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Metformin improves the glucose and lipid metabolism via influencing the level of serum total bile acids in rats with streptozotocin-induced type 2 diabetes mellitus

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Abstract. – OBJECTIVE: To study the effects of metformin on streptozotocin-induced type 2 diabetes mellitus (T2DM) in rats.

MATERIALS AND METHODS: Wistar male rats were divided into two groups: standard diet (SD, n = 20) group and high-fat diet (HFD, n = 80) group. Twenty rats in HFD group were randomly treated with metformin (EI group). After 6 weeks, among rats in HFD group, 20 rats were intraperitoneally injected with citrate buffered saline (IR group), 20 rats treated with metformin per day for 4 weeks (LI group), and 20 rats were given nothing (DM group). Rats in SD group were injected with citrate buffered saline as normal control (NC) group. Moreover, streptozotocin (STZ) was used for inducing diabetes. The metabolic parameters, such as body weight, 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: Compared with SD group, the levels of body weight, FBG, TC, LDLC, TBA and FINS and AUC (glucose) were significantly higher in HFD group. After administration of metformin, the levels of FBG, TG, TC, LDLC and TBA in DM and LI group were higher than NC group. Besides, the FBG, TG, TC, TBA and LDLC levels in EI group were higher than DM group.

CONCLUSIONS: Metformin may help to improve the glucose and lipid metabolism by influencing the level of serum total bile acids. A combination of HFD and metformin could be effective in the treatment of rats with T2DM.

Key Words:
Bile acids, Metabolism, Metformin, Type 2 diabetes, Rat models.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease, mainly characterized by an increased blood glucose concentration, insulin resistance and pancreatic beta-cell dysfunction[1]. It has been regarded as one of the most common metabolic diseases with the rate of 6.4 % in people aged 20-79 years[2] and one of the leading causes of death all over the world[3,4]. Nearly 80% of the T2DM patients come from developing countries[5]. Many factors, such as genetics, aging and life style, have been involved in the development of T2DM, diagnosis of T2DM are found to be obese[6].

There have been increasing anti-diabetic drugs such as d-limonene[7], curcumin capsules[8] and dipeptidyl peptidase-4[9] have been used to control T2DM. However, most of these anti-diabetic drugs have limited efficacy and many undesirable side effects such as drug resistance, weight gain, dropsy and high rates of secondary failure[7,10]. Therefore, the development of low toxicity, effective and economic anti-diabetic drugs is still needed and has far-reaching significance.

Metformin, a class of insulin sensitizers, is commonly used for the treatment of type 2 diabetes. While lowering the blood glucose level, metformin can cause reductin of fat mass and inhibiation of tumor cell proliferation[11,12].

Decreasing hepatic glucose production through gluconeogenesis suppression and activating peripheral glucose utilization in muscle, intestine

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and liver have been reported to be contributors to the glucose-lowering effect of metformin[13-15]. Although several possible pathophysiological mechanisms have been suggested to explain these beneficial clinical effects of metformin, detailed mechanisms of action of metformin are still not fully understood.

In this study, a rat model of T2DM was constructed with a combination of high-fat feeding and low-dose STZ intraperitoneal injection. Our study aimed to investigate the effect of metformin on treatment for rats with T2DM. Findings of this study may provide a new insight for the treatment of T2DM in future.

Materials and Methods

Animals

Total 100 Wistar male rats (8 weeks old, 200-250 g) were purchased from Shandong University Laboratory Animal Research Center. The animals were housed in standard polypropylene cages (three rats/cage) at a controlled room temperature (22 ± 2 °C) and humidity (55 ± 5%) with a 12-h light-dark cycle. The experiment was conducted under the protocol approved by Shandong University and was performed in strict compliance with the Animal Welfare Act and guidelines established by the Institutional Animal Care and Use Committee of the University.

Grouping and Treatment

After 1 week of dietary accommodation, the rats were randomly divided into 2 groups: Standard diet (SD, n = 20) group and High-fat diet (HFD, n = 80) group. For SD group, rats were fed a diet containing 6% fat, 64% carbohydrate and 23% protein. For HFD group, the diet of rats consists of 25% fat, 48% carbohydrate, and 20% protein. Then 20 rats in HFD group which were randomly selected to have metformin therapy at 500 mg/kg body weight from the beginning to the end of the experiment were considered as early intervention (EI) group. After 6 weeks, all the rats received oral glucose tolerance test (OGTT) to detect the extent of insulin resistance. 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.

Moreover, 20 rats in SD group and 20 rats successfully induced IR in HFD group were then given an intraperitoneally injection of 0.1 M citrate buffered saline (PH 4.2) at 35 mg/kg body weight, and were respectively considered as normal control (NC) group and IR group. In our study, diabetes was induced in other successfully induced IR rats by a single injection of 0.1 M citrate buffered saline (PH 4.2) containing streptozotocin (STZ, Sigma Chemical Co, MO, USA) at 35 mg/kg body weight overnight. All animals continued their original diets for the duration of the study. Three days after the injection of STZ, blood was collected from the tail vein, FBG was detected every week to monitor dynamic changes in all rats. The diagnosis of diabetes was made according to fasting glucose level (greater than 200 mg/dL). General characters of rats such as mental status, coat color, water-intake and food-intake in different groups were observed. Then rats in EI group were given metformin from the beginning to the end of the experiment. 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 was defined as another 20 rats in HFD group that were given nothing. Food intake and body weight were measured every day. 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, such as fasting blood glucose (FBG), fasting insulin concentration (FINS), total cholesterol (TC), total triglycerides (TG), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and total bile acid (TBA) were measured before and after the injection of STZ. FBG was detected by Accu-Chek Performa (Roche Diagnostics, Mannheim, Germany) and FINS was measured with the Immulite Analyzer (Diagnostic Products Corp., Los Angeles, CA, USA). TC and TG were measured in fasting blood sample by enzyme end-point method, HDLC and LDLC were detected by the PTA-Mg^{2+} method, the extent of IR and TBA was measured by the enzymatic colorimetric assay.

Calculations of Indexes

Estimation of glucose tolerance, insulin-secreting ability of beta-cell made from oral glucose tolerance test data were performed using AUC (glucose), AUC (insulin) and AUC (insulin)/AUC (glucose), respectively. Calculations of the indexes were made according to the following equations:

Table I. 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[a]	358.09 ± 18.97[b]
FBG (mmol/L)	4.29 ± 0.77	5.48 ± 0.95[aa]	5.12 ± 0.97[a]
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[a]	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[a]	0.37 ± 0.05[b]
TBA (μmol/L)	8.31 ± 1.24	11.78 ± 1.36[a]	10.12 ± 1.19
FINS (mIU/L)	9.38 ± 1.86	13.84 ± 3.20[a]	8.38 ± 2.09
AUC (glucose, mmol/L)	24.18 ± 3.45	47.92 ± 6.93[aa]	33.85 ± 2.02[aa bb]
AUC (insulin, mmol/L)	54.32 ± 6.06	58.39 ± 9.10	45.16 ± 12.75[b]
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. [a]$p < 0.05$ *vs*. SD group; [aa]$p < 0.01$ *vs*. SD group; [b]$p < 0.05$ *vs*. HFD group; [bb]$p < 0.01$ *vs*. HFD group.

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 analyzed by the unpaired Student's *t*-test or one-way ANOVA, with the Tukey-Kramer post hoc test, respectively. A value of $p < 0.05$ was considered statistically significant.

Results

Effect of Metformin on Metabolic Parameters in Wistar Rats

Effect of metformin on metabolic parameters in Wistar rats was shown in Table I. Compared with SD group, the levels of body weight, FBG, TC, LDLC, TBA and FINS and AUC (glucose) were significantly higher in HFD group ($p < 0.05$). Moreover, FBG and AUC (glucose) levels in EI group were significantly higher than SD group ($p < 0.05$). Besides, the levels of body weight, LDLC and AUC (insulin) and AUC (glucose) in EI group were significantly lower than that in HFD group ($p < 0.05$).

Effect of Metformin on OGTT

After treated with metformin for 6 weeks, the serum glucose levels at 0, 30, 60, and 120 min in HFD group were markedly higher than those in SD group ($p < 0.01$, Figure 1A). The serum glucose levels at 30, 60, and 120 min in EI group were significantly lower than HFD group ($p < 0.01$) but higher than SD group ($p < 0.05$). Serum insulin level at 0 and 60 min in HFD group was higher than SD group, and at 30 min was lower compared to SD group ($p < 0.05$, Figure 1B).

Stability of Experimental Diabetic Model

The levels of FBG in all groups were measured every week after injection of STZ/citrate buffered saline (Figure 2). FBG level was over 11.1 mmol/L (200 mg/dL) in DM group all the time. Five weeks after FBG was detected, FBG levels in DM and LI group were higher than that in NC group ($p < 0.05$). Besides, FBG levels in EI and LI group were lower than that in DM group ($p < 0.05$).

General Characters of rats in Various Groups

The general characters of rats in NC group were good. There was no obvious change in water intake and urine volume during the experiment; the color and luster of hair were fine; food intake and body weight increased persistently and steadily. For rats in DM group, flagging spirit and lags in response and action were present, with the typical symptom of polyphagia, polyuria, loose stools and loss of body weight; the hair was color-disordered and lackluster. In contrast, there are improvements in spirit, response, action, food intake, water intake, urine volume, body weight and hair in rats treated with metformin.

Table II. 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$^{*\#}$	412.85 ± 37.71*	489.1 ± 42.35$^{\#}$	463.61 ± 66.40
FBG (mmol/L)	3.66 ± 1.10	4.57 ± 2.01$^{\#}$	12.78 ± 4.64*	5.41 ± 1.82$^{\#}$	10.19 ± 2.75$^{*\#}$
TG (mmol/L)	0.75 ± 0.44	0.81 ± 0.35	2.12 ± 1.07*	1.54 ± 0.98$^{*\#}$	1.74 ± 1.09*
TC (mmol/L)	1.12 ± 0.45	1.64 ± 0.82	6.48 ± 2.93*	2.71 ± 0.93$^{*\#}$	5.39 ± 1.61*
HDL-C (mmol/L)	0.75 ± 0.29	0.71 ± 0.34	1.19 ± 0.23*	0.99 ± 0.17	1.05 ± 0.41*
LDL-C (mmol/L)	0.31 ± 0.17	0.64 ± 0.23	3.96 ± 1.18*	1.13 ± 0.56$^{*\#}$	3.15 ± 0.63*
TBA (μmol/L)	9.83 ± 1.96	10.46 ± 2.35	14.81 ± 3.51*	42.97 ± 11.23$^{*\#}$	19.92 ± 5.18$^{*\#}$

Data were expressed as mean ± SD. $^{*}p < 0.05$ *vs.* NC group; $^{\#}p < 0.05$ *vs.* DM group.

Effects of Metformin on Metabolic Parameters After Treated with STZ

The levels of metabolic parameters in different groups after treated with STZ were shown in Table II. The FBG, TG, TC, HDLC, LDLC and TBA levels in DM and LI group were significantly higher than 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 ± 11.23 and 19.92 ± 5.18 *vs.* 14.81 ± 3.51, $p < 0.05$).

Discussion

Although many anti-diabetic drugs have been used for the treatment of diabetes, potent ones with high efficiency and low side effects are necessary. In the study, we established a rat model with T2DM by high fat feeding and low-dose STZ injection, which was recognized as type T2DM model previously[16]. With this model, we found that the TBA levels in EI and LI group were significantly higher than that in DM group, suggesting the effects of metformin on rats with T2DM.

Bile acid (BA) is the end product of cholesterol breakdown and involves in the predominant pathway for eliminating excess cholesterol from the human body[17]. By interacting with multidrug transporters directly, bile acids can thus influence the drug transnport[18]. BAs are shown to be involved in the regulation of glucose, fatty acid, lipoprotein synthesis, metabolism, transport, and energy metabolism via activating specific nuclear receptors (farnesoid X receptor, pregnane X receptor, and vitamin D receptor), G-protein coupled receptor TGR5, and cell signaling pathways[19]. Moreover, the homeostasis of BA is recently reported to be altered in T2DM, although the available data are not fully consistent[20]. In our study, we used STZ

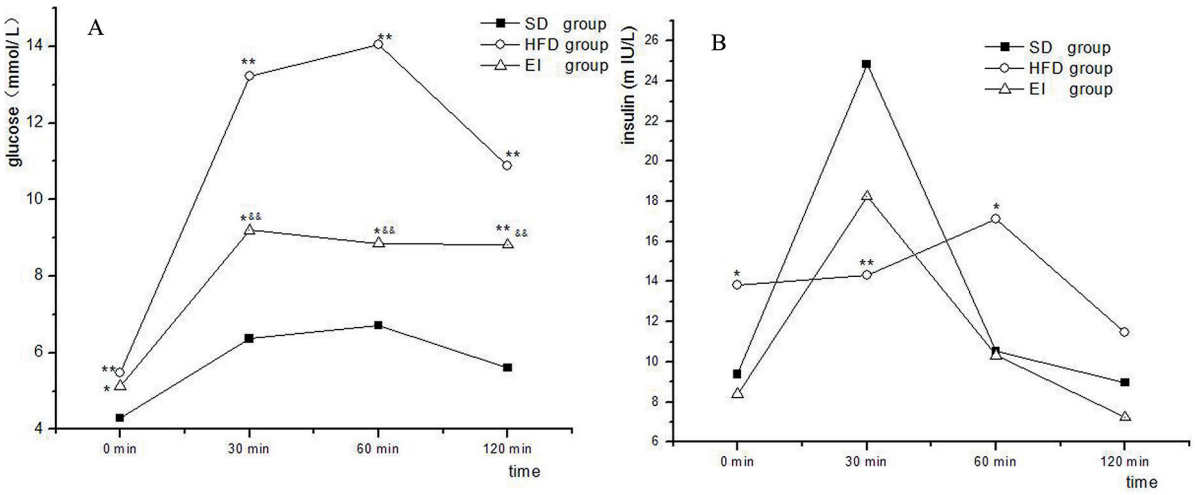

Figure 1. Effect of metformin on oral glucose tolerance (OGTT) in rats. ***A,*** The level of serum glucose. ***B,*** The level of serum insulin. Data were expressed as mean ± SD. $^{*}p < 0.05$ *vs.* SD group; $^{**}p < 0.01$ *vs.* SD group; $^{\&\&}p < 0.01$ *vs.* HFD group.

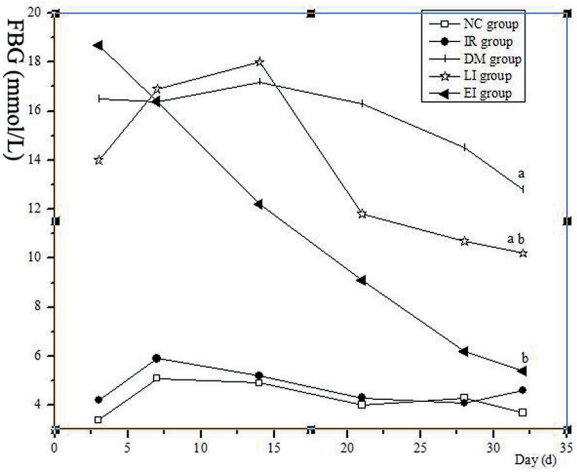

Figure 2. The FBG levels after injection with STZ. $^{a}p < 0.05$ *vs.* NC group; $^{b}p < 0.05$ *vs.* DM group.

induced-to diabetic models to further explore the effect of metformin. After successfully inducing diabetic models, diabetic rats in EI and LI group displayed a significantly elevated serum TBA level compared with NC group (Table II), as well as DM group. All these findings suggest that metformin may have an important effect on BA metabolism in rats with T2DM.

Metformin is regarded as the most widely prescribed drug for the treatment of T2DM because it not only lowers blood glucose concentrations without causing overt hypoglycemia but also leads to a significant decrease in plasma fasting insulin levels[21]. Owen et al[22] also reported that metformin exerted anti-diabetic effects via inhibition of complex 1 of the mitochondrial respiratory chain. Lien et al[22] have reported that metformin could induce the phosphorylation of nuclear BA receptor farnesoid X receptor (FXR), disturb BA homeostasis, and deteriorate liver injury through AMPK-FKR crosstalk. Another study also has showed that metformin is involved in gut-based pharmacology, including alteration of BA recirculation in patients with T2DM (Napolitano et al., 2014). Moreover, Maida et al[20] recently demonstrated that metformin induced islet incretin receptor gene expression and acutely increased plasma levels of GLP-1 (glucagon-like peptide-1). It was also reported that in human with prior gastric bypass, TBAs were inversely correlated with 2-h post-meal glucose and fasting triglycerides, and positively correlated with adiponectin and peak glucagon-like peptide-1 (GLP-1)[24]. Besides, metformin is shown to induce the phosphorylation of nuclear BA receptor farnesoid X receptor (FXR) which plays a key role in maintaining bile acid and cholesterol homeostasis[25,26]. Activation of the nuclear receptor FXR was also reported to improve hyperglycemia and hyperlipidemia in diabetic mice[26]. Taken together, it can be therefore speculated that metformin have important effects on bile acids, thus to affect glucose and lipid metabolism.

Conclusions

We observed 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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Conflict of interest

The authors declare no conflicts of interest.

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