ORIGINAL RESEARCH

Bile Acid Injection Regulated Blood Glucose in T2DM Rats Via the TGR5/GLP-1 Rather than FXR/FGF15 Pathway

Xing Zhu, MM; Zhen Chen, MM; Bin Zhang, MM; Siqi Xie, MM; Mingchang Wang, MM

ABSTRACT

Background and Aims • Type 2 Diabetes mellitus (T2DM) has reached an epidemic status worldwide. Targeting bile acid signaling has therapeutic potential for treating T2DM. However, the effect of bile acid on T2DM and related mechanisms remains unclear. Here, we explored the role of bile acid in T2DM and elucidated the mechanisms involved.

Methods • We established an STZ-induced rat model of T2DM and divided it into an bile acid-treated group and saline control group according to the random number table method. We incubated the bile acid-treated group with human bile acid via middle small intestine intubation and the saline control group was incubated with the same amount of normal saline. We compared the fasting body mass, fasting blood glucose (FBG), 2-hour postprandial blood glucose (2h-PG), fasting plasma insulin (FINS), fasting plasma triglyceride (TG), cholesterol, and total bile acid levels between the two groups one week before surgery and one to four weeks after surgery. Mechanically, Western blot, IHC, and ELISA assays were employed to detect the effect of bile acid on the TGR5/GLP-1 and FXR/FGF15 pathways.

Results • Bile acid injection could increase the FINS level and decrease the 2h-PBG level of T2DM rats. In addition, bile acid injection did not affect FBG, fasting body mass, TG, CH, and total bile acid. At the same time, bile acid injection could activate the TGR5/GLP-1 pathway but could not influence the FXR/FGF15 pathway.

Conclusion • Bile acids treatment promotes glucose homeostasis in the STZ-induced T2MD rat model via the following mechanism by activating the TGR5/GLP-1 signaling pathway rather than FXR/FGF15 pathway to improve glucose tolerance and thus achieve glucose homeostasis. The bile acid/TGR5/GLP-1 signaling pathway may be a crucial mechanism of controlling the blood glucose of T2DM rats, and TGR5/GLP-1 pathway may constitute novel targets for treating T2DM. (*Altern Ther Health Med.* 2024;30(12):480-485).

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic disease with multiple systemic complications.[1] The obesity epidemic is developing into an increase in the prevalence of T2MD by 54% by 2030.[2] T2DM patients have an increased risk for cardiovascular disease (CVD), the leading cause of death in Western countries.[3] Generally, treatments for T2DM include diet control, moderate exercise, hypoglycemic and lipid-lowing agents. Despite the therapeutic advantages of the T2DM treatment, multiple drugs can produce unexpected adverse effects. Considering the pathogenesis of T2DM, bile acids have become the essential resource of new agents for T2DM treatment.[4] Bile acid is an essential participant in glucose and lipid metabolism, and its dysregulation will lead to diabetes, dyslipidemia, and other metabolic diseases, suggesting that its pathway needs to be strictly controlled.[5,6] Although recent research has extensively observed bile acid, little is known about its regulatory networks, especially blood glucose regulation endogenous mechanisms. Herein, we explored the function of bile acid treatment and the related mechanisms in T2DM rats.

Bile acids are cholesterol-derived metabolites that promote the intestinal absorption and transport of dietary lipids.[7] Lately, bile acids have also determined as vital signaling molecules regulating glucose, lipid, and energy metabolism via interacting with the nuclear hormone farnesoid X receptor (FXR) and Takeda G protein receptor 5 (TGR5) in several organs.[8,9] FXR deficiency mice have improved hepatic triglycerides, cholesterol, a proatherogenic lipid profile, decreased bile acid pool, and promoted fecal bile acid

production, suggesting FXR is a significant regulator of bile acid and lipid metabolism.[10-12] The function of FXR in the regulation of glucose metabolism is debatable. TGR5 plays an essential role in the regulation of glucose homeostasis. Stimulation of TGR5 activates the secretion of glucagon-like peptide-1 (GLP-1) from enteroendocrine L-cells to generate insulin secretion from β-cells and improves insulin sensitivity.[13] Imbalances in bile acid metabolism and signaling are linked to obesity and T2DM. However, treating T2DM patients with bile acid sequestrants or bariatric surgery in severely obese patients shows remarkable improvements in glycemic response due to changes in the bile acid profile and pathway.[14,15]

This research indicated that the bile acid signaling pathway plays a crucial role in glucose regulation, but the effect of bile acid supplementation on glucose regulation and related mechanisms remains unclear. In this study, we explored the role of bile acid in T2DM and elucidated the mechanisms involved.

MATERIALS AND METHODS

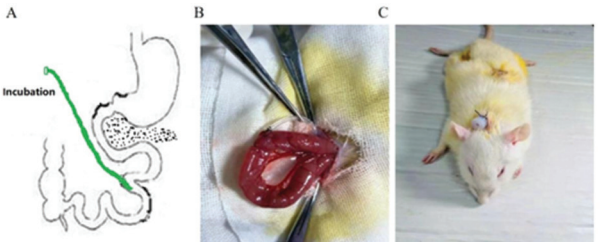
Materials

We established an STZ-induced rat model of T2DM and divided it into an bile acid-treated group and saline control group (10 rats/group) according to the random number table method. SD rats were obtained from the Shanghai SLAC laboratory animal Co. Ltd, and 40 rats were raised in the barrier facility at the experimental animal center of Fujian Medical University. Rats were fed under such conditions: ambient temperature with 20°C to 25°C and humidity with 30% to 50%. FINS ELISA kit was obtained from Shenzhen Anti Biotechnology Co. Ltd (AT0689). TG ELISA kit was obtained from Switzerland ALEXIS Company (YB-E12309). CH ELISA kit was obtained from Nanjing SenBeiJia Biological Technology Co., Ltd. (SBJ-R0194). GLP-1 ELISA kit was purchased from Wuhan Elabscience Biotechnology Company (E-EL-R3007). In enteral FGF15 ELISA kit was obtained from Wuhan USCN Co. Ltd (CEL154Ra). A Bile Acid Assay Kit (Colorimetric, ab239702) was obtained from Abcam. Rabbit anti-FXR antibody was purchased from Beijing Bioss (bs-5528R). Rabbit anti-TGR5 and goat anti-ABST antibodies were obtained from Abcam (ab72608 and ab134837). Rabbit anti-CYP7A1 antibody was purchased from Wuhan ABclonal (A10615). Rabbit anti-ASBT antibody was obtained from Abcam (ab82170). Streptozotocin (STZ) was obtained from Sigma (S0130). The Animal Welfare Committee of Fujian Medical University reviewed and approved this study protocol, approval number IACUC FJMU2022-0511.

Rat Model

The rats were fed adaptively for one week and then given a high-sugar and high-fat diet (60% normal diet, 10% lard, 10% egg yolk powder, and 20% sucrose) for four weeks. After four weeks, STZ solution was intuitionally injected at 25mg/kg dissolved in 0.1mmol/L citric acid-sodium citrate buffer at pH=4.0, injected once a day for two days. Blood samples from the tail vein were collected 72 hours and 1 week after injection to detect random blood glucose. The model was determined to be successful when random blood glucose ≥16.7mmol/L was screened twice.

Figure 1. Schematic diagram of small intestine intubation. A: The schematic diagram of small intestine intubation. B: Image of small intestine intubation of Rats. C: Schematic diagram of the end of small intestinal intubation in rats.



Small intestine catheterization

Rats were fed fasting and water abstinence a day before surgery, and isoflurane was continuously inhaled during surgery. Incubation of the middle and lower small intestine: 1.7mm*0.6mm (outer diameter 1.7mm, inner diameter 0.6mm)(mm: millimetre) capillary silicone tube was inserted into the 5# scalp needle and fixed, and then washed with normal saline after soaking and disinfecting with iodophor. The capillary silicone tube end was placed 45cm away from the ileocecal part. The needle end of the scalp was drawn from the subcutaneous tunnel from the abdominal wall to the posterior neck and fixed (Figure 1). Starting from the 6th day after surgery, 1.5mL of bile was injected from the posterior neck switch in the experimental group, and 1.5ml of normal saline was injected in the control group. Human bile was extracted from the common bile duct T tube after drainage, taken for 5 consecutive days, mixed evenly, centrifuged, and divided into EP tubes, and stored in a refrigerator at -20 for later use. The bile acid concentration was 12960 ummol/L, and the bile bacteria culture was negative. In the experimental group after the operation, one rat died due to intestinal leakage, and two rats were unable to continue because the tube was bitten. In the control group after the operation, one rat died due to intestinal obstruction, one rat died due to a progressive increase of blood glucose complicated with infection, and one rat was bitten and unable to continue.

Determining the fasting body mass

Following an overnight (12h) fast, an electronic body mass meter was employed to measure the body mass of rats in the two groups 1week before surgery and 1 to 4 weeks after the injection of bile or normal saline.

Fasting blood glucose (FBG) and 2-hour postprandial blood glucose (2h-PBG) measurement

Following an overnight (12h) fast, blood samples were collected from rat through the tail vein. The fasting blood glucose level was measured using a microglycemic meter 1

week before surgery and 1 to 4 week after the injection of bile or normal saline.

After two hours of free eating, blood samples were collected from the rat through the tail vein. The fasting blood glucose level was measured using a microglycemic meter 1 weeks before surgery and 1 to 4 week after the injection of bile or normal saline.

ELISA assay

The ELISA experiment employed to detect the expression level of fasting plasma insulin (FINS), TG, CH, total bile acid, portal vein plasma GLP-1, and FGF15. Following an overnight (12h) fast, blood sampless were collected from rat through the orbit into EDTA tube. The fasting plasma insulin (FINS) level was detected using FINS ELISA kit 1 week before surgery and 5 weeks after the injection of bile or normal saline. The procedures were performed according to the instructions of the ELISA kit.

Following an overnight (12h) fast, the rats were allowed to eat and drink freely for an hour, blood sample were collected from rats through the portal into tubes with DDP4 inhibitor and EDTA, the levels of GLP-1 and FGF15 were examined by ELISA assay 5 weeks after the injection of bile or normal saline. The procedures were performed according to the instructions of the ELISA kit.

Western blot assay

Total protein was extracted from the right lobe of the liver of rats in each group, and the protein concentration was determined by BCA kit. Proteins were transferred to the PVDF membrane by gel electrophoresis and blocked with 5% solution of fat-free milk for 2 h, incubated with the GAPDH and CYP7A1 antibodies overnight at 4°C, washed with TBST for 30 minutes, then the PVDF membrane was incubated with secondary antibody diluent for 2 h, and washed with TBST for 30 minutes. An enhanced chemiluminescent (ECL) luminescence solution was used to visualize the bands, and GAPDH was used as a reference for data analysis.(Analysed by Image J software)

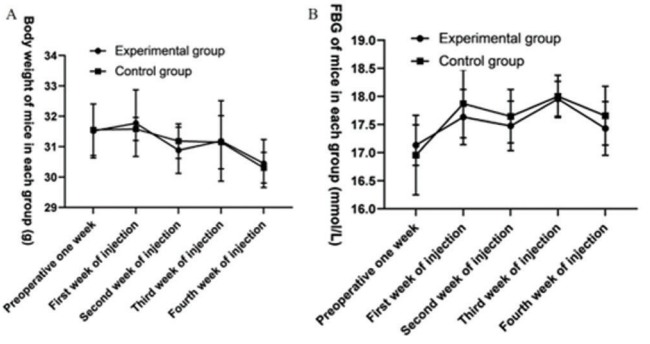
Immunohistochemical

Immunohistochemical (IHC) analysis was performed using the streptavidin-peroxidase (SP) method. The tissue of the terminal ileum was paraffin-embedded and sectioned, and then dewaxed, rehydrated, and antigen retrieved Tris-EDTA buffer, pH 9.0, in a steamer for 10 min. The TGR5, FXR, and ABST antibodies at a dilution of 1:50 were used for detection. The staining was evaluated by scanning the section under low magnification (twofold) and confirmed under high magnification (twofold). 0–4 semi-quantitative system was used to evaluate the expressions.

Statistical analysis

All statistical analyses were performed with SPSS statistics software, version 19.0 (SPSS, Chicago, IL), and the data were presented as mean±SD. Body weight, FBG, 2h-PBG, TG, CH, and total bile acid were compared using repeated measures analysis of variance (ANOVA) with a Bonferroni post-t test. One-way ANOVA and independent tests were used to compare other parameters. $P < .05$, and all P values were two-tailed.

Figure 2. Bile acid treatment had no effect on FBG and fasting body mass. A: One week before surgery and at the 1st, 2nd, 3rd, 4th week after injection of bile acid or normal saline, the FBG of experiment and control group were not significantly affected. B: One week before surgery and at the 1st, 2nd, 3rd, 4th week after injection of bile acid or normal saline, the fasting body mass of experiment and control group were not significantly affected.



Note: Significant P values marked by: [a]$P < .05$ and [b]$P < .001$.

Abbreviations: Experiment group, bile acid-treated group; Control group, saline control group.

RESULTS

Bile acid treatment did not affect FBG and fasting body mass

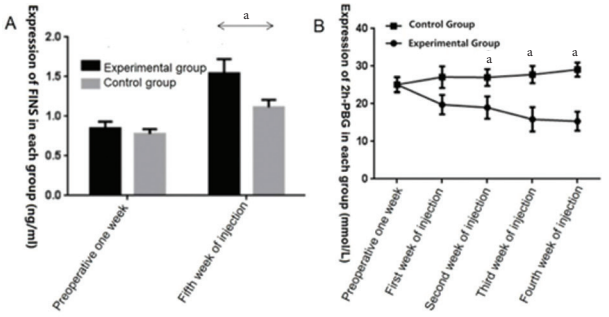
To verify the effect of bile acid on blood glucose and body weight, we compared the fasting blood glucose (FBG) and fasting body mass (MATERIALS AND METHODS) between the two groups one week before surgery and 1st to 4th weeks after the injection of bile acid or normal saline. Bile acid treatment did not significantly affect FBG or fasting body mass at any measured time point post-treatment compared to controls (Figure 2) ($P > .05$).

Bile acid supplementation could decrease the blood glucose level of rats

To elucidate the function of bile acid in blood glucose regulation, we detected the fasting plasma insulin (FINS) and 2-hour postprandial blood glucose (2h-PBG) of rats one week before surgery and 1st to 4th weeks after the injection of bile acid or normal saline. The results showed that the FINS level of the experiment group was significantly higher than that of control group after 5 weeks injection of bile acid or normal saline (Figure 3A) (1.545±1.782 *vs* 1.114±0.935, $P < .05$), but there was no statistically significant of FINS between the two groups one week before surgery (Figure 3A) (0.847±0.880 *vs* 0.780±0.226, $P > .05$);

As to 2h-PBG, at the second, third, and fourth week of injection, the 2h-PBG level of experiment group was

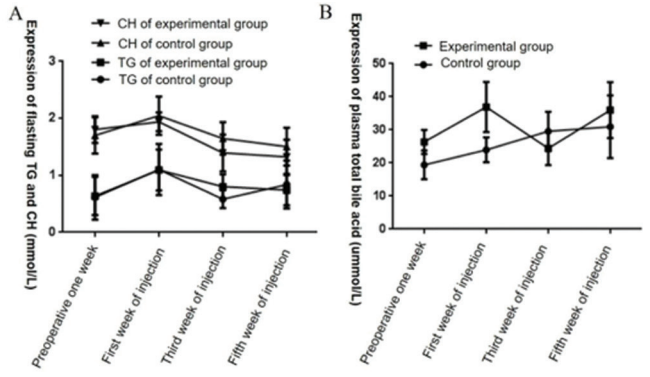
Figure 3. Bile acid supplementation could decrease the blood glucose level of rats. A: There was no statistically significant of FINS between the two groups one week before surgery, but a higher level of experiment group than that of control group after 5 weeks injection of bile acid or normal saline. B: One week before surgery, the 2h-PBG level of experiment and control group had no statistically significant and at the second, third, and forth week of injection, the 2h-PBG level of experiment group were significantly decreased compared with that of control group, and also significantly lower than that of one week before surgery at the third and fourth week of injection.



Note: Significant *P* values marked by: [a]$P < .05$ and [b]$P < .001$.

Abbreviations: Experiment group, bile acid-treated group; Control group, saline control group.

Figure 4. Bile acid treatment had no effect on blood lipid and total bile acid in T2DM rats. There was no statistical significance of TG, CH, and total bile acid between the experiment and control group one week before surgery and after the injection of bile acid or normal saline at the 1st, 3rd, and 5th weeks.



Note: Significant *P* values marked by: [a]$P < .05$ and [b]$P < .001$.

Abbreviations: Experiment group, bile acid-treated group; Control group, saline control group.

significantly decreased compared with that of the control group (25.1±5.2 vs. 27.0±5.9; 15.9±8.5 *vs.* 27.8±6.0; 15.4±6.7 *vs.* 29.1±5.0, all $P < .05$), and also significantly lower than that of one week before surgery at the third and fourth week of injection (Figure 3B) (19.0±7.8 *vs*25.1±5.2, $P > .05$; 15.9±8.5 *vs.* 25.1±5.2; 15.4±6.7 *vs.* 25.1±5.2, all $P < .05$). One week before surgery, the experiment group and the control group had no statistical significance (25.1±5.2 vs 25.1±5.3, $P > .05$); At the second, third, and fourth week of injection, the 2h-PBG level of the control group and one week before surgery had no statistically significant (Figure 3B) (27.0±5.9 vs 25.1±5.3; 27.8±6.0 *vs.* 25.1±5.3; 29.1±5.0 *vs.* 25.1±5.3, all $P > .05$).

These results clarified that bile acid treatment could increase the FINS level and decrease the 2h-PBG level of rats with T2MD, suggesting its important role in blood glucose regulation.

Bile acid treatment did not affect blood lipid and total bile acid in T2DM rats

To verify whether bile acid supplementation is involved in blood lipid regulation in T2DM rats, plasma triglycerides (TG), cholesterol (CH), and total bile acid were inspected one week before surgery and 1st, 3rd and 5th weeks after injection of bile acid or normal saline. As shown in Figures 4A and 4B, bile acid treatment did not significantly alter plasma triglycerides, cholesterol, or total bile acid levels compared to baseline or control values at any time point ($P > .05$). These analyses suggested that bile acid supplementation did not affect blood lipid regulation and total bile acid.

Bile acid treatment modulated blood glucose in T2DM rats via TGR5/GLP-1 pathway rather than the FXR/FGF15 pathway

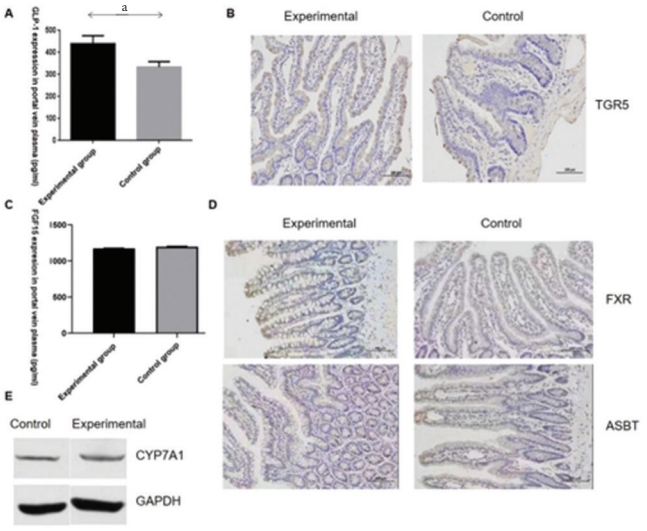
The GLP-1 and TGR5 expression significantly in the bile acid-treated group but not saline control group in the ELISA assay and IHC experiment.

TGR5/GLP-1 and FXR/FGF15 pathway are the main components of bile acid signaling.[16,17] To clarify the mechanism of bile acid regulating blood glucose in T2DM rats, we tested the effect of bile acid injection on the expression of the TGR5/GLP-1 pathway and FXR/FGF15 pathway. The result of the ELISA assay showed that the expression status of GLP-1 in the portal at one hour postprandial in the experiment group was significantly higher than that in the control group at 5th week after injection (Figure 5A) ($P < .05$). As shown in Figure 5B, IHC experiment demonstrated that TGR5 in the terminal ileum of the experiment group was significantly higher than that of the control group at 5th weeks after injection ($P < .05$).

There were no significant changes between the bile acid-treated group and saline control group in the ELISA assay and IHC experiment about the FXR/FGF15 pathway.

Western blot and ELISA experiments were employed to assess the effect of bile acid on the expression of FXR/FGF15 pathway. ELISA assay suggested that the expression level of FGF15 in the portal at one hour postprandial in the experiment group was no significant difference compared with that of the control group at 5th weeks after injection (Figure 5C) ($P > .05$). IHC and Western blot experiment showed that CYP7A1 in the liver and FXR, ABST in the terminal ileum of the experiment group were no significant difference compared with that of the control group 5th weeks after injection (Figure 5D, 5E) ($P > .05$).

Figure 5. Bile acid treatment modulated blood glucose in T2DM rats via TGR5/GLP-1 pathway rather than the FXR/FGF15 pathway. A: The expression status of GLP-1 in portal at one hour postprandial in experiment group was significantly higher than that in control group at 5th weeks after injection. B: IHC experiment demonstrated that TRG5 in the terminal ileum of experiment group was significantly higher than that of control group at 5th weeks after injection. C: ELISA assay suggested that the expression level of FGF15 in portal at one hour postprandial in experiment group was no significance difference compared with that of control group at 5th weeks after injection. D: IHC showed that FXR, ABST in terminal ileum of experiment group were no significance difference compared with that of control group 5th weeks after injection. E: Western blot experiment showed that FXR, CYP7A1 in liver and FXR, ABST in terminal ileum of experiment group were no significance difference compared with that of control group 5th weeks after injection.



Note: Significant *P* values marked by: [a]$P < .05$ and [b]$P < .001$.)

Abbreviations: Experiment group, bile acid-treated group; Control group, saline control group.

DISCUSSION

T2DM has reached an epidemic status worldwide. T2DM patients are insulin-resistant and glucose-intolerant in skeletal muscle, adipose tissue, and liver. Consequently, as the disease progresses, hyperglycemia can cause blindness, kidney failure, atherosclerosis, stroke, and liver diseases.[18] Alterations of bile acid homeostasis and dysbiosis can cause diabetes.[19] In this sense, several bile acids have been determined as potential therapeutic targets to cure T2MD. Therefore, identifying the potential role of bile acid in glucose regulation and involved mechanisms is essential for improving clinical treatment. In our study, BA injection did not affect fasting blood glucose and fasting body weight but could increase FINS level and reduce 2h-PBG in T2DM rats. At the same time, BA injection had no significant effect on blood lipid levels in T2DM rats, and bile acids injection could increase the expression of TGR5 and GLP-1, indicating that bile acids might regulate glucose metabolism in T2DM rats via the TGR5/GLP-1 pathway.

Bile acids are a diverse class of cholesterol-derived, amphipathic molecules that act as detergents to accelerate the digestion and absorption of dietary lipids and as hormones with systemic endocrine effects.[20]

Recently, research has determined that bile acids are signaling molecules that influence some nuclear receptors, especially FXR and TGR5. These bile acid-activated receptors affect glucose and lipid metabolism.[21-23] Both conjugated and nonconjugated bile acids can bind FXR, with chenodeoxycholic acid (CDCA) being the most promising receptor antagonist.[24] FXR is ubiquitously expressed in tissues and organs, including the liver, gut, white adipose tissues, and heart, endowing bile acid-mediated regulation of multiple physiological affections.[25] FXR forms a heterodimer with retinoic X receptor (RXR), consequently inhibiting the expression of CYP7A1, the rate-limiting enzyme in BA biosynthesis, resulting in decreased hepatic transition of cholesterol to bile acids.[15] CYP7A1 overexpression in obese mice induces weight decrease and inhibits glucose intolerance, insulin resistance, dyslipidemia, and liver steatosis, indicating that hepatic expression of CYP7A1 reduces metabolic derangements related to obesity.[26,27] Knockout of FXR in obese mice resulted in weight loss and enhanced glycemic response and insulin sensitivity.[28]

On the contrary, lean mice lacking FXR manifest dyslipidemia related to impaired insulin sensitivity and attenuated glucose tolerance.[11] Hepatic bile acid-FXR signaling regulates postprandial glucose status via reduced liver gluconeogenesis and hepatic glycogen synthesis abduction. Mice research demonstrated that after eating, bile acids secretion leads to bile acid-FXR signaling in the liver, resulting in the activation of glycogen storage and suppression of hepatic glycolytic and lipogenic gene expression, such as carbohydrate-responsive element-binding protein (ChREBP) and sterol responsive element–binding protein 1 (SREBP1c).[11,29]

Significantly, multiple mice types of research indicate that the FXR pathway does not directly influence liver insulin sensitivity but affects peripheral insulin sensitivity in adipose tissue and skeletal muscle.[30] These studies favor a crucial role of the hepatic FXR signaling pathway regulating whole-body glucose and energy homeostasis. In our research, BAs did not affect the expression of FXR, FGF15, ASBT, and CYP7A1, suggesting that bile acid injection did not regulate blood glucose in T2MD rats through the FXR pathway.

TGR5 is a G protein-coupled receptor that s in several organs and tissues,including the intestine, gallbladder, brown and white adipose tissues, skeletal muscle, brain, and pancreas.[31] Bile acid stimulation of TGR5 results in cAMP secretion, which, on the other hand, stimulates protein kinase A (PKA) signaling in multiple tissues and cells.[32] Stimulation of TGR5 via bile acids facilitates GLP-1 production from intestinal L cells.[33] This peptide functions

on the pancreatic β cells and modulates glucose-stimulated insulin production. TGR5 pathway in intestinal L cells is indicated to promote mitochondrial oxidative phosphorylation, a rise in the ATP/ADP ratio, subsequent closure of the ATP-dependent potassium channel (KATP), and improved mobilization of intracellular calcium, resulting in GLP-1 production and enhancement in glucose homeostasis.[34] These results suggest that TGR5 could be a potential target for developing drugs for metabolism-related diseases. Our study found that bile acids injection could increase the expression of TGR5 and GLP-1, indicating that bile acids might regulate glucose metabolism in T2DM rats via the TGR5/GLP-1 pathway.

In conclusion, we found that bile acids treatment promotes glucose homeostasis in the STZ-induced T2MD rat model via the following mechanism: activating the TGR5/GLP-1 signaling pathway rather than FXR/FGF15 pathway to improve glucose tolerance and thus achieve glucose homeostasis. These results indicated that bile acid treatment is a promising therapeutic method to counteract glucose homeostasis disturbance in T2MD progression.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Xing Zhu: Writing and experiments; Bin Zhang, Siqi Xie, and Mingchang Wang: Data analysis; Zhen Chen: Participate in some experiments; Siqi Xie: Project design and thesis revision; Mingchang Wang: Project design and thesis revision

ACKNOWLEDGMENTS

Five-week-old SPF SD rats were housed at the Animal Experimental Center of Fujian Medical University.

DATA AVAILABILITY

Some or all data, models, or codes generated or used during the study are available from the corresponding author by request.

ETHICS STATEMENT AND CONSENT TO PARTICIPATE

The Animal Welfare Committee of Fujian Medical University reviewed and approved this study protocol, approval number IACUC FJMU2022-0511. All applicable institutional and/or national guidelines for the care and use of animals were followed.

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