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Biliary diversion increases resting energy expenditure leading to decreased blood glucose level in mice with type 2 diabetes

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Keywords
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ABSTRACT

Aims/Introduction: Type 2 diabetes mellitus is a group of metabolism abnormalities in carbohydrates and energy. Our aim was to investigate resting energy expenditure (REE) and blood glucose changes after biliary diversion in mice with diabetes.

Materials and Methods: Male mice with diabetes were randomly divided into biliary diversion and sham groups. REE was detected by indirect calorimetry, the levels of fasting blood glucose, total bile acids and triiodothyronine were analyzed. After mice were killed, the weight amount of brown adipose tissue (BAT) and gastrocnemius was measured, and the expression level of G protein-coupled bile acid receptor and type 2 iodothyronine deiodinase in BAT and gastrocnemius were examined.

Results: The two groups of mice were pair-fed, the bodyweights ($P < 0.001$) and the fasting blood glucose level ($P < 0.001$) in the biliary diversion group significantly decreased 24 weeks after surgery. The intraperitoneal glucose tolerance test ($P = 0.035$) and oral glucose tolerance test ($P = 0.027$) showed improvement in glucose tolerance after surgery. The REE level significantly increased 24 weeks after surgery ($P = 0.005$), the levels of total bile acids ($P = 0.014$) and triiodothyronine ($P < 0.001$) increased at the 24th postoperative week. The weight ratio of BAT ($P = 0.038$) and gastrocnemius ($P = 0.026$) in the biliary diversion group were higher than that in the sham group. The expression of G protein-coupled bile acid receptor in BAT ($P < 0.001$) and gastrocnemius ($P = 0.003$) were upregulated after surgery, and the type 2 iodothyronine deiodinase expression also increased in BAT ($P = 0.015$) and gastrocnemius ($P = 0.015$).

Conclusions: The REE level increased and the glucose metabolism improved in mice with diabetes after biliary diversion.

INTRODUCTION

Type 2 diabetes mellitus is still one of the most serious chronic disease that threaten public health[1]. It is a group of metabolic disorders, and energy metabolism disturbance is its important pathological feature in type 2 diabetes mellitus[2]. Normally, body energy mainly comes from the breakdown of carbohydrates and fatty acids in food, energy intake and expenditure maintain a dynamic balance under the fine regulation. In type 2 diabetes mellitus, the energy intake is higher than that of healthy people[3], but energy utilization is impaired, studies show that insulin resistance changes the expressions of genes involved in energy metabolism pathways[4]. Energy metabolism disorder can further lead to a series of behavioral, psychological and disease problems, such as fatigue, depression, immune diseases and even tumors.

In recent years, improvement of the metabolism in type 2 diabetes mellitus patients after metabolic surgery has been widely recognized[5–7]. Biliopancreatic diversion, which diverts the bile and pancreatic juice to the distal small intestine, is commonly recognized as the most effective procedure to improve diabetes[8]. Our previous study showed mere biliary diversion (BD) of biliary jejunostomy also improves glucose in type 2 diabetes mellitus patients[9], and indicated the possible role of increased bile acids (BAs) after surgery; however, the mechanism among them is still not clear.

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Resting energy expenditure (REE) changes could affect bodyweight and plasma glucose levels[10,11]. To our knowledge, there are few reports about REE change in type 2 diabetes mellitus patients after BD so far. An in-depth study of the relationship between energy metabolism and type 2 diabetes mellitus helps to clarify its pathogenesis, and therefore, we carried out a model of BD from the gallbladder to the jejunum in diabetic mice[12] that resulted in a similar effects to biliary jejunostomy. The present study focused on the energy change after BD, to verify the improvement of glucose, and investigated the possible role of postoperative energy changes on glucose. We hope that a better understanding of energy metabolism will lead to finding a new therapeutic strategy for the prevention and treatment of type 2 diabetes mellitus.

MATERIALS AND METHODS

Animals

For the present study, 8-week-old male *db*/*db* mice weighting 20–24 g were purchased from National Rodent Laboratory Animal Resources, Shanghai, China. The *db*/*db* mice developed typical clinical symptoms of diabetes, such as polydipsia, polyphagia and polyuria, which was an ideal model of type 2 diabetes mellitus. The mice were housed in a 12-h light/dark cycle (lights off at 10.00 hours) in a climate-controlled environment (18–22°C temperature and 50% humidity) in the animal center of Peking Union Medical College Hospital (Beijing, China). All mice had free access to tap water, and they were fed with a standard chow diet before the operations. After 1 week of acclimation, the mice were randomly divided into the BD group and the sham group, and biliary diversion procedures or sham surgery were carried out, respectively. All experiments and surgical preparations were carried out in accordance with the laboratory animal ethics committee of our hospital and the UK Animals (Scientific Procedures) Act.

Surgeries and postoperative care

The mice were fasted overnight and anesthetized with isoflurane. The abdominal skin was wiped with 75% alcohol and the abdominal cavity was accessed through a small medial laparotomy. The procedure was carried out using a ×15 surgical microscope, the common bile duct was ligated horizontally below the cystic duct opening using 9-0 needles with thread. Biliary diversion of the gallbladder to the jejunum (approximately 10 cm distal to the ligament of Treitz) was created by continuous suture using 9-0 needles with thread, and the anastomosis was 2–3 mm long. Abdominal wall was closed with 5-0 nylon suture, which was removed 7 days after the procedure. Sham operation involved the same incisions without the ligation of common bile duct and anastomosis of the gallbladder to the jejunum (Figure 1). All mice received analgesia postoperatively, and to ensure adequate healing of anastomosis, they were not allowed to eat or drink until 24 h after surgery. Approximately 24 h after surgery, mice started to drink. Regular chow was started on the third postoperative day. Early operative mortality (1 week postoperatively) was 50–70%, mostly due to anastomotic leakage. Mice survival over 1 week without complications was defined as a successful operation.

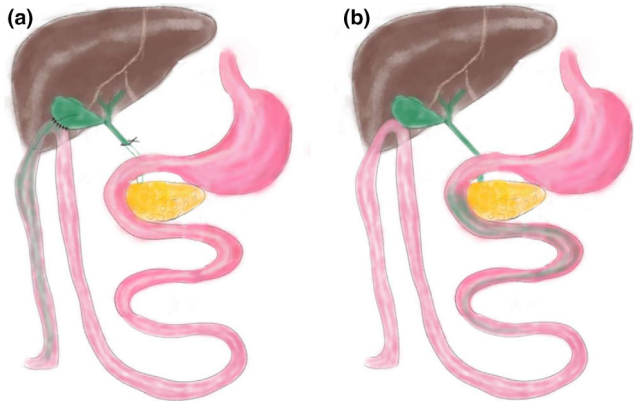

Figure 1 | Sketch image of biliary diversion of gallbladder to jejunum and sham surgery. (a) A sketch of biliary diversion of the gallbladder to the jejunum. The common bile duct was ligated and the gallbladder anastomoses with the distal jejunum, so bile did not flow through the duodenum and proximal jejunum. (b) A sketch of sham surgery. It involved the same incision without the anastomoses, and the physiological pathway of bile was not changed.

Food intake and bodyweight

Food intake and bodyweight were measured every 2 weeks. Two groups were pair-fed to reduce the potential impact of food intake and activity on energy metabolism. The mice were given 5% fat rat chow diet (Keao Xieli, Beijing, China) and had free access to water. Bodyweight was measured after overnight fasting. Then, the mice had free access to chow for 1 day, and the maximum 24-h food intake was measured in two groups. After that, the intake was calculated by subtracting the weight of the remaining food from the weight of the food provided. The mice were pair-fed thereafter.

Intraperitoneal glucose tolerance test and oral glucose tolerance test

The intraperitoneal glucose tolerance test (IPGTT) was carried out before surgery, and at the 8th, 16th and 24th weeks postoperatively. After fasting overnight, all mice received 2 g/kg glucose by intraperitoneal injection, and blood glucose was measured 0, 30, 60, 90 and 120 min after a glucose administration with a One Touch glucometer(Roche, Mannheim, Germany). The oral glucose tolerance test (OGTT) procedure was similar to that of IPGTT, except the glucose was received by oral administration.

Resting energy expenditure

We assessed energy metabolism of mice by indirect calorimetry. REE was calculated by the oxygen consumption (VO_2) under

thermoneutrality (30.0 ± 0.5°C) and post-absorptive conditions. After an overnight fast (10 h), individual mice were housed in an airtight glass bottle (1,000 mL), which was covered with sodium hydroxide at the bottom and connected to a U-type liquid pressure gauge. After half an hour of rest, 10 mL of oxygen was injected into the airtight glass bottle, then there was a pressure difference in the U-type liquid pressure gauge. As mice consumed oxygen, and the carbon dioxide they exhaled was absorbed by sodium hydroxide, the pressure difference in the pressure gauge gradually disappeared. When the water column on both sides reached the same level, the time taken to consume 10 mL oxygen was recorded; the measurement was repeated three times and the average was taken. REE was calculated using the equation: REE (kJ/m^2 h) = Q / S (Q is the quantity of heat per hour, S is the surface area), Q (kJ/h) = VO_2 (L/h) · 20.22 (VO_2 is the oxygen consumption per hour, and the corresponding oxygen heat value was 20.22 kJ/L). Body surface area was calculated using the Meeh–Rubner equation[13]: S (m^2) = 0.091 · W $(kg)^{2/3}$ (W is weight).

Biochemical tests

Blood glucose was measured through the tail vein every 2 weeks with a One Touch glucometer (Roche). Blood samples were collected by piercing the submaxillary vein monthly. At the end of the study period, the mice were anesthetized with isoflurane and blood samples were collected by eyeball extraction. After centrifugation at 1000 *g* at 4°C for 15 min, the plasma samples were separated immediately and stored at −80°C until further analysis. Enzyme-linked immunosorbent assay kits were used to measure insulin (Youersheng, Wuhan, China), glucagon-like peptide-1 (GLP-1; Youersheng), triiodothyronine (T3; Youersheng), and total bile acid (TBA; Youersheng). The homeostasis model assessment-insulin resistance (HOMA-IR) was calculated using the formula: HOMA-IR = FPG (mmol/L) × insulin (mIU/mL) / 22.5[14]. The levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured with a fully automatic biochemical analyzer (Roche).

Body compositions

All mice were euthanized after being weighed at the 24th postoperative week, and brown adipose tissue (BAT) of the scapular area, and gastrocnemius of the lower extremities were dissected and obtained. The samples were weighed immediately and stored at −80°C until further investigations.

Western blotting

Membrane G protein-coupled bile acid receptors (GPBAR1/TGR5) were extracted from fresh muscle and adipose tissue samples, and measured by Western blotting. Proteins were resolved by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and transferred onto 0.45-μm polyvinylidene fluoride membranes. Membranes were blocked with blocking buffer for 2 h and probed with anti-TGR5 antibody (Abcam, Cambridge, UK) at 1:10,000 dilution and anti-β-Actin (Cell Signaling Technology, Danvers, MA, USA) at 1:10,000 dilution overnight at 4°C. After washing with Wash Buffer three times, the membranes were incubated for 1 h at room temperature with goat anti-rabbit antibody (Zsgb-bio, Beijing, China) at 1:10,000. The relative concentration of protein was quantified by densitometry using the Versa Doc 1000 Imaging System and Quantity One 4.4 software (Bio-Rad, Hercules, CA, USA).

Ribonucleic acid extraction and real-time polymerase chain reaction

Total ribonucleic acid was extracted from the fresh muscle and adipose tissue samples using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer. Complementary deoxyribonucleic acid was synthesized by M-MLV reverse transcriptase (Invitrogen) from 5 μg of total ribonucleic acid. Real-time polymerase chain rection assay was carried out to detect the level of type 2 iodothyronine deiodinase (D2). Primers for D2 were from TSINGKE Biological Technology, forward primer 5′-AATTATGCCTCGGAGAAGA CCG-3′, reverse primer 5′-GGCAGTTGCCTAGTGAAAGGT-3′.

Statistical analysis

We carried out the statistical analysis using the SPSS Statistics software (version 24.0; IBM, Armonk, NY, USA) and drafted histograms using the GraphPad software (version 7.0; GraphPad Prism, La Jolla, CA, USA). Quantitative data are shown as the mean ± standard deviation, repeated-measures ANOVA was used to compare the differences between preoperative and postoperative, multivariate analysis was used to compare the differences between the BD group and sham group, the differences of body composition and grey value were determined by the unpaired *t*-test. *P*-values <0.05 were considered statistically significant.

RESULTS

Food intake and bodyweight

The food intake and bodyweights of the mice are shown in Figure 2. There were no significant differences in the maximum 24-h food intake ($P = 0.65$) or bodyweight ($P = 0.58$) before surgery. Two groups of mice were pair-fed, and there were also no significant differences in the maximum 24-h food intake between the two groups after surgery ($P = 0.83$). Whereas, the weights of the mice in the BD group were less than those of mice in the sham group beginning in the 10th week after surgery. At the 24th postoperative week, the weight in the BD group was 55.6 ± 1.3 g and that in the sham group was 59.9 ± 1.2 g ($P < 0.001$).

Effect on energy metabolism

REE was measured under standard conditions with indirect calorimetry. There was no significant difference between two groups before surgery (BD: 221.0 ± 13.3 kJ/m^2 h vs sham:

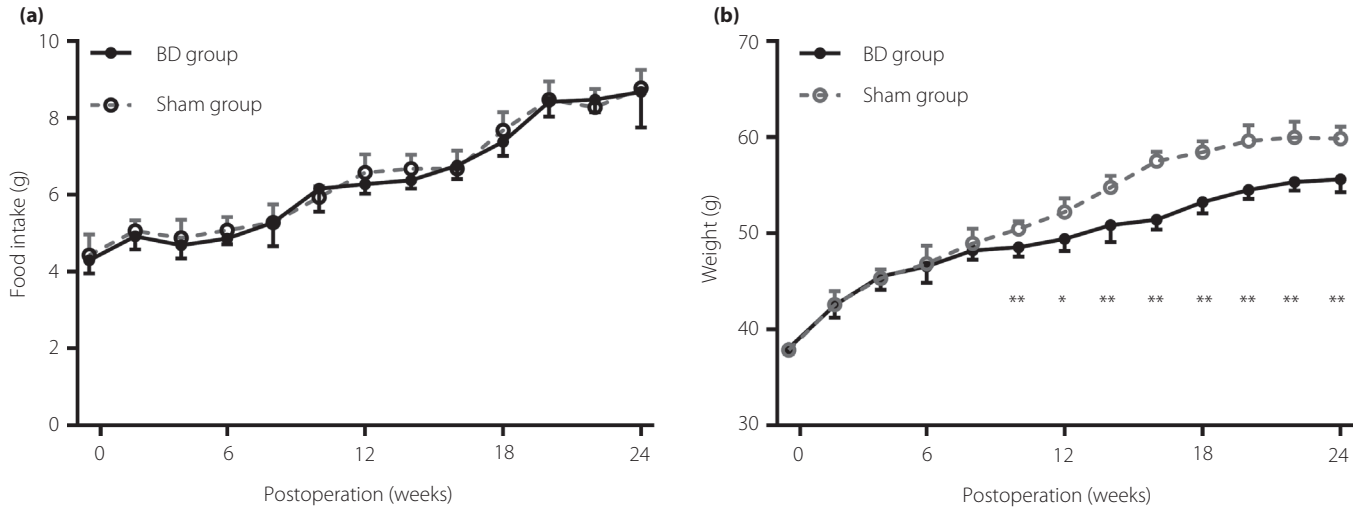

Figure 2 | The food intake and weights of mice in the biliary diversion (BD) and sham groups. (a) Food intake of mice in the two groups. There was no significant difference between the groups. (b) The bodyweights of mice in two groups. The bodyweights of mice in the BD group were less than those of mice in the sham group beginning in the 10th postoperative week. BD $n = 8$, sham $n = 9$. The data are presented as the mean ± standard deviation, $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the sham group.

223.3 ± 14.2 kJ/m^2 h, $P = 0.78$). After biliary diversion, REE increased in different degrees from the 4th postoperative week, except for the 8th and 10th weeks. The REE increased to 264.4 ± 16.7 kJ/m^2 h at the 24th week in BD group compared with the baseline level ($P = 0.004$), and higher than that in the sham group (211.8 ± 34.3 kJ/m^2 h, $P = 0.005$; Figure 3).

TBA and T3 tests

The TBA concentration was not different between the two groups before surgery (BD: 2.0 ± 0.5 μmol/L vs sham: 1.9 ± 0.2 μmol/L, $P = 0.49$). After biliary diversion, the TBA level significantly increased beginning in the 8th postoperative week, except for the 16th week; it increased to 4.6 ± 0.6 μmol/L in the BD group at the 24th postoperative week ($P = 0.015$) compared with the baseline, which was also higher than that in the sham group (2.6 ± 1.3 μmol/L, $P = 0.014$; Figure 4a). The fecal TBA level in BD group was 18.9 ± 4.3 μmol/day/100 g before surgery, it increased to 25.4 ± 6.5 μmol/day/100 g at the 24th postoperative week ($P = 0.026$), and was higher than that in the sham group (17.6 ± 17.2 μmol/day/100 g, $P = 0.034$).

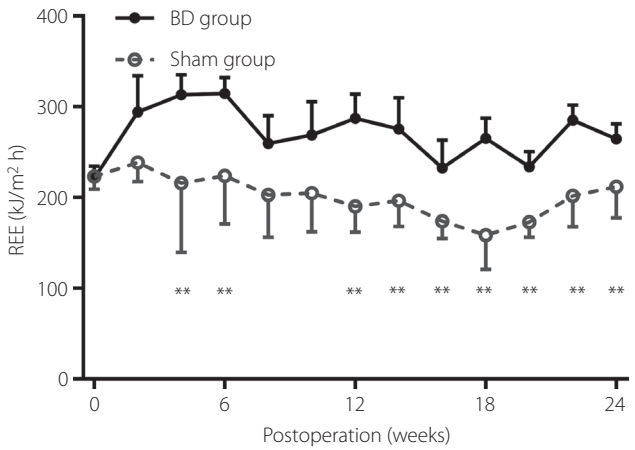

Figure 3 | Change of resting energy expenditure (REE) after biliary diversion in diabetic mice. The level of REE increased significantly in the biliary diversion (BD) group beginning in the 4th postoperative week, except for the 8th and 10th weeks. BD $n = 8$, Sham $n = 9$. The data are presented as the mean ± standard deviation, $^{**}P < 0.01$.

There was no significant difference of T3 level between the two groups before surgery (BD: 1.7 ± 0.8 ng/mL vs sham: 1.4 ± 0.6 ng/mL, $P = 0.58$). After biliary diversion, the T3 level increased beginning in the 4th postoperative week, except for the 8th week, it increased to 2.6 ± 0.4 ng/mL in the BD group at the 24th postoperative week ($P = 0.007$), and was higher than that of the sham group (1.3 ± 0.5 ng/mL, $P < 0.001$; Figure 4b).

Changes of fast blood glucose and glucose tolerance

There were no significant differences in fasting blood glucose (FBG) between the two groups before surgery (BD: 16.9 ± 1.1 mmol/L vs sham: 16.6 ± 0.6 mmol/L, $P = 0.68$). At the 24th postoperative week, the FBG in the BD group decreased to 13.9 ± 2.4 mmol/L compared with the baseline ($P = 0.002$), and was lower than that of the sham group (25.9 ± 2.2 mmol/L, $P < 0.001$; Figure 4c).

There was no difference in the area under the curve (AUC) of IPGTT before surgery (BD: 70.7 ± 5.5 mg/dL min vs sham: 72.9 ± 4.7 mg/dL min, $P = 0.76$). The BD group had significantly improved clearance of i.p.-injected glucose compared with that of the sham group. The AUC of IPGTT in the BD

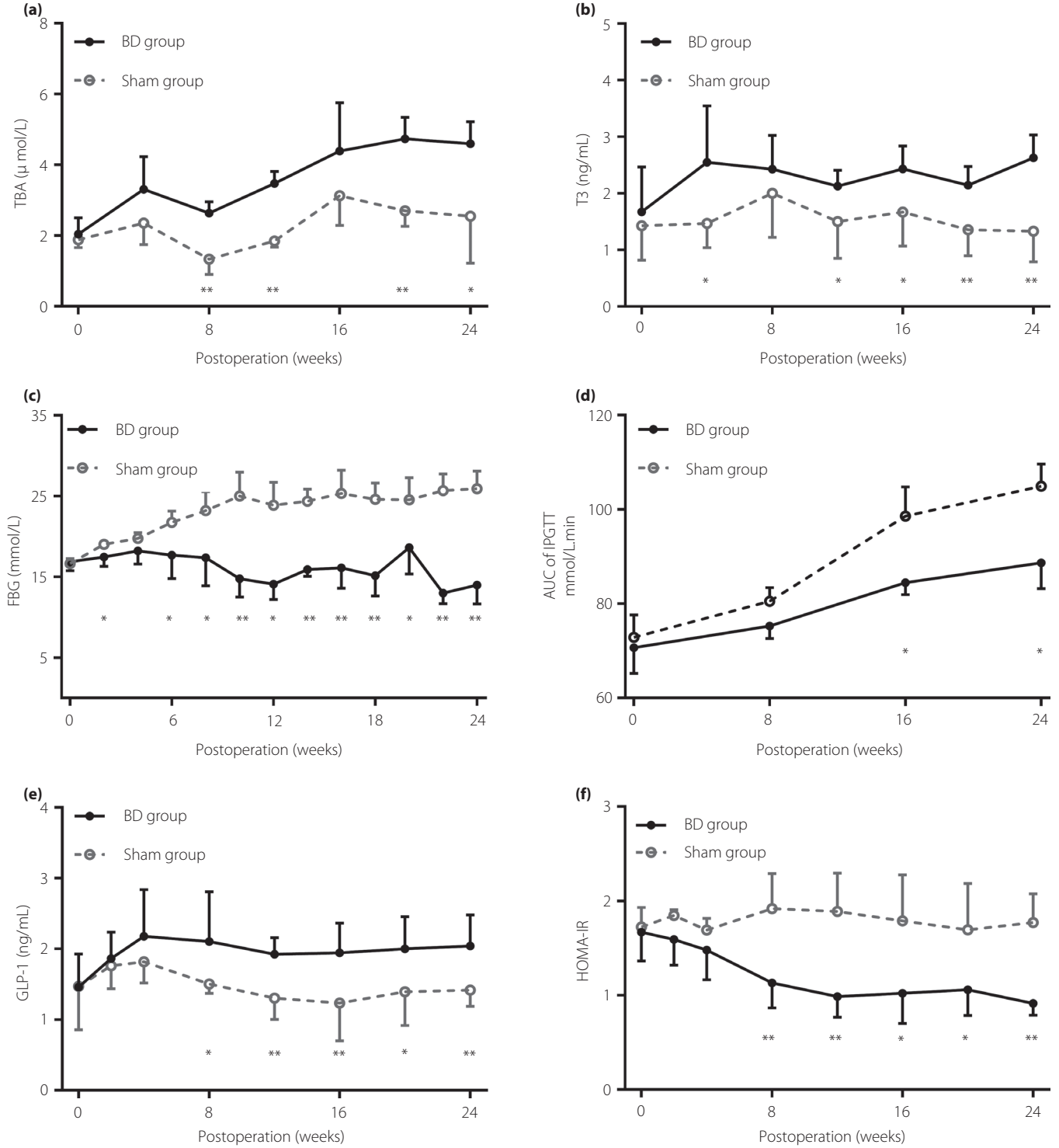

Figure 4 | Biochemical tests and changes of glucose metabolism. (a) The total bile acid (TBA) curve showing that the TBA level increased significantly beginning in the 8th postoperative week, except for the 16th week. (b) Triiodothyronine (T3) curve showing that the T3 level increased beginning in the 4th postoperative week, except for the 8th week. (c) The fast blood glucose (FBG) curve showing that FBG level decreased significantly in the biliary diversion (BD) group beginning in the 2nd postoperative week, except for the 4th week. (d) Changes of area under the curve (AUC) value of the intraperitoneal glucose tolerance test (IPGTT), the tolerance of glucose improved after BD at the 16th and 24th postoperative weeks. (e) Glucagon-like peptide-1 (GLP-1) curve showing that the GLP-1 level increased significantly beginning in the 8th postoperative week. (f) Homeostasis model assessment-insulin resistance (HOMA-IR) curve showing that the value of HOMA-IR decreased from the 8th postoperative week. BD $n = 8$, sham $n = 9$. The data are presented as the mean ± standard deviation, $^{*}P < 0.05$, $^{**}P < 0.01$.

group (82.1 ± 6.5 mg/dL min) was not different from that of the baseline ($P = 0.13$), but was significantly less than that of the sham group (104.9 ± 4.7 mg/dL min, $P = 0.035$) at the 24th postoperative week (Figure 4d). The AUC of OGTT between the two groups before surgery showed no difference (BD: 68.7 ± 6.1 mg/dL min vs sham: 67.6 ± 3.1 mg/dL min, $P = 0.88$). At the 24th postoperative week, the AUC of OGTT in the BD group (72.1 ± 6.5 mg/dL min) was similar to the baseline ($P = 0.64$), but was less than that of the sham group (89.7 ± 7.6 mg/dL min, $P = 0.027$).

Changes of insulin, GLP-1 and HOMA-IR

There was no significant difference of the insulin level between the groups after surgery. The GLP-1 level before surgery was not different between the two groups (BD: 1.5 ± 0.5 ng/mL vs sham: 1.5 ± 0.6 ng/mL, $P = 0.97$). After surgery, the GLP-1 level in the BD group increased to 2.0 ± 0.4 ng/mL at the 24th postoperative week ($P = 0.023$), which was also higher than that in the sham group (1.4 ± 0.2 ng/mL, $P = 0.002$; Figure 4e).

The average value of HOMA-IR in the BD group before surgery was 1.7 ± 0.3, and it was 1.7 ± 0.2 in the sham group with no difference ($P = 0.74$). After BD, the value of HOMA-IR in the BD group decreased from the 8th postoperative week and decreased to 0.9 ± 0.1 at the 24th postoperative week ($P < 0.001$), which was also less than that in the sham group (1.8 ± 0.3, $P < 0.001$; Figure 4f).

Effects on lipid metabolism

There were no differences in the level of TG ($P = 0.91$), TC ($P = 0.86$), HDL-C ($P = 0.75$) and LDL-C ($P = 0.89$) before surgery. At the 24th postoperative week, the TC level (BD: 2.3 ± 0.3 mmol/L vs sham: 3.4 ± 0.4 mmol/L, $P < 0.001$) and LDL-C level (BD: 0.13 ± 0.05 mmol/L vs sham: 0.21 ± 0.04 mmol/L, $P = 0.002$) were lower than that of the sham group. The TG level (BD: 0.38 ± 0.09 mmol/l vs 0.18 ± 0.17 mmol/L, $P = 0.009$) and HDL-C level (BD: 2.1 ± 0.5 mmol/L vs 1.6 ± 0.2 mmol/L, $P = 0.014$) were higher than that of the sham group.

Body composition

All mice were euthanized at the 24th postoperative week, the ratio of weight in BAT and gastrocnemius in the BD group were higher than that in the sham group. The ratio of the BAT was 2.5 ± 0.6 mg/g in the biliary diversion group, higher than that in the sham group (2.0 ± 0.4 mg/g, $P = 0.038$). The ratio of the gastrocnemius was 1.8 ± 0.4 mg/g in the BD group, higher than that in the sham group (1.3 ± 0.7 mg/g, $P = 0.026$; Figure 5a).

Expression of TGR5 and D2 in adipose tissue and gastrocnemius

The expression of TGR5 in the adipose tissue and gastrocnemius was measured by western blotting (Figure 5b). The relative expression level of adipose TGR5 in the BD group (0.7 ± 0.1) was higher than that in the sham group (0.4 ± 0.1, $P < 0.001$). The relative expression level of muscle TGR5 in the BD group (0.8 ± 0.1) was higher than that in the sham group (0.4 ± 0.3, $P = 0.003$).

The D2 expression in the adipose tissue and gastrocnemius was measured by real-time polymerase chain reaction (Figure 5c). The relative D2 expression of adipose in the BD group (4.6 ± 2.5) was higher than that in the sham group (2.3 ± 1.3, $P = 0.029$). The relative D2 expression level of gastrocnemius in the BD group (1.5 ± 0.5) was higher than that in the sham group (0.9 ± 0.4, $P = 0.015$).

DISCUSSION

Energy changes after biliary diversion in type 2 diabetes mellitus and the underlying mechanism have not been well investigated. Previous studies showed that gastrointestinal reconstruction and the following hormonal changes could decrease energy intake and bodyweight[15,16], increase sympathetic nerve stimulation of peripheral tissues, and increase the oxidized amounts of glucose and lipid[17]. Although, one study also showed that massive weight loss can dramatically decrease the REE[11]. Therefore, the change of REE after gastrointestinal reconstruction is still controversial and, to our knowledge, there are few reports about REE in diabetic mice after biliary diversion so far.

We chose decompensated diabetic mice to observe the effect of biliary diversion on energy metabolism, because there are many control mechanisms for energy balance, which might compensate the change caused by biliary diversion. The compensatory mechanism of glucose in the diabetic model is not well developed, which might reflect the potential causality. The present results showed that FBG and bodyweight decreased significantly after BD surgery. Mice were pair-fed, the food effect was negligible, and there was not fatty diarrhea and constipation after BD, the fecal properties between two groups were similar; furthermore, although bodyweight in the BD group was lower than that in the sham group, it was still increased over the time in both groups, so there was no significant malabsorption and the weight loss was not related to defecation. In addition, the level of REE and BAs increased in the BD group after surgery, which might be responsible for the improvement of FBG and the weight loss.

BAs are synthesized from cholesterol in the liver, stored in the gallbladder and secreted into the intestine when a meal is ingested. They account for catabolism of approximately 50% of the daily cholesterol output, but 95% of BAs are reabsorbed and transported back to the liver through the portal vein. This system is known as enterohepatic circulation[18]. Studies have shown that the TBA level increased remarkably after biliary diversion in mice and human[9,12], probably due to the higher expression of 7-α hydroxylase[19]. Some kinds of the component in TBA might play a key role in metabolic changes, such as chenodeoxycholic acid, which was reported to be the most effective signaling molecular for activation of BAT[20]. BAs also

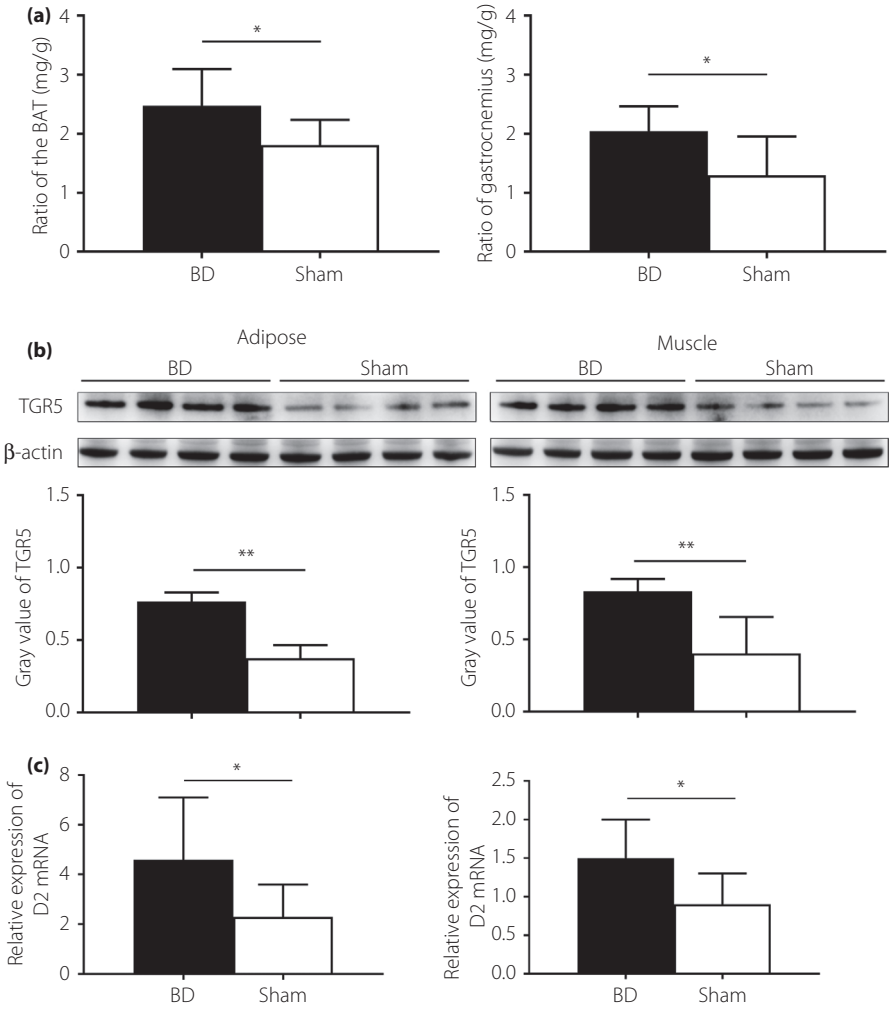

Figure 5 | Weight of brown adipose tissue (BAT) and gastrocnemius, and expression of (TGR5). (a) Ratio of BAT and gastrocnemius; the relative content of BAT and gastrocnemius in the biliary diversion (BD) group were higher than that in the sham group after surgery. (b) The western blot analysis of TGR5 (upper) and the relative expression level of TGR5 in the adipose tissue and gastrocnemius were higher than those in sham group after BD. The gray value of TGR5 expression is also shown (lower). (c) The real-time polymerase chain reaction showing type 2 iodothyronine deiodinase (D2) expression in adipose tissue and gastrocnemius was higher than those in the sham group after BD. The data are presented as the mean ± standard deviation, $*P < 0.05$, $**P < 0.01$. mRNA, messenger ribonucleic acid.

circulated faster after biliary diversion, because rhythmic functions of the gallbladder acting as a reservoir of bile and contractile pump were missing, BAs continuously secreted into the small intestine and influenced enterohepatic circulation. As a result, the BA pool might circulate more quickly and fecal bile loss is increased. The elevated and rapidly circulated BAs were the trigger for other subsequent metabolic changes.

BAs not only facilitate the intestinal digestion and absorption of dietary fat, steroids, drugs, and lipophilic vitamins[21], but it has been gradually identified that they could regulate various hormones and receptors, and modulate whole body metabolism as signaling molecules[22]. TGR5, which is expressed ubiquitously, is one of the widely investigated receptors of BAs[23]. Previous studies of TGR5 focus more on the enteroendocrine L cells in the ileum, which could stimulate the secretion of GLP-1 after activated by BAs, thereby improving liver and pancreatic function, and increasing insulin sensitivity and glucose tolerance[13,14,17]. In the present study, we found FBG was decreased after biliary diversion, glucose tolerance was enhanced and HOMA-IR was decreased. Improvement of glucose tolerance and insulin resistant should contribute to the decreased blood glucose; in addition, we hypothesized that increased energy expenditure resulting from the continuous activation of TGR5 was another reason for the glucose improvement.

TGR5 is highly expressed in BAT and skeletal muscle, which are two important organs for thermogenesis in both cold-exposed humans and rodents[24]. Activation of TGR5 in BAT and

skeletal muscle can directly promote mitochondrial uncoupling and regulate glucose homeostasis[20,25]. In BAT, activation of TGR5 by BAs induces D2 activation, which converts the inactive thyroid hormone thyroxine (T4) to active triiodothyronine (T3), thereby increasing BAT activity and energy expenditure in BAT[26], and promoting white adipose tissue browning[20,27]. Similarly, a study showed that after the stimulation of taurocholic acid in human skeletal muscle myoblasts, D2 activity was significantly and dose-dependently enhanced[26]. In the present study, we confirmed that TGR5 and D2 upregulated in adipose tissue and gastrocnemius after biliary diversion, and the T3 level increased significantly. In combination with the results of TBA and REE, we supposed that the increase of REE after biliary diversion was mainly achieved by the BAs–TGR5–D2–T3 pathway.

Furthermore, the present study also showed not only that TGR5 expression was elevated in BAT and gastrocnemius after biliary diversion, but the weight of BAT and gastrocnemius was also increased. There are two mainly heat-product ways to balance body temperature: (i) shivering thermogenesis comes from the contraction of skeletal muscles; and (ii) non-shivering thermogenesis comes from BAT. Normally, skeletal muscle produces approximately 20% of body energy, while that can be as high as 90% during vigorous exercise. In BAT, there are plenty of mitochondria, which can produce a large quantity of energy under certain conditions, this effect was specific for BAT and not observed in white adipose tissue. A study showed that continuous stimulation of BAs promoted white adipose tissue browning or energy consumption increasing[20]. We confirmed the increased amount of BAT and gastrocnemius, and bodyweight loss after biliary diversion.

The present study also showed that the levels of TG and HDL-C increased after BD, whereas the levels of LDL-C and TC decreased. It seemed that with the improvement in glucose metabolism, lipid metabolism also partially improved. Diabetic dyslipidemia and fatty liver are common comorbidities for diabetes, and have received much attention in recent years, but the underlying mechanism is still unclear[28]. We hypothesized that the improvement in insulin resistance leads to the partial alleviation of dyslipidemia, as insulin is involved in the production and secretion of several lipids[29]. A previous study also showed significant reductions in hepatic steatosis result from higher BAs level after BD in diet-induced obesity mice; and genes expression potentially regulated by BAs, such as lipoprotein lipase and BA transporters, were significantly upregulated[19].

Several metabolic surgeries, such as Roux-en-Y gastric bypass, duodenal-jejunal bypass and biliopancreatic diversion, have been shown to be beneficial for the control of diabetes. Although BD is not a classical metabolic surgery so far, it has fewer complications compared with the aforementioned surgeries, as there is less anastomosis and a short operative duration. More importantly, BD, which only involves the diversion in bile, could better investigate the mechanism of BAs and metabolic surgery. Furthermore, it is a new choice and more suitable for type 2 diabetes mellitus patients with benign biliary tract disease, such as bile duct cyst or recurrent refractory bile duct stones, not only treating the primary disease, but also improving the metabolic homeostasis without additional procedures.

The present findings suggested that BAs increased after biliary diversion, thereby continuously activating TGR5 in BAT and skeletal muscle, leading to increased REE and decreased FBG in diabetic mice. There were several limitations, more hormones and downstream mechanism need to be deeply investigated, and prolonged intervention studies are warranted. The improvement of glucose was a result of multiple mechanisms, the present study showed that increased REE probably induced by elevated BAs seems to be one of the effective methods. An in-depth understanding of energy metabolism in diabetes could help improve the procedure of metabolic surgery with fewer complications.

The REE level increased and the glucose metabolism improved in mice with diabetes after biliary diversion.

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DISCLOSURE

The authors declare no conflict of interest.

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