diabetes Society of the Chinese Medical Association [17]. Subjects with NGT were determined by measuring fingertip glucose levels: FBG < 5.6 mmol/L (100 mg/dL), and 2-h oral glucose tolerance test (2h-OGTT) < 7.8 mmol/L (140 mg/dL).

Inclusion and exclusion criteria for donors

Stool samples were collected from healthy individuals from Chinese Kazaks, a Chinese minority ethnic group. The inclusion and exclusion criteria for donors were based on previous publications [18–22]. The inclusion criteria were as follows: (1) age 20–40 years old with NGT and normal body mass index (18.5–24.9 kg/m^2) [23]; (2) individuals descended from at least three generations of lineal relatives of Kazak ethnicity; (3) residents of the same areas for >10 years; (4) long-term healthy lifestyle, including good eating habits and regular bowel evacuation habits; (5) healthy mental state; (6) no chronic diseases, such as constipation or diarrhea, irritable bowel syndrome, inflammatory bowel disease, autoimmune diseases, or malignant diseases; (7) no family history of diseases, including diabetes, obesity, high blood pressure, hepatitis, etc., among family members in the same household; (8) had not taken antibiotics, probiotics, or any other medication for 3 months. The exclusion criteria, included (1) pregnant or lactating; (2) history of mental illness or substance abuse; (3) undergone surgery or presented other emergency conditions; (4) obvious impairment of liver and kidney function, diarrhea, biliary tract infection history, enteritis or other gastrointestinal diseases, or blood system or other endocrine system disease; (5) history of insulin treatment or ketoacidosis; and (6) diagnosed with type 1 or type 2 diabetes or a special type of diabetes, such as gestational diabetes mellitus.

Preparation of donor fecal fluid

Based on the inclusion and exclusion criteria for donors, one healthy individual from the Kazak ethnic group with NGT was selected as the fecal donor. Fresh feces from this donor were collected in the morning and sealed in aseptic containers and quickly transferred to aseptic biosafety cabinets on ice. Approximately, 50 g of fecal material was placed into sterile tubes and homogenized in 250 mL of sterile normal saline. The slurry was passed through 2.0-, 1.0-, 0.5-, and 0.25-mm stainless-steel filters to remove undigested food and small particulate matter. The fecal liquid that was passed through the 0.25-mm sieve was centrifuged at 6000 × *g* for 15 min and then resuspended in 125 mL of sterile normal saline [24]. Finally, the fecal bacteria slurry was diluted with 0.1 M phosphate-buffered saline (PBS) (pH 7.2) containing 10% sterile medical glycerin. The resulting fecal bacteria liquid was aliquoted into 5-mL sterilizing cryopreservation tubes and preserved at −80 °C.

Animal groups and sample collection

Eight-week-old male *db/db* and *db/m* mice were purchased from Changzhou Kavins Experimental Animal Co., Ltd. (Changzhou, China). Mice were housed in an specific pathogen-free environment at 21 ± 2 °C and 45% ± 5% humidity and maintained on a light/dark cycle for 12/12 h. A total of 15 *db/m* mice and 27 *db/db* mice were used in the study. After 1 week of adaptive feeding, three *db/m* mice and three *db/db* mice were killed at week 0. The remaining mice were divided into three groups: one group of *db/m* + PBS (12 mice in total) (PBS was used to dissolve fecal bacteria from KNGT) and two groups of *db/db* mice. The *db/db* groups were randomly divided into the *db/db* + PBS group (control) (12 mice in total) and the *db/db* + KNGT group (12 mice in total) (*db/db* mice treated with fecal bacteria from the same KNGT person throughout intervention). Mice were treated with either 0.2 mL of PBS or 0.2 mL of fecal solution daily. Blood and fecal samples were collected at five time points (0, 4, 6, 8, and 10 weeks). To measure metabolic parameters during animal experiments, ~10 μL of blood was collected from each mouse, and the blood was added to 90 μL of an anticoagulant (the anticoagulant was prepared by adding 0.1 g of sodium fluoride to 0.9% sodium chloride solution) and centrifuged at 10 500 × *g* for 1 min. Feces were collected and placed in sterilized cryopreservation tubes and then stored at −80 °C. Three mice randomly selected from each group were anesthetized with sodium pentobarbital (35 mg/kg body weight) and killed at the 0- and 8-week time points. Colonic tissue samples were collected into cryotubes and stored at −80 °C.

Detection of bacterial colonization in mice

Frozen fecal bacteria solutions were thawed for 2–4 h in an ice bath prior to FMT. The fecal bacteria solution was then stained with 10 μg/mL CM-Dil fluorescent dye (Thermo Fisher Scientific, Waltham, MA, USA) for 30 min at 37 °C. Each *db/db* + KNGT mouse was gavaged with 0.2 mL of the stained fecal bacteria solution [25]. Mice gavaged with the same amount of PBS were used as controls. Fresh feces of treated and control mice were collected in sterile tubes on days 3, 7, and 14, respectively, after intragastric administration. The feces were examined by using an Olympus IX73 microscope (Olympus, Tokyo, Japan). Detection of fluorescent signals in the fecal bacteria solution of *db/db* + KNGT mice on day 14 confirmed successful colonization of KNGT fecal bacteria in the *db/db* mice.

Plasma glycolipid analysis

FBG and postprandial glucose (PPG) were measured by using the glucose oxidase method [26]. Total cholesterol (TC) and triglyceride (TG) levels were analyzed by using COD-PAP and GPO-PAP (Biosino Biotechnology Co., Ltd., Beijing, China) according to the manufacturer's instructions. Plasma levels of high-density lipoprotein–cholesterol (HDL–c) and low-density lipoprotein–cholesterol (LDL–c) were determined by using enzyme-linked immunosorbent assay (ELISA) kits (SenBeiJia Biological Technology Co., Ltd., Nanjing, China) according to the manufacturer's instructions.

Intestinal microbiome analysis

Extraction of bacterial total DNA. Approximately, 0.2 g (range 0.18–0.22 g) of mouse fecal samples were placed in a 2-mL collection tube. Total DNA was extracted by using a DNA

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Table 1. Real-time PCR primers of intestinal target bacteria.

Intestinal bacteria real-time PCR system				
Target bacteria	Target gene	Amplicon length (bp)	Primer sequences (5′–3′) (F, forward; R, reverse)	Tm (°C)
Desulfovibrio [40]	*16S rRNA*	135	F: CCGTAGATATCTGGAGGAACATCAG R: ACATCTAGCATCCATCGTTTACAGC	55
Clostridium coccoides [33]	*16S rRNA*	440	F: AAATGACGGTACCTGACTAA R: CTTTGAGTTTCATTCTTGCGAA	55
Akkermansia muciniphila [41]	*16S rRNA*	280	F: AGAGGTCTCAAGCGTTGTTCGGAA R: TTTCGCTCCCCTGGCCTTCGTGC	58

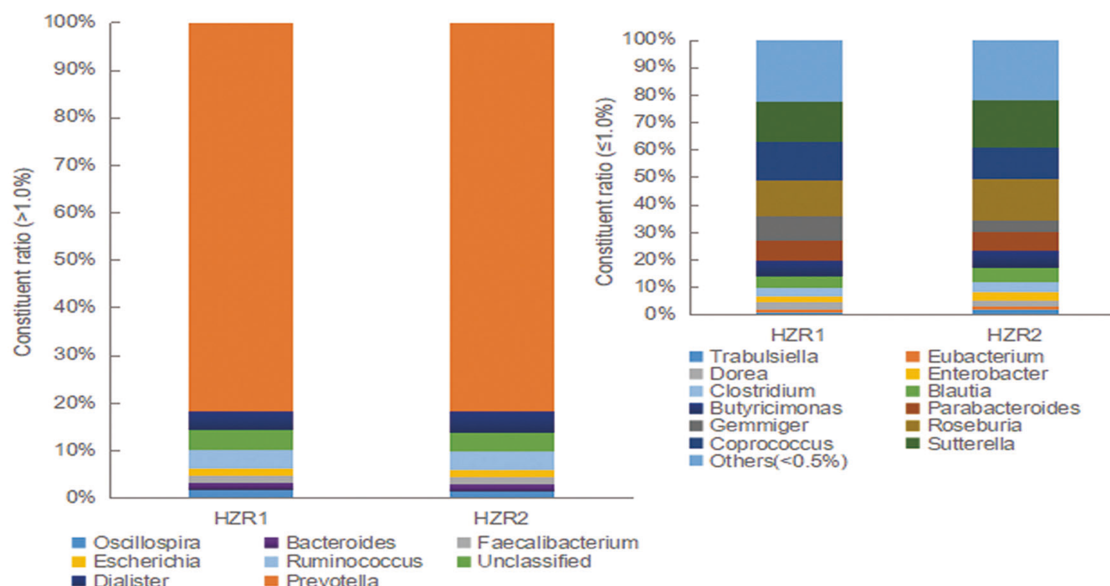

Fig. 1 Prepared feces from the donors were divided into several 5-mL cryopreservation tubes and fed to the *db/db* mice one tube per day. Two tubes were randomly selected to determine the consistency of the composition of the daily administered fecal bacteria. HZR1: fecal bacteria of KNGT tube #1; HZR2: fecal bacteria of KNGT tube #2.

extraction kit (Qiagen, Germany). The absorbance at A_{260}/A_{280} was measured by using a spectrophotometer to determine the concentration and purity of the extracted DNA.

High-throughput Illumina sequencing of the V4 variable region of 16S rRNA. The extracted DNA samples were amplified by using a set of universal primers targeting the hypervariable V4 region of *16S rRNA*. The forward primer was 5′-AYTGGGYDTAAAGNG-3′, and the reverse primer was 5′-TACNVGGGTATCTAATCC-3′ [27]. To distinguish samples, seven nucleotide sequence tags were randomly added to the forward sequence of the universal primer to form a barcoded fusion primer for polymerase chain reaction (PCR) amplification. The gene bank was constructed according to standard protocols. The Illumina MiSeq sequencing platform and the PE250 sequencing strategy were used for sequencing.

Real-time PCR of intestinal target bacteria. The hypervariable regions of the target bacteria *16S rRNA* were amplified by using specific primers and a thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR products were extracted from a 2% agarose gel and further purified by using the AxyPrep DNA Gel Extraction Kit and used as standard material for real-time PCR (RT-PCR). RT-PCR reactions were conducted by using the SYBR® Premix EX Taq II kit (TaKaRa, Dalian, China). RT-PCR reactions were performed in triplicate in a 25-μL mixture containing 1 μL of each primer, 12.5 μL of Taq DNA polymerase, 8.5 μL of nuclease-free water, and 50 ng of template DNA. The PCR products were amplified by the specific primers of the target bacteria (Table 1).

Western blot analysis

Total proteins were extracted from mouse colon tissue cells by using RIPA lysis buffer. Protein concentrations were determined by using the bicinchoninic acid assay. Protein lysates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% acrylamide) and transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 5% nonfat milk for 2 h at room temperature and washed three times with washing buffer (1 × Tris-buffered saline with Tween 20). Membranes were incubated with anti-HDAC3 (Affinity, cat. No. Ab-AF0733) diluted 1:1000 at 4 °C overnight, followed by incubation with anti-rabbit horseradish peroxidase-conjugated IgG (Bioss, cat. No. bs-0295G) at room temperature for 1 h. The light intensities of the protein band signals relative to those of the actin signal were calculated by using ImageJ analysis software.

Statistical analysis

Data were analyzed by using SPSS 19.0 statistical software (SPSS, Inc., Chicago, IL, USA). Parameter differences among the three groups (*db/m* + PBS, *db/db* + PBS, and *db/db* + KNGT) were evaluated by using one-way ANOVA for normally distributed variables and the Kruskal–Wallis test for non-normally distributed variables. All values are expressed as the means ± standard

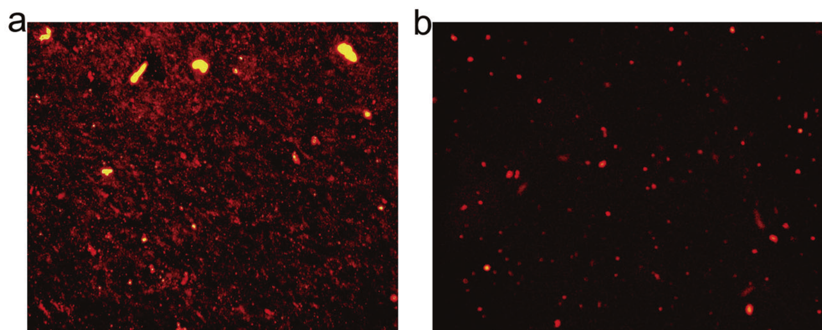

Fig. 2 Staining of live bacteria in fecal samples from KNGT and mice. a Fecal bacteria from KNGT. **b** Fecal bacteria from the intestinal tracts of mice, indicating colonization of KNGT bacteria on day 14.

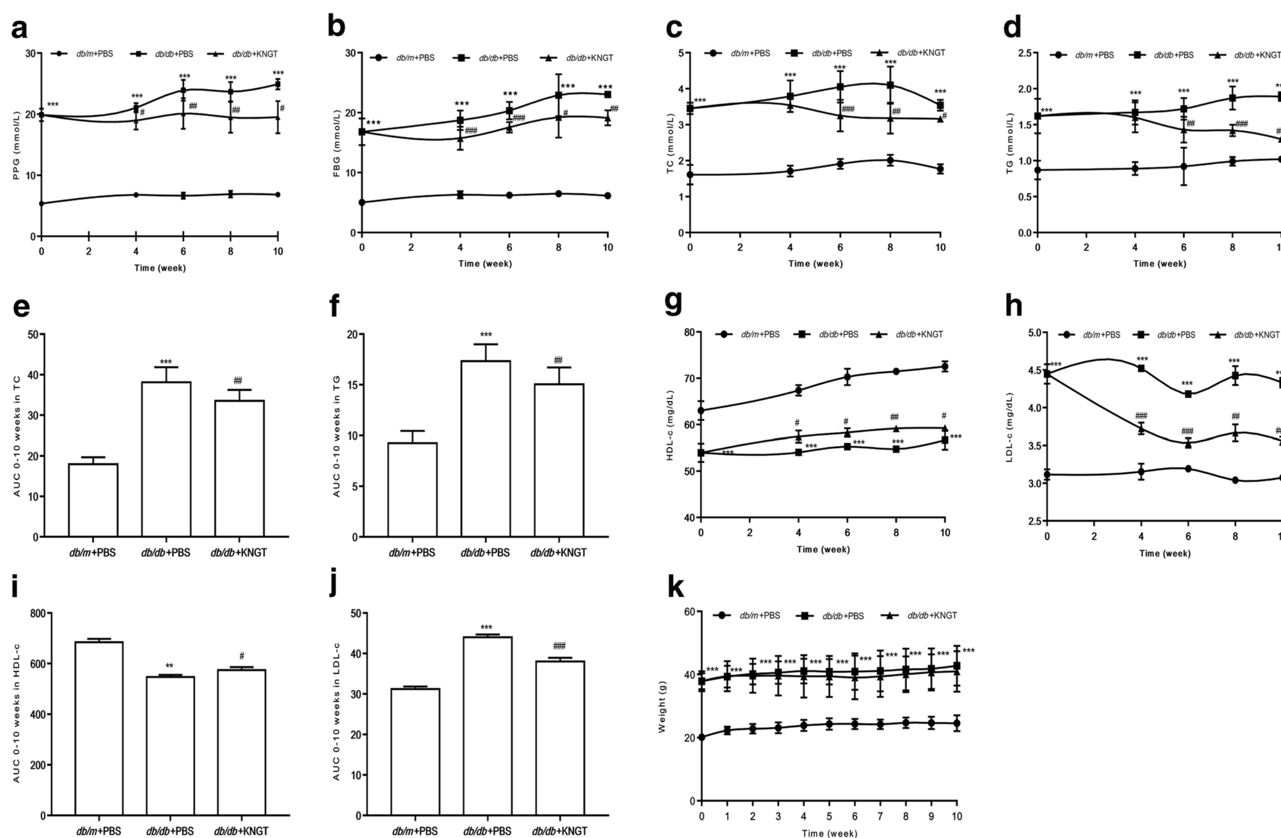

Fig. 3 Changes in body weight and plasma glycolipid levels in *db/db* mice treated with fecal bacteria from KNGT. PPG (**a**), FBG (**b**), TC (**c**), TG (**d**), HDL–c (**g**), LDL–c (**h**), and body weight (**k**) in mice treated with KNGT. Area under the blood lipid-time curve (AUC) of TC (**e**), TG (**f**), HDL–c (**i**), and LDL–c (**j**) in mice treated with KNGT for 10 weeks. Differences between the groups were analyzed by using one-way ANOVA. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the *db/m* + PBS mice. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with the *db/db* + PBS mice.

deviation. Pearson's correlation coefficient was used to evaluate associations between variables. P values < 0.05 were considered significant.

RESULTS

Homogeneity of the fecal bacteria liquid fed to mice

Two fecal bacteria tubes were randomly selected from the prepared fecal bacteria liquid of KNGT to determine the microbiota. No significant difference in *16S rRNA* sequences between the two samples was found (Fig. 1). The relative abundances of *Desulfovibrio*, *Clostridium*, and *A. muciniphila* in the fecal sample of the KNGT were 1.654%, 3.562%, and 0.382%, respectively.

Fecal bacteria from KNGT successfully colonized the intestinal tract of *db/db* mice

Fluorescence microscopy of fecal bacteria from KNGT showed red fluorescent signals (Fig. 2a), indicating that the fecal bacteria were successfully labeled by the fluorescent dye CM-DiI. Fluorescent signals were observed in feces collected from mice on day 14 after intragastric administration (Fig. 2b). These results suggest that the fecal bacteria from KNGT successfully colonized the intestinal tract of *db/db* mice.

Changes in the phenotypic parameters of *db/db* mice

Fecal bacteria of KNGT inhibit increases in blood glucose levels in db/db *mice.* Blood samples collected from *db/db* mice after FMT showed that treatment with fecal bacteria from KNGT significantly

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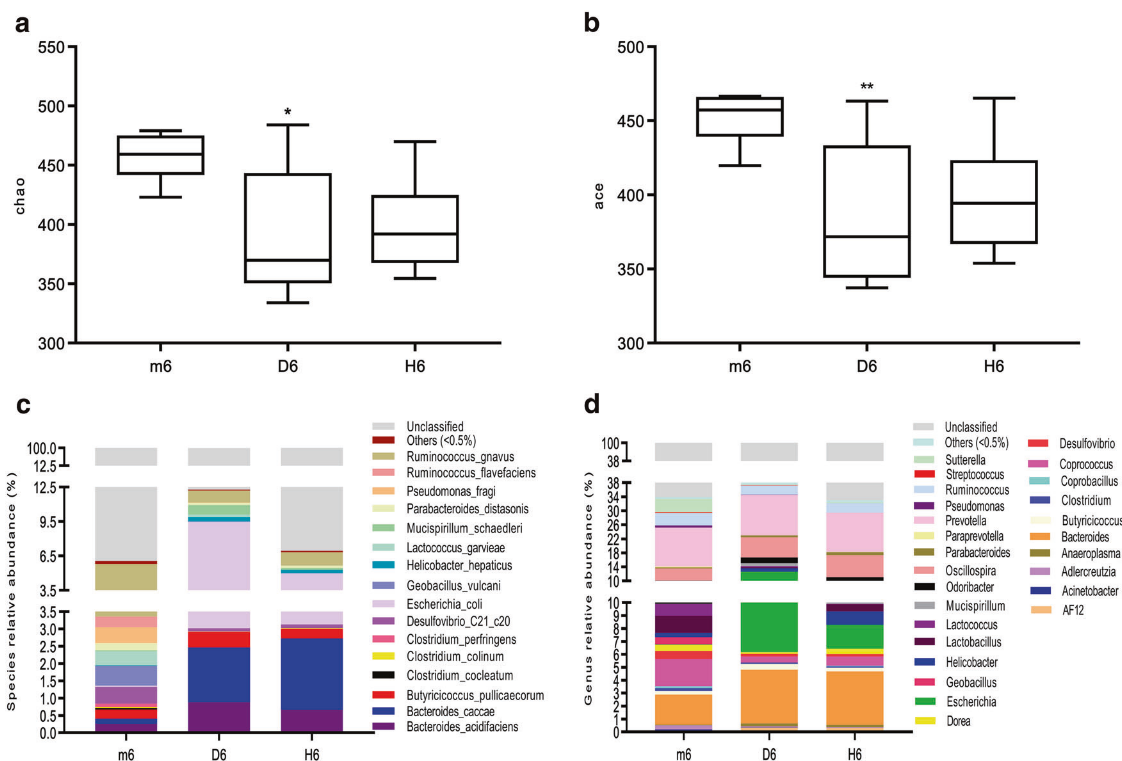

Fig. 4 Total OTU richness was evaluated by chao (**a**) or ace (**b**). The results were analyzed by using one-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the *db/m* + PBS mice. Relative abundance ratio of the intestinal microbiome at the species (**c**) and genus (**d**) levels. Data are presented as the percentage of species, and the results were obtained by using the Kruskal–Wallis test. The same color in m6, D6, and H6 represents the same species. m6: *db/m* + PBS; D6: *db/db* + PBS; H6: *db/db* + KNGT. Mice were tested at 6 weeks.

lowered plasma PPG (Fig. 3a) and FBG (Fig. 3b) levels in *db/db* mice from week 4. These results strongly suggest that fecal bacteria from KNGT can inhibit increases in blood glucose levels in *db/db* mice.

Fecal bacteria from KNGT regulate blood lipid levels in db/db *mice*. The TC and TG levels (Fig. 3c, d) in *db/db* + KNGT mice were markedly lower compared with the levels in the *db/db* + PBS mice 6 weeks after FMT, although both the TC and TG levels were much higher in the *db/db* + PBS compared with levels in the *db/m* + PBS mice. Consistent with the TC and TG levels, the TC and TG AUCs were significantly decreased (Fig. 3e, f) in the *db/db* + KNGT mice. The HDL–c levels in the *db/db* + KNGT mice were markedly higher compared with those in the *db/db* + PBS mice (Fig. 3g), and the LDL–c levels were significantly lower (Fig. 3h). The AUC of HDL–c was significantly increased (Fig. 3i) and the AUC of LDL–c was significantly decreased in the *db/db* + KNGT (Fig. 3j). However, we did not observe any significant change in body weight in the *db/db* + KNGT mice compared with that of the *db/db* + PBS mice during the 10 weeks (Fig. 3k).

Changes in the intestinal microbiome in *db/db* mice treated with fecal bacteria from KNGT

Richness and composition of the intestinal microbiome. The two α-diversity indexes, chao and ace, were used to estimate OTU richness. The indexes of *db/db* + PBS mice were significantly lower compared with those of *db/m* + PBS mice (chao: $P = 0.013$; ace: $P = 0.008$). The indexes increased slightly but not significantly after *db/db* mice were treated with fecal bacteria from KNGT, suggesting that FMT of KNGT may help increase or recover the species richness in *db/db* mice (Fig. 4a, b).

The identified OTUs were further classified by using the database of species (http://greengenes.secondgenome.com/). Compared with the *db/m* + PBS mice, the relative abundances of *Helicobacter_hepaticus*, *Escherichia_coli*, *Bacteroides_acidifaciens*, *Butyricicoccus_pullicaecorum*, *Escherichia*, *Odoribacter*, and *Butyricicoccus* were higher in the *db/db* + PBS mice. Their false-discovery rates (FDRs) were 0.035, 0.134, 0.134, 0.134, 0.136, 0.136, and 0.161, respectively. These bacteria were downregulated 6 weeks after FMT treatment in the *db/db* mice. The FDRs were 0.969, 0.755, 1, 0.755, 0.876, 0.994, and 0.876, respectively. Compared with the *db/m* + PBS mice, the relative abundances of *Sutterella* and *Paraprevotella* were decreased in the *db/db* + PBS mice (with FDRs of 0.094 and 0.161, respectively) and upregulated after FMT treatment for 6 weeks (with FDRs of 0.994 and 0.994, respectively). These results suggest that fecal bacteria from KNGT can improve the composition of the intestinal microbiome in *db/db* mice (Fig. 4c, d).

β-diversity analysis showed some differences between *db/m* + PBS and *db/db* + PBS samples in species composition and species abundance. However, there was little difference in species composition and species abundance between samples of *db/db* + PBS and *db/db* + KNGT mice.

FMT changes the number of intestinal target bacteria in mice. Three bacterial species that correlate with diabetic risk factors (*Desulfovibrio*, *C. coccoides*, and *A. muciniphila*) were chosen as the target bacteria to further understand the effect of FMT from KNGT on the intestinal microbiome of *db/db* mice. RT-PCR analysis of these three species showed that the levels of *Desulfovibrio* (Fig. 5a) and *C. coccoides* (Fig. 5b) in the feces of the *db/db* + PBS mice were higher compared to levels in the *db/m* + PBS mice, while *A. muciniphila* (Fig. 5c) was lower. Compared with the *db/db* + PBS mice, the levels of *Desulfovibrio* ($P = 0.027$) and *C. coccoides* ($P = 0.005$) in the *db/db* + KNGT mice were significantly lower 4 weeks following FMT, and the

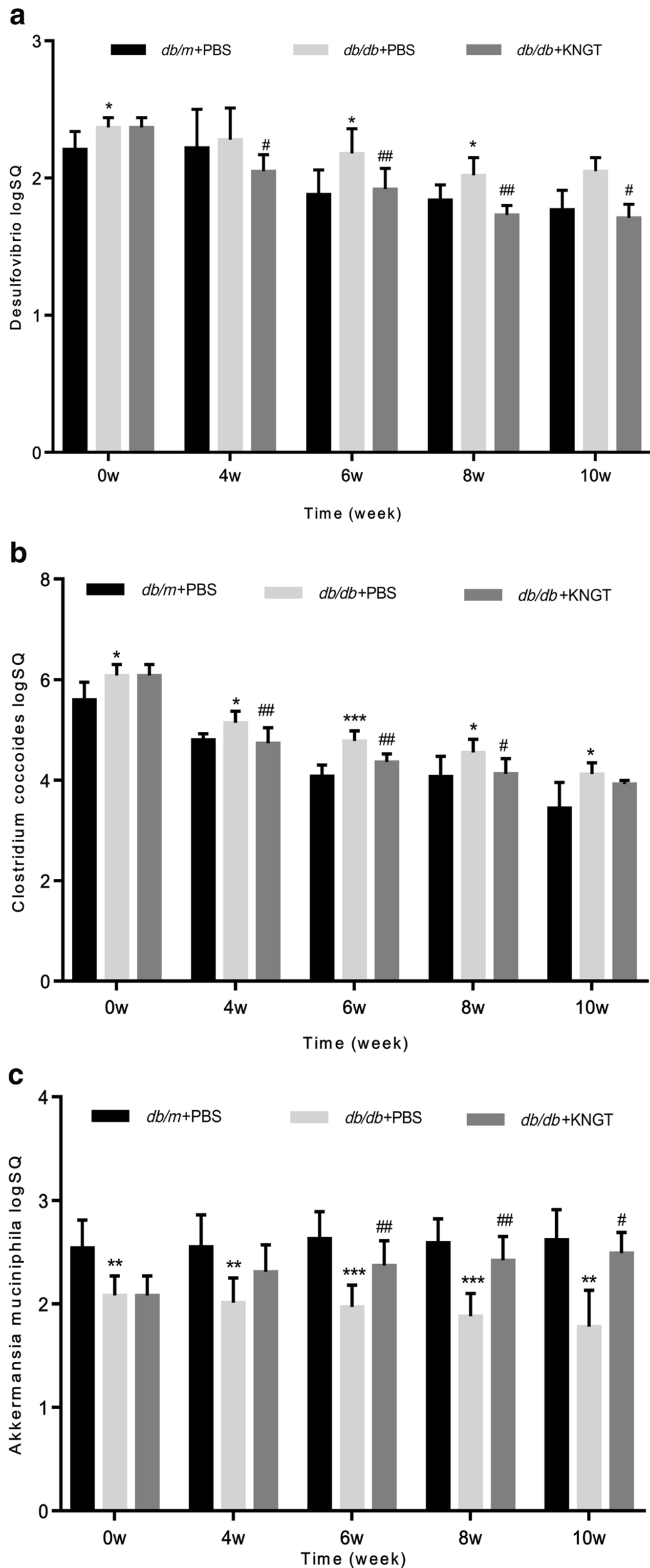

Fig. 5 Quantification of intestinal target bacteria in mice. SQ represents the starting template quantity of *Desulfovibrio* (**a**), *Clostridium coccoides* (**b**), and *Akkermansia muciniphila* (**c**) at different time points. The results were analyzed by using one-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with *db/m* + PBS mice. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with *db/db* + PBS mice.

level of *A. muciniphila* was significantly higher 6 weeks following FMT ($P = 0.008$).

HDAC3 expression is upregulated in the *db/db* + KNGT mice

Western blot analysis showed that HDAC3 expression was decreased in the colonic samples of *db/db* + PBS mice compared with expression in *db/m* + PBS mice. FMT treatment for 8 weeks recovered the HDAC3 expression level of *db/db* mice to a level similar to that of the *db/m* mice (Fig. 6).

Correlation analysis

Pearson correlation analysis between target bacteria and glycolipid metabolism at 8 weeks showed that the level of *C. coccoides* was positively correlated with TC and TG levels ($r = 0.512$, $P = 0.030$; $r = 0.645$, $P = 0.004$, respectively) (Fig. 7a, b). The level of *A. muciniphila* was negatively correlated with PPG, FBG, TC, and TG levels ($r = -0.621$, $P = 0.006$; $r = -0.668$, $P = 0.002$; $r = -0.656$, $P = 0.003$; $r = -0.761$, $P < 0.001$, respectively) (Fig. 7c–f).

Pearson correlation analysis between HDAC3 expression and glycolipid metabolism at 8 weeks showed that the expression of HDAC3 was negatively correlated with PPG, TC, and TG levels ($r = -0.669$, $P = 0.049$; $r = -0.778$, $P = 0.014$; $r = -0.772$, $P = 0.015$, respectively) (Fig. 7g–i).

Correlation analysis between target bacteria and HDAC3 at 8 weeks showed that the level of *A. muciniphila* was positively correlated with HDAC3 expression ($r = 0.620$, $P = 0.037$) (Fig. 7j).

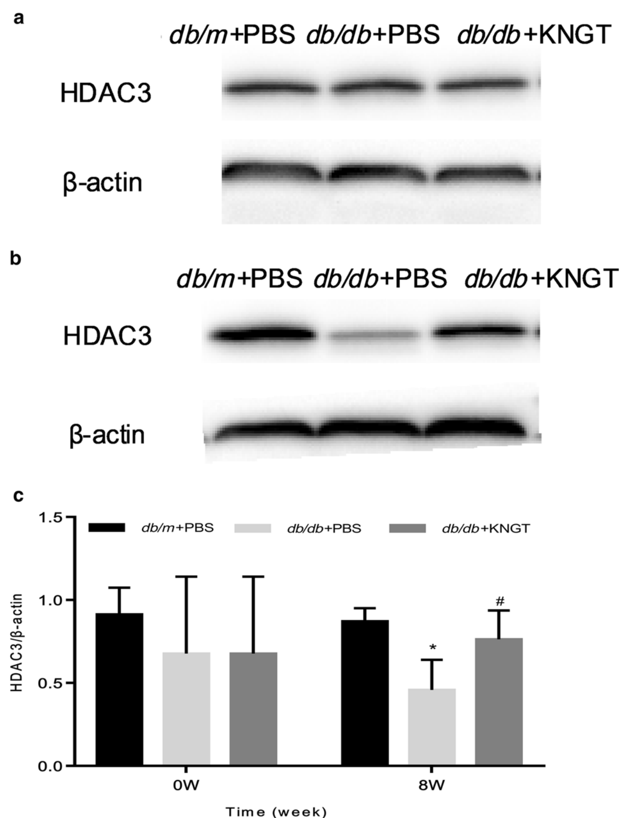

Fig. 6 HDAC3 protein expression in mouse colonic samples. Total protein was extracted from mouse colon specimens at different time points (**a**: 0 weeks, **b**: 8 weeks), and the levels of HDAC3 were analyzed by using Western blot. The signal intensity was quantified by using densitometry and is expressed relative to β-actin expression (**c**). Values are the mean ± S.D. $^{*}P < 0.05$ compared with *db/m* + PBS mice. $^{\#}P < 0.05$ compared with *db/db* + PBS mice.

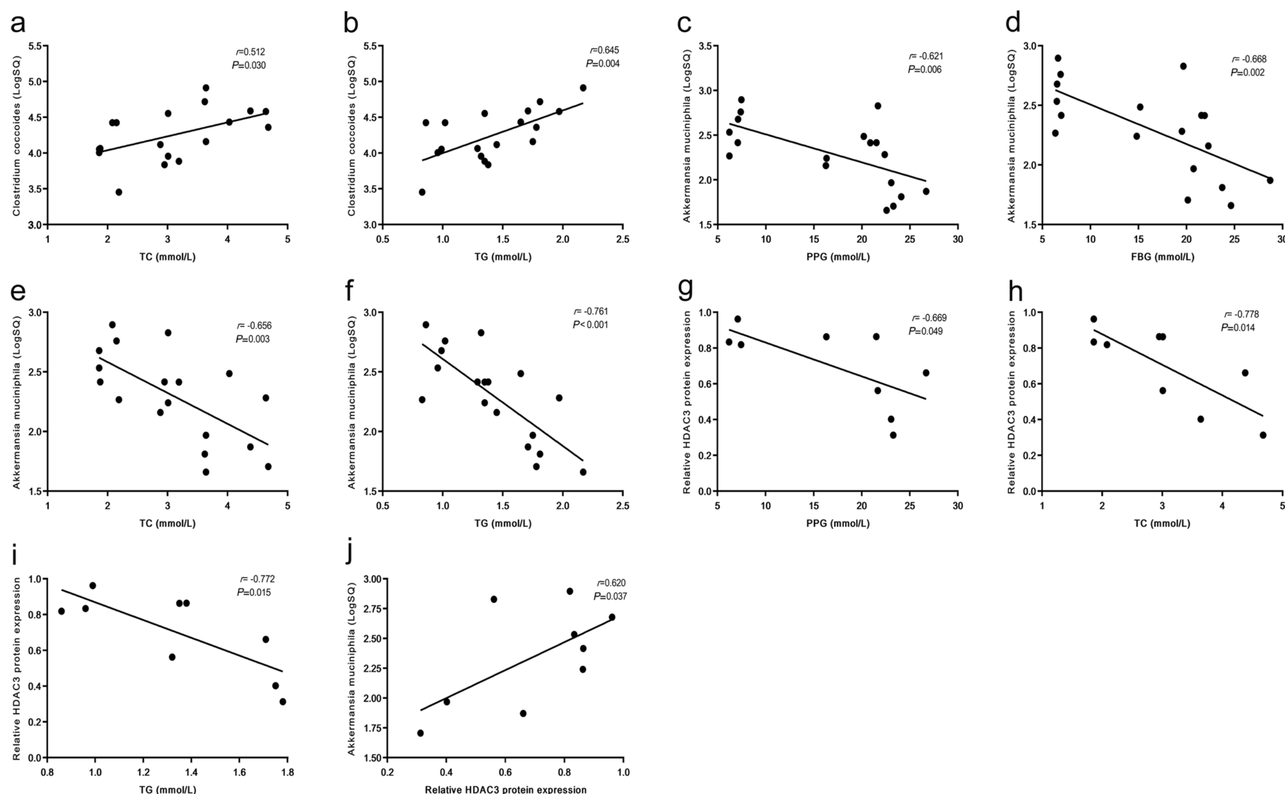

Fig. 7 Correlation between intestinal target bacteria and glycolipid metabolism parameters in *db/db* mice treated with fecal bacteria from KNGT on week 8 (**a**–**f**). Correlation between HDAC3 expression and glycolipid metabolism parameters (**g**–**i**). Correlation analysis between intestinal target bacteria and HDAC3 expression (**j**). The results were analyzed by using Pearson's correlation analysis. *P* values < 0.05 were considered significant.

DISCUSSION

Genetic and environmental factors can lead to T2DM. Changes in the intestinal microbiome composition attributed to age and diet are also an important factor that can induce diabetes [28]. Our previous study showed that the prevalence of T2DM in Kazaks was 1.56%, which was lower than that of other ethnic groups [29]. Therefore, we hypothesized that *db/db* mice given fecal symbiotic bacteria solution of KNGT by intragastric administration (FMT of KNGT) could improve the symptoms of diabetes. The core flora will be further explored to provide a new target for the treatment of diabetes.

In this study, *16S rRNA* gene sequencing showed that the composition of the intestinal microbiome changed and that the microbiome richness decreased significantly in the *db/db* mice compared with that of the *db/m* mice. The species abundance was also different between *db/m* + PBS and *db/db* + PBS samples. However, the α- and β-diversity analysis showed little difference after FMT from KNGT. We speculate that this was likely caused by the small sample size.

Desulfovibrio spp. are the dominant bacteria of the sulfate-reducing bacteria in the human colon and can promote interleukin-6 (IL-6) and IL-8 secretion and induce an inflammatory response [30], which may be related to the chronic low-grade inflammation of T2DM. *Desulfovibrio* increased in the intestinal tracts of mice with impaired glucose tolerance, especially in mice fed a high-calorie diet [31]. *C. coccoides* is the main community of Firmicutes. Most of the bacteria in this group can help the host absorb energy from the diet, increasing the occurrence of metabolic diseases, such as obesity and diabetes [32]. Obesity and type 2 diabetes are also associated with circulating insulin and glucose levels. A study in humans found that the abundance of *C. coccoides* in feces samples negatively correlated with insulin levels [33]. A higher abundance of *C. coccoides* causes lower insulin levels, which may lead to higher FBG and therefore increase the risk of obesity and diabetes [32, 33].

RT-PCR based on the *16S rRNA* gene was performed to further accurately analyze the target bacteria. The results showed that the levels of *Desulfovibrio* and *C. coccoides* in the feces of the *db/db* mice were higher than those of the *db/m* mice, and the levels were significantly reduced when the *db/db* mice were treated with fecal bacteria from KNGT. Our results also showed a positive correlation between the level of *C. coccoides* and glycolipid levels in mice, suggesting that *C. coccoides* may be a primary cause of obesity in diabetic mice.

It has been reported that the level of *A. muciniphila* negatively correlates with the levels of diabetic risk factors, such as blood glucose and TG [34]. The number of *A. muciniphila* decreases in T2DM mice. However, this decrease could be restored by probiotic therapy [35]. In this study, the level of *A. muciniphila* was lower in the feces of *db/db* mice compared with the level in the *db/m* mice, and the level significantly increased in *db/db* mice when treated with fecal bacteria from KNGT, which is consistent with previous studies. Moreover, there was a significant negative correlation between *A. muciniphila* and glycolipid levels in mice, suggesting that *A. muciniphila* is the main intestinal probiotic that improves metabolism in diabetes.

HDAC3 is one of the four members of the human class I histone deacetylases that regulate gene expression by deacetylating histones. *A. muciniphila* leads to increased expression of HDAC3 [11], which can activate brown fat cells to burn sugar and fat, thereby increasing metabolism and allowing animals to lose weight and counteract diabetes [36]. In this study, the results showed that the level of *A. muciniphila* was increased, and that HDAC3 protein expression was upregulated in *db/db* mice treated with FMT from KNGT. We also showed that HDAC3 upregulation correlated with glycolipid metabolism. Our study suggests that *A. muciniphila* may affect the metabolism of the animal by regulating HDAC3 expression.

FMT has been recognized as a common and effective strategy to change the composition of the intestinal microbiome. It has been demonstrated that intragastric administration of *Prevotella copri* induces insulin resistance and aggravates glucose intolerance in mice [37]. FBG levels were significantly reduced in diabetic patients treated with complex probiotics [38]. Probiotic supplementation also improves glucose tolerance and decreases fat content [39].

In conclusion, our study demonstrates that FMT from individuals with NGT significantly improves the intestinal microbiome composition and reduces plasma glycolipid levels in *db/db* mice. Specifically, we found an increase in the level of *A. muciniphila*, which correlated with HDAC3 expression. Our study suggests that fecal bacteria from KNGT could be used as a potential source for FMT to treat T2DM patients.

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AUTHOR CONTRIBUTIONS

YW, LLL, and PPZ designed the study. PPZ, XH, QWL, and XHZ conducted the studies. PPZ analyzed the data. PPZ, JJL, YW, and LLL wrote the paper.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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