



# Metformin Influences Bile Acids to Improve the Glucose and Lipid Metabolism in Rats with Streptozotocin-induced Type 2 Diabetes Mellitus

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### Abstract

**Objective:** To explore the association between metformin and bile acids in rats with streptozotocin-induced type 2 diabetes mellitus (T2DM).

**Materials and Methods:** 100 wistar male rats were randomly divided into two groups: SD group rats (n=20) were fed with standard diet and HFD group rats (n=80) were fed with high-fat diet. 80 HFD group rats were divided into 4 other group: IR group, EI group, LI group and DM group. IR group (n=20) were intraperitoneally injected with citrate buffered saline after 6 weeks of high-fat diet. DM group (n=20) were intraperitoneally injected with citrate buffered saline and streptozotocin (STZ) after 6 weeks of high-fat diet. EI group (n=20) were treated with metformin from the beginning to the end of the experiment. LI group (n=20) were treated with metformin per day for 4 weeks from the building of type 2 diabetes. Fasting blood glucose (FBG), fasting insulin concentration (FINS), total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDLC) and total bile acid (TBA) were measured.

**Results:** The levels of FBG, TG, TC, LDLC and TBA in DM and LI group were higher than NC group after administration of metformin. The FBG, TG, TC, TBA and LDLC levels in EI group were higher than DM group.

**Conclusions:** Metformin may influence the levels of bile acids to improve the metabolism of glucose and lipid.

### Keywords

Bile acids; Metabolism; Metformin; Type 2 diabetes; Rat models

**Abbreviations:** T2DM: Type 2 Diabetes Mellitus; STZ: Streptozotocin; FBG: Fasting Blood Glucose; FINS: Fasting Insulin Concentration; TC: Total Cholesterol; TG: Total Triglycerides; LDLC: Low-Density Lipoprotein Cholesterol; TBA: Total Bile Acid; NC: Normal Control; OGTT: Oral Glucose Tolerance Test; IR: Insulin Resistance; EI: Early Intervention; LI: Late Intervention; AUC: Area Under Curves; LDLC: Low-Density Lipoprotein Cholesterol

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### Introduction

The prevalence of type 2 diabetes mellitus (T2DM), which is characterized by an increased blood glucose concentration, insulin resistance and pancreatic beta-cell dysfunction has reached epidemic proportions worldwide and represents an important threat to global public health systems [1]. On the basis of available clinical evidence, metformin has been affirmed as the first-line therapy for patients with T2DM [2]. Although a few pathophysiological mechanisms have been proposed to explain these beneficial clinical effects of metformin, detailed mechanisms of action of metformin are still not fully understood, especially its relationship with bile acids.

We investigated the correlation between metformin and bile acids in T2DM-induced rats.

### Materials and Methods

#### Animals

A total of 100 Wistar male rats (8 weeks old, 200-250 g) were purchased from Shandong University Laboratory Animal Research Center. The animals were housed in a standard condition.

#### Grouping and treatment

All rats (n=100) were randomly divided into 2 groups: Standard diet (SD, n=20) group and High-fat diet (HFD, n=80) group. SD group rats were fed with a diet of low fat and were considered as normal control (NC) group. HFD group rats were fed with a diet of high fat. After 6 weeks, all rats received oral glucose tolerance test (OGTT) to detect the extent of insulin resistance (IR). The 20 rats successfully induced IR were then given an intraperitoneally injection without streptozotocin (STZ) and were considered as IR group. Other 20 rats induced IR were given an intraperitoneally injection with streptozotocin (STZ) in order to establish type 2 diabetes rats-DM group. Early intervention (EI) group rats were separated from HFD group and were assigned metformin administration at 500 mg/kg body weight from the beginning to the end of the experiment. Late intervention (LI) group was established in another 20 rats in HFD group with metformin (500 mg/kg body weight) per day for 4 weeks from the building of type 2 diabetes. DM group rats were not given metformin at any time. For OGTT, the rats were orally administered with glucose 2.2 g/kg body weight. Blood samples were then collected from the retro orbital plexus at 0, 30, 60, and 120 minutes after the glucose load and used for the measurement of glucose and insulin. The area under curves (AUC) of glucose and insulin were calculated. At the end of the experiment, blood samples were collected from the retro-orbital plexus of all rats under ether anesthesia after an overnight fast. The metabolic parameters including fasting blood glucose (FBG), fasting insulin concentration (FINS), total cholesterol (TC), total triglycerides (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDLC) and total bile acid (TBA) were measured before and after the injection of STZ.

#### Calculations of indexes

Calculations of the indexes were made according to the following equations:

AUC (glucose) = 0.5 × BG (0 min) + BG (30 min) + 1.5 × BG (60 min) + BG (120 min)

AUC (insulin) = 0.5 × INS (0 min) + INS (30 min) + 1.5 × INS (60 min) + INS (120 min)

AUC (insulin)/AUC (glucose) = AUC (insulin)/AUC (glucose)

### Statistical analysis

The data were analyzed with the Statistical Package for the Social Sciences Version 18.0 (SPSS Inc., Chicago, IL, USA). Normal distribution data were described as means ± SD. Statistical analysis between or among groups was conducted by the unpaired Student's t-test or one-way ANOVA, respectively. A value of  $p < 0.05$  was considered statistically significant.

## Results

### Effect of metformin on metabolic parameters in Wistar rats

Table 1 showed that compared with SD group, the levels of body weight, FBG, TC, LDLC, TBA and FINS and AUC (glucose) were significantly higher in HFD group ( $p < 0.05$ ). FBG and AUC (glucose) levels in EI group were higher than SD group ( $p < 0.05$ ). The levels of body weight, LDLC and AUC (insulin) and AUC (glucose) in EI group were significantly lower than that in HFD group ( $p < 0.05$ ).

### Effects of metformin on metabolic parameters after treated with streptozotocin

Table 2 showed that the levels of FBG, TG, TC, HDLC, LDLC and TBA in DM group and LI group were significantly higher than that in NC group ( $p < 0.05$ ). Moreover, the FBG, TG, TC and LDLC levels in EI group were markedly higher than DM group ( $p < 0.05$ ). Besides,

after administration of metformin, TBA levels in EI and LI group were higher than that in DM group ( $42.97 \pm 11.23$  and  $19.92 \pm 5.18$  vs.  $14.81 \pm 3.51$ ,  $p < 0.05$ ).

## Discussion

It is well known that metformin has very critical actions in decreasing blood glucose by increasing peripheral glucose uptake and reducing hepatic gluconeogenesis. In our study, we found that the levels of body weight, LDLC, FBG, AUC (insulin), AUC (glucose) and AUC (glucose) were improved in EI group than DM group, suggesting that metformin improves metabolism of rats with T2DM.

Bile acids are well known to have lipid-lowering effects and control liver triglycerides by mechanisms that are not fully understood. Many recent studies have suggested that FXR and TGR5 regulate glucose metabolism [3]. In our previous study, we demonstrated that FXR might inhibit gluconeogenesis to improve glucose tolerance and insulin sensitivity [4]. In present study, we establish diabetic models by using STZ to further explore the association between metformin and bile acids, suggesting that metformin may have an important effect on BA metabolism in rats with T2DM. It has been proved that the effects of metformin on glucose-lowering are related to its function of increasing the concentration of circulating GLP-1 [5]. Researches show that bile acids can increase the release of circulating GLP-1 in T2DM patients. The possible mechanism may be that bile acids activate TGR5 and FXR in order to promote glucose homeostasis [6]. Rohed suggested that gallbladder emptying played a physiological role in human GLP-1 release and the GLP-1 stimulatory effect of metformin can be potentiated by the presence of bile acids in humans [7]. Taken together, it can be therefore speculated that metformin have important effects on bile acids, thus to affect glucose and lipid metabolism.

Table 1: Effect of metformin on metabolic parameters in Wistar rats.

Parameters	SD group	HFD group	EI group
Body weight (g)	367.13 ± 20.41	430.92 ± 31.49 <sup>a</sup>	358.09 ± 18.97 <sup>b</sup>
FBG (mmol/L)	4.29 ± 0.77	5.48 ± 0.95 <sup>aa</sup>	5.12 ± 0.97 <sup>a</sup>
TG (mmol/L)	0.63 ± 0.10	0.97 ± 0.51	0.75 ± 0.24
TC (mmol/L)	1.52 ± 0.13	2.08 ± 0.16 <sup>a</sup>	1.95 ± 0.09
HDLC (mmol/L)	1.19 ± 0.14	1.26 ± 0.12	1.35 ± 0.16
LDLC (mmol/L)	0.20 ± 0.07	0.51 ± 0.07 <sup>a</sup>	0.37 ± 0.05 <sup>b</sup>
TBA (μmol/L)	8.31 ± 1.24	11.78 ± 1.36 <sup>a</sup>	10.12 ± 1.19
FINS (mIU/L)	9.38 ± 1.86	13.84 ± 3.20 <sup>a</sup>	8.38 ± 2.09
AUC (glucose, mmol/L)	24.18 ± 3.45	47.92 ± 6.93 <sup>aa</sup>	33.85 ± 2.02 <sup>aa bb</sup>
AUC (insulin, mmol/L)	54.32 ± 6.06	58.39 ± 9.10	45.16 ± 12.75 <sup>b</sup>
AUC (insulin)/AUC (glucose)	2.25 ± 0.03	1.22 ± 0.01	1.34 ± 0.01

Data were expressed as mean ± SD. FBG: fasting blood glucose, TG: total triglycerides, TC: total cholesterol, HDLC: high density lipoprotein cholesterol, LDLC: low density lipoprotein cholesterol, TBA: total bile acid, FINS: fasting insulin concentration, AUC: area under curve. <sup>a</sup> $p < 0.05$  vs. SD group; <sup>aa</sup> $p < 0.01$  vs. SD group; <sup>b</sup> $p < 0.05$  vs. HFD group; <sup>bb</sup> $p < 0.01$  vs. HFD group.

Table 2: Effect of metformin on metabolic parameters in different groups.

Parameters	NC group	IR group	DM group	EI group	LI group
Body weight (g)	482.53 ± 35.74	565.12 ± 42.31 <sup>#</sup>	412.85 ± 37.71 <sup>*</sup>	489.1 ± 42.35 <sup>#</sup>	463.61 ± 66.40
FBG (mmol/L)	3.66 ± 1.10	4.57 ± 2.01 <sup>#</sup>	12.78 ± 4.64 <sup>*</sup>	5.41 ± 1.82 <sup>#</sup>	10.19 ± 2.75 <sup>#</sup>
TG (mmol/L)	0.75 ± 0.44	0.81 ± 0.35	2.12 ± 1.07 <sup>*</sup>	1.54 ± 0.98 <sup>#</sup>	1.74 ± 1.09 <sup>*</sup>
TC (mmol/L)	1.12 ± 0.45	1.64 ± 0.82	6.48 ± 2.93 <sup>*</sup>	2.71 ± 0.93 <sup>#</sup>	5.39 ± 1.61 <sup>*</sup>
HDL-C (mmol/L)	0.75 ± 0.29	0.71 ± 0.34	1.19 ± 0.23 <sup>*</sup>	0.99 ± 0.17	1.05 ± 0.41 <sup>*</sup>
LDL-C (mmol/L)	0.31 ± 0.17	0.64 ± 0.23	3.96 ± 1.18 <sup>*</sup>	1.13 ± 0.56 <sup>#</sup>	3.15 ± 0.63 <sup>*</sup>
TBA (μmol/L)	9.83 ± 1.96	10.46 ± 2.35	14.81 ± 3.51 <sup>*</sup>	42.97 ± 11.23 <sup>#</sup>	19.92 ± 5.18 <sup>#</sup>

Data were expressed as mean ± SD. <sup>\*</sup> $p < 0.05$  vs. NC group; <sup>#</sup> $p < 0.05$  vs. DM group.

## Conclusions

Our study demonstrates that metformin may help to improve the glucose and lipid metabolism by influencing the level of serum TBAs. A combination of HFD and metformin could be effective on treatment of rats with T2DM.

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