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# Type 2 diabetes mitigation in the diabetic Goto–Kakizaki rat by elevated bile acids following a common-bile-duct surgery

*Feng Gao*[a], *Xu Zhang*[b], *Lanlan Zhou*[c], *Shixiang Zhou*[a], *Yue Zheng*[b], *Jiani Yu*[b], *Weimin Fan*[a], *Yunxia Zhu*[b,*], *Xiao Han*[b,*]

[a] *Department of Orthopedic Surgery, the First Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China*
[b] *Key Laboratory of Human Functional Genomics of Jiangsu Province, Jiangsu Diabetes Center, Nanjing Medical University, Nanjing, Jiangsu, China*
[c] *Department of rheumatism, the First Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China*

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ABSTRACT

*Objective.* Elevated plasma bile acids after bariatric surgery are thought to explain type 2 diabetes mellitus (T2DM) remission. Bile acids can bind to and activate the nuclear receptor farnesoid-X receptor (*FXR*) by regulating lipid and glucose metabolism. We performed a surgical procedure (ligation of the common bile duct and external biliary drainage [LBD]) in the diabetic Goto–Kakizaki (GK) rat in order to investigate its effect on bile acids metabolism and T2DM mitigation.

*Material/Methods.* LBD surgery and sham control surgery were performed on diabetic GK rats. The concentrations of total bile acids and blood glucose were analyzed by an automatic analyzer. Intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT) were used to monitor blood glucose level. Expression of genes involved in bile acid metabolism (*FXR*, *CYP7A*, et al.) and glycolipid metabolism (*G6Pase*, *PEPCK*, et al) was analyzed using qRT-PCR. The protein levels of pAKT, AKT and pGSK3β were tested by western blot. The morphological alterations of the liver and epididymal fat were monitored by H&E staining.

*Results.* LBD increased plasma total bile acids, improved hepatic insulin sensitivity, and eventually mitigated T2DM, whereas food intake and body weight were unaltered. Post-LBD, the levels of total bile acids were elevated from 24.80 ± 7.12 to 61.44 ± 6.40 and the concentration of fast blood glucose was decreased from 204.7 ± 11.06 mg/dL to 109.3 ± 5.4 mg/dL. IPGTT and ITT showed that LBD operation improved insulin sensitivity in GK rats. Clusters of FXR signaling target genes were altered in the liver, such as *FXR*, *CYP7A*,

*Abbreviations:* T2DM, type 2 diabetes mellitus; FXR, farnesoid-X receptor; LBD, ligation of the common bile duct and external biliary drainage; GK, Goto–Kakizaki; RYGB, Roux-en-Y gastric bypass; VSG, vertical sleeve gastrectomy; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase;IPGTT, intraperitoneal glucose tolerance test; ITT, insulin tolerance test; H&E, hematoxylin and eosin; GLP-1, glucagon like peptide-1; AUC, area under the curve; SHP, small heterodimer partner; CYP7A1, cholesterol 7-alpha-hydroxylase; CYP27A1, cholesterol 27-hydroxylase gene; OATP, organic anion transporting polypeptide; NTCP, Na+-taurocholate-cotransporting polypeptide; SREBP1C, sterol regulatory element-binding protein-1c gene; FASN, fatty acid synthase; ACC1, acetyl-CoA carboxylase 1; PPARγ, peroxisome proliferator-activated receptor γ; CPT1α, carnitine palmitoyltransferase 1α; PGC1α, PPAR gamma co-activator 1α; ER, endoplasmic reticulum; CHOP, CCAAT/enhancer-binding protein (C/EBP) homologous protein; TBA, total bile acids; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TP, total protein; ALB, albumin; A/G, albumin/globin; Cr, creatinine; UA, uric acid (UA).

* *Corresponding authors at:* Key Laboratory of Human Functional Genomics of Jiangsu Province, Jiangsu Diabetes Center, Nanjing Medical University, Nanjing, Jiangsu 210029, China. Tel.: +86 25 86862898; fax: +86 25 86862731.
E-mail addresses: zhuyx@njmu.edu.cn (Y. Zhu), hanxiao@njmu.edu.cn (X. Han).

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*G6Pase* and *PEPCK*. These contributed to sustained bile acid homeostasis, and they ameliorated hepatic endoplasmic reticulum (ER) stress, increased energy expenditure, and reduced gluconeogenesis, resulting in a substantial improvement in hepatic insulin sensitivity. LBD also significantly reduced epididymal fat tissue and decreased the size of adipocytes.

*Conclusion*. These results demonstrate that the elevated bile acids observed in LBD-operated GK rats link insulin sensitivity improvement to T2DM mitigation, recapitulating the metabolic effects of bariatric surgery. Our investigation establishes a model for a focused study of bile acids in the context of bariatric surgery that may contribute to the identification of therapeutics for T2DM.



## 1. Introduction

Type 2 diabetes mellitus (T2DM) is a heterogenous metabolic disorder characterized by insulin resistance and impaired insulin secretion. It affects more than 371 million people worldwide and accounted for an estimated 12.9 million deaths globally in 2010 [1–3]. The current management of T2DM is focused on pharmacological and behavioral interventions. Until the emergence of bariatric surgeries, T2DM was considered a chronic and lifelong disease, without complete resolution [4].

Bariatric surgeries, such as Roux-en-Y gastric bypass (RYGB) surgery and vertical sleeve gastrectomy (VSG) surgery, were first developed to treat morbid obesity [5–7]. However, they are now also used to improve obesity-associated disorders, such as T2DM [8–12]. Remarkably, 40% of obese T2DM patients who underwent bariatric surgery achieved entire resolution of symptoms within one year [13]. Despite the dramatic outcomes with bariatric surgeries, the underlying mechanisms remain unclear.

Conventional opinion holds that bariatric surgeries, including RYGB and VSG, lead to weight loss and resolution of T2DM because of gastric mechanical restriction and intestinal malabsorption [14,15]. Therefore, the antidiabetic effect of bariatric surgeries has been interpreted as a conceivable result of the treatment of obesity. However, blood glucose control often occurs within days before achieving significant weight loss [16,17], challenging the theory that T2DM resolution is a secondary result of the treatment of obesity. Additionally, T2DM resolution postsurgery can be reproduced in nonobese individuals [18,19].

Accumulating evidence points to the involvement of bile acids and bile acid signaling in the resolution of symptoms in T2DM following bariatric surgeries [20–23]. Besides assisting mechanical digestion and the absorption of lipids, bile acids act as signal molecules by binding to the nuclear farnesoid-X receptor (FXR) [24,25]. An increase in the intracellular level of bile acids results in transcriptional activation of FXR, which regulates bile acid synthesis and excretion [26,27]. FXR regulates clusters of target genes, thus modulating various metabolic processes, including normal glucose homeostasis [28]. Insulin and D-glucose regulate the gene level of *FXR*[29]. FXR activation improved hyperglycemia in diabetic mice [30–32]. FXR disrupted mice displayed glucose intolerance and insulin resistance [28]. Studies demonstrated that the hypoglycemia effect of FXR signaling was associated with improved insulin sensitivity via the regulation of gluconeogenic genes, including phosphoenolpyruvate carboxykinase (*PEPCK*), glucose-6-phosphatase (*G6Pase*), and fructose 1,6-bis phosphatase [30,33,34].

Successful RYGB and VSG outcomes were reported to be associated with a significant increase in plasma total bile acids in humans and rodent models [35,36]. However, the relationship between the elevated bile acids and metabolic outcomes was still unclear. To specifically define the role of bile acids, we designed a surgery called ligation of the common bile duct and external biliary drainage (LBD) to elevate plasma total bile acids. After the LBD surgery, we investigated the antidiabetic effect of bile acids in the nonobese diabetic Goto–Kakizaki (GK rat).

## 2. Materials and Methods

### 2.1. Animals and Diets

Male GK rats (12–13 weeks old; n = 14 per group) were purchased from Changzhou Cavens Laboratory Animal Company. They were kept under routine laboratory conditions in the Animal Center of Nanjing Medical University. All the rats had free access to tap water, and they were fed with a standard chow diet unless fasting. All animal procedures were carried out in accordance with the Animal Care and Use Committee of the Model Animal Research Centre of Nanjing Medical University, Nanjing, China.

### 2.2. Surgeries and Postoperative Care

All the GK rats were stochastically divided into two groups: a sham group and an LBD group. The operations were performed after the rats had fasted overnight. Under 7% chloral hydrate anesthesia, one end of an epidural catheter with an inner diameter of 0.6 mm was inserted into the common bile duct of the rats in the LBD group, and the other end was inserted through the lateral abdominal wall, crossed the posterior cervical skin and exposed *in vitro*. In the sham control rats, the abdominal cavities were cut open and then sewn up. The rats received analgesia postoperatively, and they were returned to a chow diet. In addition, all the rats had *ad libitum* access to a diet of melon seeds and oatmeal for the first week after the surgeries. The weight and food intake of the rats were measured every day in the postoperative period.