

# Effects of Sleeve Gastrectomy on Serum 12 $\alpha$ -Hydroxylated Bile Acids in a Diabetic Rat Model

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## Abstract

**Background** Diabetes mellitus is a prevalent disease that endangers human health. Bariatric surgery can effectively relieve insulin resistance with elevated serum bile acids (BAs). 12 $\alpha$ -Hydroxylated BAs were previously reported to be associated with insulin resistance. The aim of this study was to analyze changes in 12 $\alpha$ -hydroxylated BA composition and possible associated mechanisms in diabetic rats following sleeve gastrectomy (SG).

**Methods** SG and sham operations were performed in diabetic rats induced by high-fat diet feeding and streptozotocin. Body weight, food intake, oral glucose tolerance test (OGTT), insulin tolerance test (ITT), and serum BAs were analyzed at corresponding time points. Cholesterol 12 $\alpha$ -hydroxylase (CYP8B1) and transcription factor V-Maf Avian Musculoaponeurotic Fibrosarcoma Oncogene Homolog G (MAFG) expression levels were assessed by RT-PCR and western blotting.

**Results** Compared with the SHAM group, the SG group displayed significant weight loss from 6 weeks postoperatively, accompanied by decreased food intake from 4 weeks after the operation. At 2 and 12 weeks postoperatively, the areas

under the curve of OGTT and ITT were significantly decreased in the SG group. At 12 weeks post-operation, the SG group displayed elevated serum BAs, but the percentage of 12 $\alpha$ -hydroxylated BAs was reduced. Furthermore, SG rats exhibited higher MAFG and lower CYP8B1 protein and mRNA levels in the liver ( $P < 0.05$ ).

**Conclusion** The percentage of 12 $\alpha$ -hydroxylated bile acids was reduced after SG, which was relevant with the inhibition of CYP8B1 and overexpression of MAFG. These outcomes may play an important role in the improvement of insulin sensitivity following SG.

**Keywords** Bariatric surgery · Sleeve gastrectomy · Type 2 diabetes mellitus · Bile acids · Liver

## Introduction

Type 2 diabetes mellitus (T2DM) is a prevalent disease that endangers human health, and identifying methods to control the disease in a long-term and effective manner is a worldwide problem [1]. Bariatric surgery can not only effectively reduce body weight but also relieve insulin resistance rapidly and permanently, a finding that has been confirmed in clinical studies [2]. Furthermore, bariatric surgery has been included in the guidelines for treatment of T2DM [3, 4]. Sleeve gastrectomy (SG), the most widely used bariatric surgery [5], can dramatically alleviate T2DM [6], but the specific mechanisms remain unclear.

It was confirmed that after multiple bariatric surgical procedures, including SG, serum bile acid (BA) levels were elevated [7–9]. BAs, one of the main components of bile juice, are generated from cholesterol under the catalysis of several enzymes such as cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) and cholesterol 12 $\alpha$ -hydroxylase (CYP8B1). Additionally, BAs

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participate in the absorption of lipids and fat-soluble vitamins in the gut [10]. Moreover, BAs serve as signaling molecules to activate farnesoid X receptor and G-protein-coupled receptor, thereby inhibiting gluconeogenesis [11], increasing glucagon-like peptide 1 release, and improving body energy metabolism [12]. This indicates that elevated BA levels could improve insulin resistance. BAs include more than 20 components, and each component may have specific effects on body metabolism [13]. It has been demonstrated that 12 $\alpha$ -hydroxylated BAs might cause insulin resistance [14]. However, the composition changes and relevant mechanisms of 12 $\alpha$ -hydroxylated BAs after SG, which might play a significant role in the mechanism underlying SG-induced improvements in T2DM, remain unclear. The aim of our study was to analyze and explore 12 $\alpha$ -hydroxylated BA composition changes and associated mechanisms by following SG in T2DM Wistar rats.

## Materials and Methods

### Animals

Eight-week-old male Wistar rats (weight ranged 200–220 g, provided by the Laboratory Animal Center of Shandong University [Jinan, China]) were housed in a 12-h light/dark cycle under constant temperature ( $24 \pm 2$  °C) and humidity ( $60 \pm 10\%$ ) in independent ventilated cages. All rats were given access to clean water and a high-fat diet (HFD, 40% fat, Huafukang Biotech, China) for 1 month to induce insulin resistance and then were injected with streptozotocin (STZ, 35 mg/kg) (Sigma, USA) intraperitoneally. After 72 h, 20 rats with random blood glucose  $\geq 16.7$  mmol/l and that satisfied the inclusion criteria were randomly assigned to undergo SG ( $n = 10$ ) or sham procedures (SHAM,  $n = 10$ ). All study protocols were approved by the Animal Care and Utilization Committee of Shandong University.

### Surgical Procedures

Before each procedure, rats were fed 10% Ensure (Abbott, USA) for 2 days and then fasted for 12 h. SG group [15, 16]: rats were anesthetized with intraperitoneal injection of 10% chloral hydrate (3 ml/kg) before procedure. We performed an upper abdominal incision of approximately 5 cm, and the gastric omentum and lesser omentum were then dissected. After ligation and transection of the gastric omental vessels in the pylorus area, we used forceps to clamp the greater curvature in case of hemorrhage. The portion of the stomach outside the clamped area, which was approximately 70% of the whole stomach volume and included the gastric fundus, was resected. The stomach incision was sutured with 5-0 silk suture (Ningbo Medical Needle, China). The

abdomen was closed after leakage and hemorrhage was prevented. SHAM group [17]: a laparotomy was performed to expose the stomach and esophagus, and operative time was prolonged to mimic that experienced by the SG rats. Subsequently, the abdominal incision was closed.

### Weight and Food Intake

The weight and food intake were measured once per week from the very beginning until the second week post-operation. These parameters were then measured once every 2 weeks until the rats were sacrificed.

### Measurements on Remission of T2DM

**Oral Glucose Tolerance Test (OGTT)** Glucose was administered by intragastric gavage (1 g/kg) after a 12-h fast. Blood samples were obtained from the tail vein of conscious rats, and blood glucose was measured before and at 10, 30, 60, 90, and 120 min after intragastric gavage. OGTT was performed preoperatively and at 2 and 12 weeks after surgeries.

**Insulin Tolerance Test (ITT)** ITT was performed preoperatively and at 2 and 12 weeks after surgeries. After a 12-h fast, blood samples were collected from the caudal vein before and at 10, 30, 60, and 120 min after intraperitoneal injection of insulin (0.5 IU/kg).

### Serum BAs

At 12 weeks postoperatively, blood samples (1 ml) were collected from the retrobulbar venous plexus into chilled EDTA tubes containing a dipeptidyl peptidase IV inhibitor and centrifuged (3000 rpm) at 4 °C for 15 min. Serum was collected and stored for further measurements. (1) Total serum BAs were determined by the enzymatic cycling method. (2) Components of BAs, including alpha-muricholic acid ( $\alpha$ MCA), beta-muricholic acid ( $\beta$ MCA), cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA), lithocholic acid (LCA), taurine conjugates (taurine-, T-) of the aforementioned proteins, and hyodeoxycholic acid (HDCA), were measured by high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [18], which was previously used by our team in another study. The reference standards, including T $\alpha$ MCA, T $\beta$ MCA,  $\alpha$ MCA, and  $\beta$ MCA, were purchased from Steraloids (USA), while all others were from Sigma Aldrich (USA).

### Quantitative Real-Time PCR

Total RNA from rat liver was extracted using Trizol reagent (Invitrogen, USA) and reverse transcribed into cDNA in the presence of primers (TOYOBO, Japan). Quantitative real-

time reverse transcription PCR was performed in a LightCycler system (Roche Diagnostics, Germany), and the primers used were as follows:

CYP8B1: 5'-TCCATATGTCCCGGCAGGTTCTTT-3'  
(forward)

5'-TGTCAGGGTCCACCAGTTCAAAGT-3' (reverse)

MAFG: 5'-CCCAATAAAGGAAACAAGGCC-3'  
(forward)

5'-GCACCGACATGGTCACCA-3' (reverse)

GAPDH: 5'-TCCCTCAAGATTGTCAGCAA-3'  
(forward)

5'-AGATCCACAACGGATACATT-3' (reverse)

## Western Blotting

Liver protein was extracted with RIPA lysis buffer (Beyotime, China) and quantified using the BCA method. Protein from each group was equally loaded on 8% SDS-PAGE gels and separated by electrophoresis. Proteins were then transferred onto PVDF (Millipore, USA). After being blocked in 5% nonfat milk for 2 h, the membranes were incubated with primary antibodies to CYP8B1, V-Maf Avian Musculoaponeurotic Fibrosarcoma Oncogene Homolog G (MAFG), and  $\beta$ -actin (Abcam, USA) overnight at 4 °C condition. The membranes were then incubated with corresponding secondary antibodies in horseradish for 1 h. The protein bands were visualized with ECL solution (Beyotime, China) and imaged on an automated gel imaging analysis system. The gray levels were assessed with ImageJ software (National Institutes of Health, USA).

## Statistical Analysis

Data are expressed as mean  $\pm$  standard error and analyzed by SPSS version 22.0 software. The areas under the curves (AUC) of OGTT ( $AUC_{OGTT}$ ) and ITT ( $AUC_{ITT}$ ) were calculated using the trapezoidal rule. Student's *t* test was used for comparison between two groups, while Mann-Whitney *U* test was used for comparison of the BA components in two groups.  $P < 0.05$  was considered statistically significant in all cases.

## Results

SG and sham surgeries were successfully conducted in the respective groups, and all rats survived until the end of the study.

## Body Weight and Food Intake

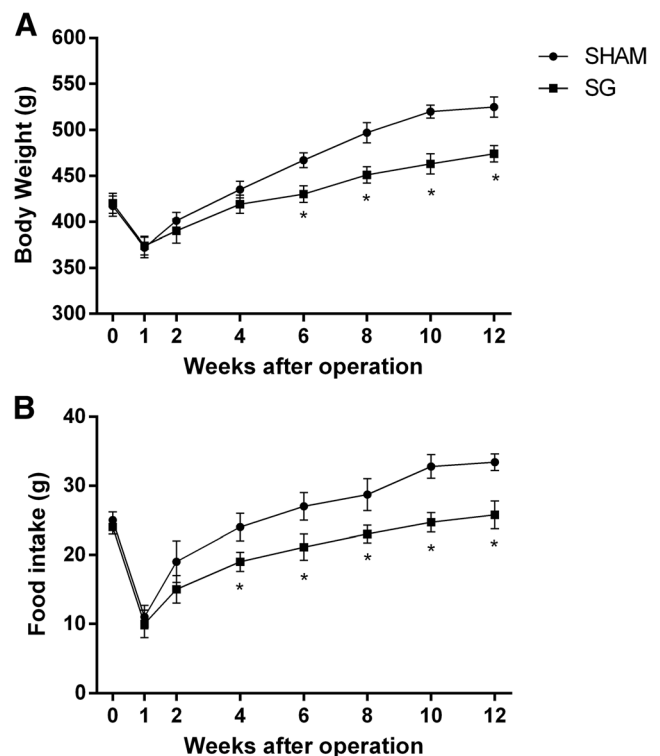
As displayed in Fig. 1, there was no statistical difference in body weight or food intake between the SG and SHAM groups at the beginning of the study ( $P > 0.05$ ). These values reached the lowest levels at 1 week after the operation. From 6 weeks post-operation, SG group weights were significantly lower than those of the SHAM group ( $P < 0.05$ , Fig. 1a). Similarly, food intake was also lower in the SG group than that of the SHAM group from 4 weeks after the operation ( $P < 0.05$ , Fig. 1b).

## Glucose Homeostasis

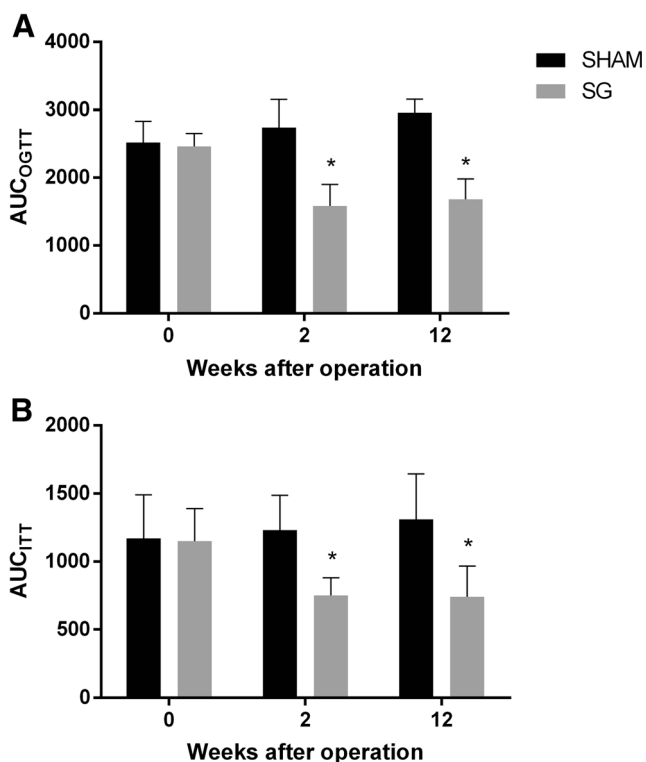
As shown in Fig. 2, there were no differences in preoperative  $AUC_{OGTT}$  or  $AUC_{ITT}$  values between the SG and SHAM groups ( $P > 0.05$ ). However, these values were significantly lower in the SG group than those in the SHAM group at 2 and 12 weeks after the operation ( $P < 0.05$ ). These findings indicated that glucose metabolism and insulin resistance in the SG group were significantly improved during the early postoperative stage.

## Serum BAs

As shown in Fig. 3, total serum BAs in the SG group were significantly higher than those in the SHAM group ( $P < 0.05$ ) at week 12 after the operation.

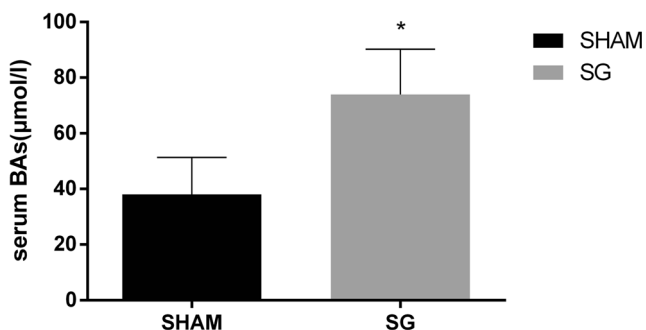


**Fig. 1** Body weight (a) and food intake (b) of rats before and after operation. \* $P < 0.05$  vs. SHAM group



**Fig. 2** AUC<sub>OGTT</sub> (a) and AUC<sub>ITT</sub> (b) of rats preoperatively and at 2 and 12 weeks postoperatively. \* $P < 0.05$  vs. SHAM group

In the SG group, the absolute value of  $\beta$ MCA, UDCA, CDCA, and all taurine-conjugated BAs increased ( $P < 0.05$ , Fig. 4a), while the percentage of CDCA, T $\alpha$ MCA, T $\beta$ MCA, TCA, and TDCA elevated and CA decreased compared with the SHAM group in serum BAs ( $P < 0.05$ , Fig. 4b). No significant differences between the two groups were shown in other kinds. According to whether belonging to 12 $\alpha$ -hydroxylated BAs, those BAs are divided into 12 $\alpha$ -hydroxylated BAs (CA, DCA, TCA, and TDCA) and non-12 $\alpha$ -hydroxylated BAs. Our study found that the concentrations of both categories were significantly elevated in the SG group ( $P < 0.05$ , Fig. 4c). However, the percentage of 12 $\alpha$ -hydroxylated BAs in total BAs was reduced in the SG group compared with that of the SHAM group ( $P < 0.05$ , Fig. 4d).



**Fig. 3** Serum TBAs were tested at 12 weeks after operation. \* $P < 0.05$  vs. SHAM group

## The Expression of MAFG and CYP8B1 in the Liver

Since we observed decreased 12 $\alpha$ -hydroxylated BA expression in the liver, we investigated CYP8B1, which can reflect 12 $\alpha$ -hydroxylated BA synthesis, and MAFG, which plays a role in CYP8B1 regulation [19]. At week 12 after operation, both MAFG messenger RNA (mRNA) and protein levels were elevated in the SG group relative to those in the SHAM group ( $P < 0.05$ , Fig. 5a, c). In contrast, CYP8B1 mRNA and protein levels were lower in the SG group ( $P < 0.05$ , Fig. 5b, c).

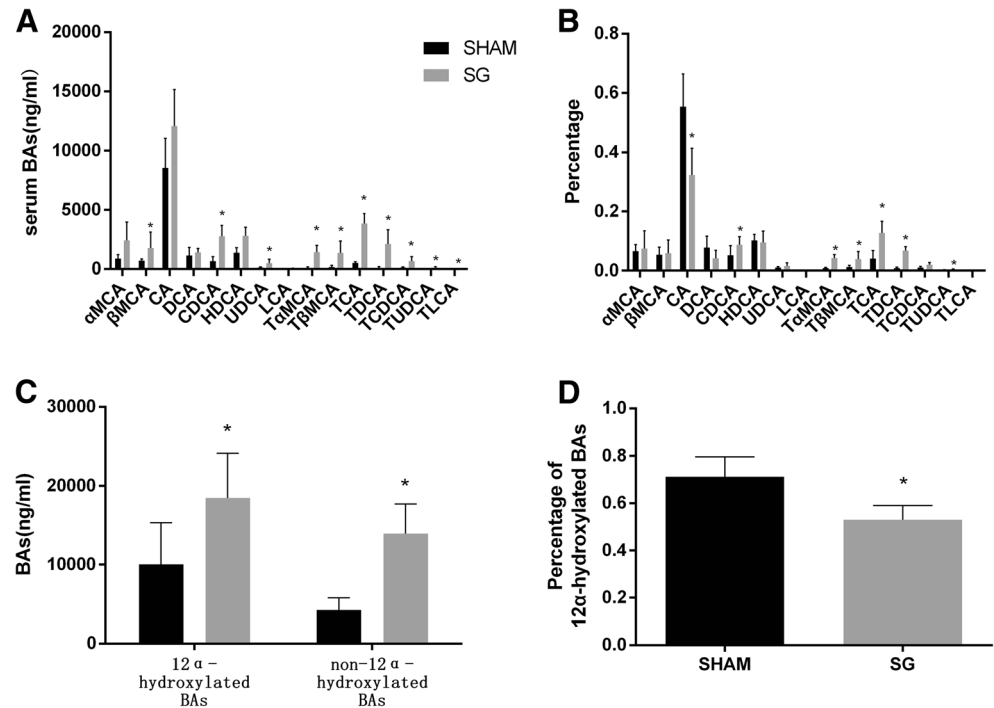
## Discussion

SG can effectively reduce body weight and induce T2DM remission, but the specific mechanisms have remained elusive. In this study, we observed that the reduction in blood glucose and improvements in insulin resistance occurred earlier than the reductions in body weight and food intake. These findings indicate that early remission effect of T2DM following SG is not dependent on the effects of weight loss, consistent with previous research [20, 21].

Serum BAs reflect whole-body BA levels. Thus, analysis of serum BAs allows us to research BA levels and function as signaling molecules in rodent models [22]. In this study, we observed that total serum BA levels were elevated following SG, consistent with our previous study [18] and Cummings' research [9]. CYP8B1 expression following the SG procedure was downregulated, consistent with a report by Myronovych et al. [23]. These findings revealed that BA synthesis metabolism was inhibited after SG procedure. This suggests that elevated total serum BA levels were not due to increased BA synthesis but rather may be due to increased intestinal BA uptake. It has also been reported that accelerated gastric emptying after SG could lead to faster BA absorption in the distal ileum, which might elevate total serum BA levels [24].

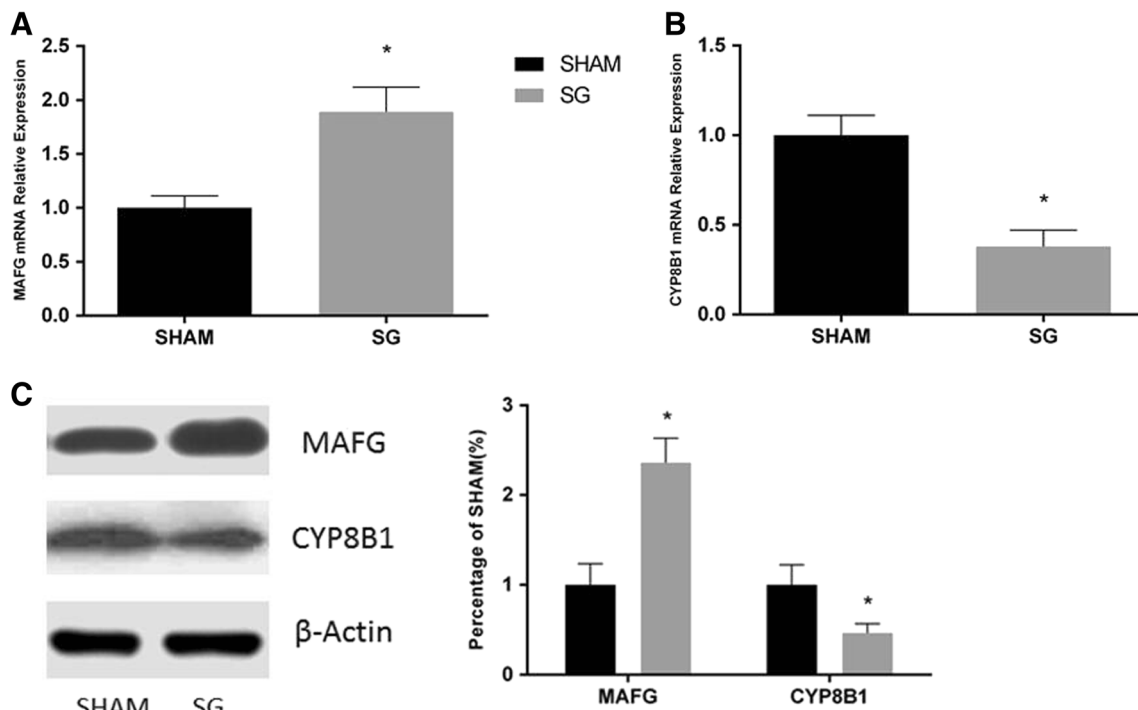
CDCA and CA are generated from cholesterol under the action of CYP7A1 and several other enzymes in human hepatic microsome, while the generation of CA requires the assistance of CYP8B1. In humans, the primary BAs were glycine- or taurine-conjugated CA and CDCA [25]. In rodents, most CDCA is transformed into MCA [26]. Over 95% of MCA and CA combine with taurine and constitute the main component of BAs in rodents [27]. DCA is a secondary bile acid generated from CA under the action of intestinal microflora. CA, TCA, DCA, and TDCA belong to the family of 12 $\alpha$ -hydroxylated BAs because of the participation of CYP8B1 in their generation process. Biddinger et al. [28] observed that serum 12 $\alpha$ -hydroxylated BA levels were increased compared with those of the control group. Haeusler's study [14] revealed that human insulin resistance was relevant with the elevated level of serum 12 $\alpha$ -

**Fig. 4** Effects of surgeries on serum BA composition. At 12 weeks after operation, absolute values (a) and relative percentage (b) of each serum BA component, and absolute values (c) and relative percentage (d) of 12 $\alpha$ -hydroxylated BAs. \* $P < 0.05$  vs. SHAM group



hydroxylated BAs. Kaur et al. [29] determined that lack of CA induced by CYP8B1 deficiency led to improved insulin resistance in *Cyp8b1*<sup>-/-</sup> mice. In summary, there was a significant correlation between 12 $\alpha$ -hydroxylated BAs and insulin resistance. Based on analysis of BA composition, we found a reduced proportion of CA in BAs but elevated absolute levels.

Additionally, we observed a decreased percentage of 12 $\alpha$ -hydroxylated BAs following the SG procedure. In our previous study [18], we observed enhanced conjugation of BAs in the liver to reveal the elevated taurine-conjugated BAs following SG in a diabetic rat model. However, this could not explain the increased absolute value but decreased percentage of



**Fig. 5** mRNA relative expression of MAFG (a) and CYP8B1 (b) and the protein expression of them (c) at 12 weeks postoperatively. \* $P < 0.05$  vs. SHAM group



12 $\alpha$ -hydroxylated BAs after surgery. These results indicate that downregulated CYP8B1 expression after SG decreased the proportion of 12 $\alpha$ -hydroxylated BAs, thus leading to a weakened role of 12 $\alpha$ -hydroxylated BAs in total BAs. These outcomes might be crucial for amelioration of postoperative insulin resistance. However, Dutia et al. [30] observed that there was a higher proportion of 12 $\alpha$ -hydroxylated BAs in total BAs 2 years after Roux-en-Y gastric bypass procedure, which was contrary to our study. However, there was lack of change in the ratio of 12 $\alpha$ -hydroxylated/non-12 $\alpha$ -hydroxylated BAs at 1 year after RYGB in patients with T2DM in a study by Sachdev and colleagues [31]. In addition, Zhang et al. [32] found the ratio unaltered after duodenal-jejunal bypass. The results are varied, and we speculate that this discrepancy may be due to the different selection of experimental subjects and surgical procedures, which remain unclear.

We observed that CYP8B1 expression was reduced following SG, but the mechanism remains unclear. De Aguiar Vallim et al. [19] reported that MAFG could inhibit BA metabolism by suppressing Cyp8b1 activity. MAFG is a member of the MAF transcription factor family and encoded by the MAFG gene. De Aguiar Vallim et al. confirmed that overexpression of liver MAFG inhibited expression of CYP8B1, decreased CA levels, and increased MCA levels in mice. Furthermore, knocking out the MAFG gene elevated the level of CYP8B1 and CA. Based on the conclusions above, we measured the expression of MAFG in rat liver after operation and found that the transcription and expression level of liver MAFG in the SG group were higher than those of the SHAM group, indicating that the expression of MAFG was elevated in diabetic rats after SG. There have been no studies investigating the correlation between the expression of MAFG and bariatric surgery to date.

In conclusion, our study demonstrated that the absolute value level of 12 $\alpha$ -hydroxylated BAs was elevated but its percentage was reduced after SG, which might be relevant with the inhibition of CYP8B1 by overexpression of MAFG. These may play an important role in the improvement of insulin sensitivity after SG.

**Compliance with Ethical Standards** All study protocols were approved by the Animal Care and Utilization Committee of Shandong University.

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Statement of Human and Animal Rights** All applicable institutional and national guidelines for the care and use of animals were followed.

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