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# Myo-inositol rescued insulin resistance and dyslipidemia in db/db mice

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**Abstract**
Myo-inositol (MI), present in a variety of foods, is essential in several important processes of cell physiology. In this study, we explored the protective effects of MI against hyperglycemia and dyslipidemia in db/db mice, a typical animal model of type 2 diabetes mellitus (T2DM). MI supplement effectively suppressed the high plasma glucose and insulin levels and markedly relieved the insulin resistance (IR) in the db/db mice, comparable to metformin's effects. In MIN6 pancreatic β cells, MI also restrained the upsurge of insulin secretion stimulated by high-concentration glucose but had no impact on the promoted cell proliferation. Moreover, MI abated the enhanced plasma triglyceride and total cholesterol levels in the db/db mice. Notably, the lipid droplet formation of mesenchymal stem cells (MSCs) from db/db mice was significantly diminished after the treatment of MI, indicating that MI could effectively inhibit the differentiation of db/db mouse MSCs into adipocytes. However, MI regretfully failed to control obesity in db/db mice. This work proved that MI significantly helped db/db mice's metabolic disorders, indicating that MI has potential as an effective adjunctive treatment for hyperglycemia and dyslipidemia in T2DM patients.

**Keywords:** db/db mice; Dyslipidemia; Hyperglycemia; Insulin resistance; Myo-inositol

**Highlights:**
- The monotherapy of myo-inositol effectively suppressed the high plasma glucose and insulin levels and markedly relieved the insulin resistance (IR) in the db/db mice, at a level comparable to the effects of metformin.
- MI abated the enhanced plasma triglyceride and total cholesterol levels in the db/db mice and diminished the lipid droplet formation of their MSCs.
- Unlike other studies, MI failed to control obesity in db/db mice.

## Introduction

Myo-inositol (MI), one of the B vitamins, is present in various foods such as wheat germ, fruits, and vegetables. It is the most prominent natural inositol (hexahydroxycyclohexane, C6H12O6) as well as a vital component and nutrient for living cells. MI and its derivatives, such as phosphatidylinositols (PI), MI polyphosphates (IPs), and phosphoinositides (PIPs), have a significant impact on several indispensable processes of cell physiology involving cell adhesion, cell signaling, and vesicular trafficking (Baldassarre et al., 2021; Dinicola et al., 2021; Gajewiak et al., 2006). In addition, MI is also used as a compatible osmolyte to control intracellular osmolarity in different tissues (Lahiri Majumder and Biswas, 2006; Lahjoujia et al., 2007). The roles of MI in various diseases of different systems have been studied. Evidence suggests that the MI level in the brain correlates with emotional instability in patients with mental and cognitive diseases. Abnormal levels of MI, as well as glutamate and glutamine, were observed in the brains of most patients with depression (Shirayama et al., 2017). Moreover, a high MI level may be related to obsessive-compulsive and panic disorders. The administration of lithium could lower the MI level in critical brain areas to achieve a therapeutic effect (Bloch et al., 2010; Seelan et al., 2009; Vaden et al., 2001). MI is also useful in cancer prevention, such as early pulmonary lesions, colon, breast, prostate, and metastatic lung cancer (Kassie et al., 2010; Vucenik and Shamsuddin, 2003, 2006).

There has been a growing interest in using MI to modulate metabolic diseases (Arefhosseini et al., 2023; Krysiak et al., 2023; Watkins et al., 2023). MI is also indicated to modulate lipometabolism. Using it in treatment could increase serum plasmalogens, decrease small dense low-density lipoprotein (LDL) in hyperlipidemic subjects, and reduce the free fatty ac-

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ids released from adipose tissues (Maeba et al., 2008). These studies mainly focused on human disorders associated with insulin resistance, such as polycystic ovary syndrome, gestation, or metabolic syndrome (Croze and Soulage, 2013). However, few studies described the effect of MI on lipometabolism in individuals with hyperglycemia. Also, several randomized, controlled studies reported that MI supplementation might reduce the incidence of gestational diabetes (GDM) in pregnant women with a family history of type 2 diabetes mellitus (T2DM) or non-obese overweight (D'Anna et al., 2013; Santamaria et al., 2016). MI seems to be a safe nutritional supplement for GDM prevention and treatment. Pintaudi et al. (2016) showed that in T2DM patients with suboptimal glycemic control already treated with glucose-lowering agents, the combination of MI and d-chiro-inositol as an add-on supplement could further decrease their fasting blood glucose and glycated hemoglobin A1c (HbA1c) levels.

However, no study has demonstrated the monotherapy of MI in T2DM and compared it with classic oral antidiabetic drugs.

Meanwhile, the underlying mechanisms of MI's effects on the modulation of glycemic and lipid profiles are still unclear. The db/db mouse is a typical animal model of T2DM, which shows phenotypes of obesity, hyperglycemia, dyslipidemia, and insulin resistance. This work aims to prove the protective effect of MI against T2DM with db/db mice and to explore its mechanism preliminarily, which may further evidence the potential benefits of MI on the early prevention and progress control of T2DM.

## Materials and methods

### *Mice*

Male BKS-Lepr –/– (db/db) mice and the lean wild-type littermates (WT) were purchased from the Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China). The mice were set in a well-air-conditioned room with a temperature of 20 ± 1 °C, relative humidity of 55 ± 5%, and 12 h light/dark cycle. The animals were maintained following the guidelines for the care and use of laboratory animals of Hunan Provincial People's Hospital. The study was approved by the Committee on the Use of Live Animals in Teaching and Research of Hunan Provincial People's Hospital. MI (Sigma-Aldrich, USA) supplement was dosed by administrating MI at 1% (wt/vol) to the mice's drinking water. The four-week-old db/db mice were divided into three groups ($n$ = 6 per group) and treated with Myo-inositol (1% in drinking water), metformin (0.25% in drinking water), and a normal diet, respectively, for eight weeks. The four-week-old WT mice were administered a normal diet. Mice with a normal diet were set as the control, including the wild-type control (WT CTL) and db/db control (db/db CTL).

The body weight and length of the mice were recorded at indicated time points. After 8 hours of fasting, the blood glucose concentrations of the mice were detected using tail vein blood with a glucometer (One-Touch Ultra, Lifescan) every week. The plasma lipid (total cholesterol and triglyceride) concentrations and insulin levels were determined using commercially available kits (Asan Pharm. Co., Ltd.). The homeostatic model assessment of insulin resistance (HOMA-IR) was determined to assess insulin resistance using the following simplified equation: HOMA-IR = fasting plasma glucose (FPG) (mmol/l) × Fasting plasma insulin (mIU/l)/22.5. Plasma triglyceride and total cholesterol were measured in 12-week-old mice that had been treated for eight weeks.

### *Cell culture*

Mouse MIN6 beta-cells (clone 8, ATCC, USA, CCL-226) were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, USA) containing 10% fetal bovine serum (FBS) and 100 units/ml penicillin/streptomycin. The MIN6 cells were seeded in plates and then treated with 2.8 or 16.8 mmol/l glucose solution plus 1 mmol/l MI. After 72 h culturing, the effect of MI on cell viability was analyzed by 3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl Tetrazolium Bromide (MTT) assay (protocol from Roche Applied Science). The optical density (OD) values of MTT were detected at 595 nm and compared. The insulin secretion of MIN6 cells was determined and normalized by the cell number (ng/1000 cells).

Primary MSCs were isolated from the bone marrow and cultured using a standard protocol (Helfrich and Ralston, 2003). To induce the differentiation of MSCs into adipocytes, the adipocytic differentiation medium, which contained α-MEM, 10% FBS supplemented with 10 μg/ml insulin (Sigma-Aldrich, St. Louis, MO, USA), and 1 μM dexamethasone (Sigma-Aldrich, St. Louis, MO, USA) was applied. On the 28th day after differentiation, adipocytes containing lipid droplets were stained red by the Oil Red O solution (0.3% Oil Red O in 60% isopropanol). The staining area was measured using Image J (National Institute of Health, USA). The extent of differentiation was assessed by the ratio to the area of the WT CTL group.

### *Statistics*

Data were represented as mean ± standard deviation (SD). Statistical analyses were optimized by one-way analysis of variance (ANOVA) or student's $t$-test with SPSS 26.0 (SPSS Inc., USA) for significant differences at $p < 0.05$.

## Results

### *Effects of MI on FPG, plasma insulin, and HOMA-IR in db/db mice*

As illustrated in Fig. 1a, the db/db mice (db/db CTL) presented impaired glucose metabolism by significant hyperglycemia compared with WT mice (WT CTL, FPG normally under 10 mmol/l). Their FPG rose with time from the age of 4 weeks and constantly remained high above 30 mmol/l after the age of 7 weeks. Their fasting insulin and calculated HOMA-IR were relevantly and markedly elevated (Fig. 1b, 1c), indicating prominent insulin resistance in db/db mice. Notably, the MI supplement in db/db mice effectively suppressed hyperglycemia to a level ranging from 12.6 to 18.9 mmol/l, decreased serum insulin levels from 8.43 ng/ml to 5.92 ng/ml (–29.8%), and consequently markedly relieved insulin resistance. These actions were similar to what the traditional anti-hyperglycemic agent metformin did (Fig. 1).

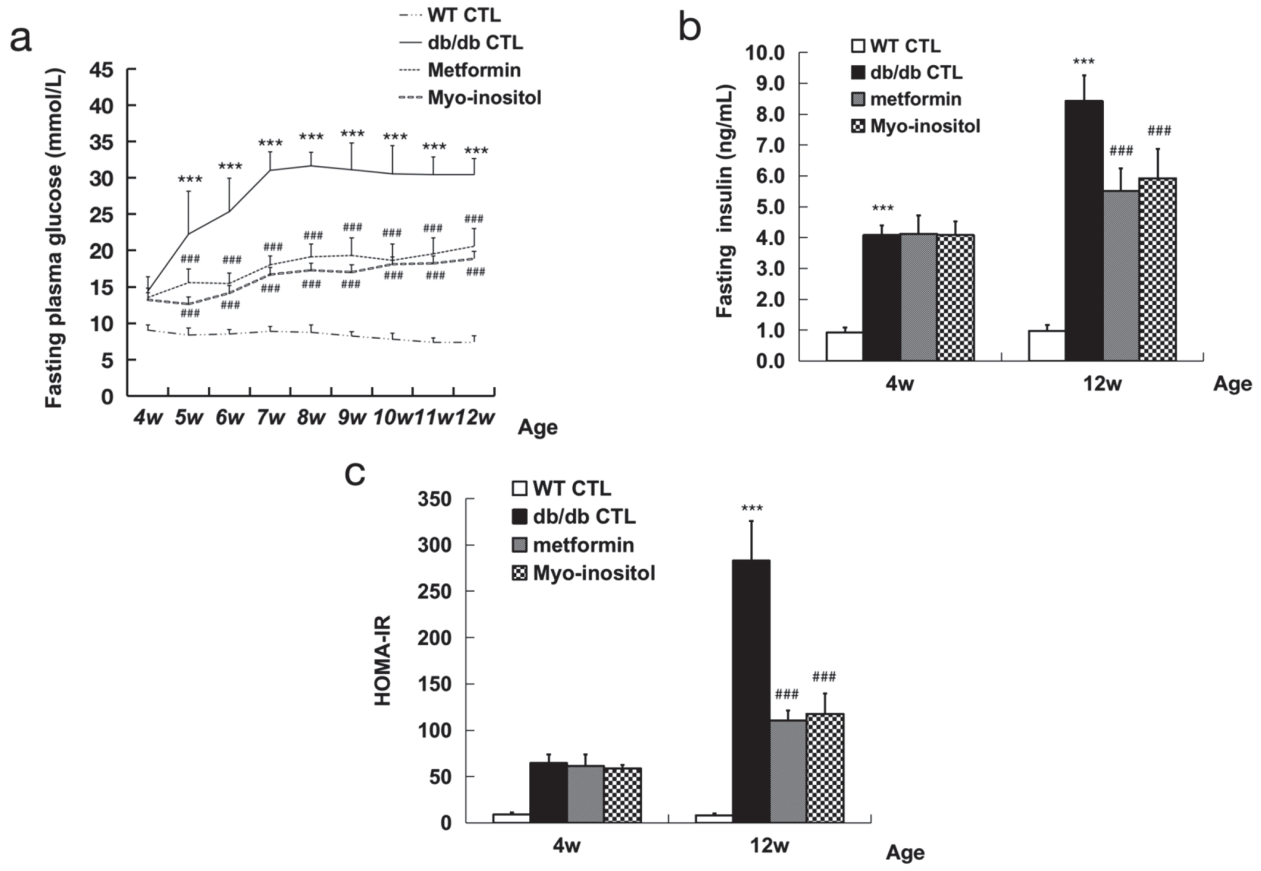


**Fig. 1.** Effects of MI on FPG (**a**), fasting insulin (**b**), and HOMA-IR (**c**) in db/db mice. Mice were treated with Myo-inositol (1% in drinking water), metformin (0.25% in drinking water), or a normal diet from the age of 4 to 12 weeks. Mice with a normal diet were set as the control, including the wild-type control (WT CTL) and db/db control (db/db CTL). *** $p < 0.001$ vs WT CTL and ### $p < 0.001$ vs. db/db CTL at the respective time point. Data are presented as mean ± SD ($n = 6$).

### *Effects of MI on proliferation and insulin secretion of MIN6 beta-cells*

To further delineate the effect of MI on insulin secretion, we employed the MIN6 pancreatic β cell line. After 72 h culturing, the cell proliferation of MIN6 cells was significantly promoted by 16.8 mmol/l glucose rather than 2.8 mmol/l glucose, which could not be altered by the MI supplement (Fig. 2a). The 16.8 mmol/l glucose also stimulated nearly 3-fold insulin secretion in MIN6 cells (Fig. 2b). This upsurge of insulin secretion induced by high-concentration glucose could be restrained by the MI supplement. However, the MI treatment did not change the basic insulin secretion of those islet β cells incubated in low-concentration glucose (Fig. 2b). These results suggested that MI protected islet function by containing the high-glucose-induced insulin secretion rather than the cell proliferation of pancreatic β cells.

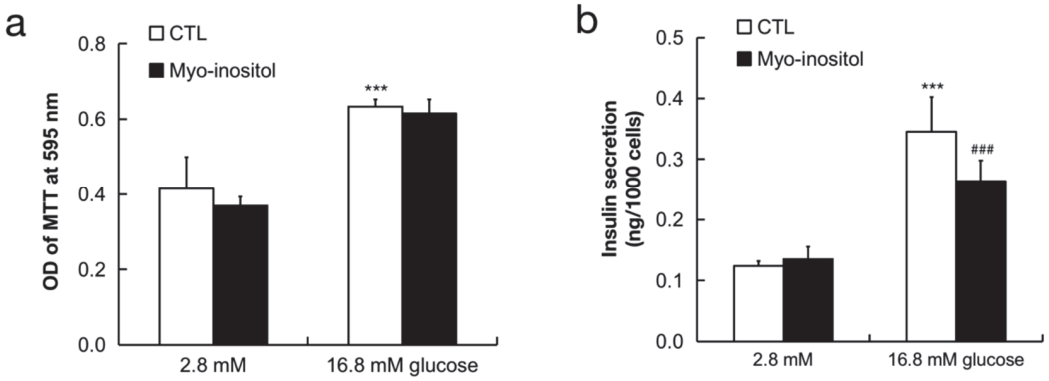


**Fig. 2.** Effects of MI on cell proliferation (**a**) and insulin secretion (**b**) of MIN6 beta-cells after low- or high-concentration glucose inducement. The MIN6 cells were treated with 2.8 or 16.8 mmol/l glucose solution for 72 h. The cells without 1 mmol/l MI were set as the control (CTL). *** $p < 0.001$ vs CTL with 2.8 mM glucose; ### $p < 0.001$ vs CTL with 16.8 mM glucose. Data are presented as mean ± SD of one representative experiment of 3 independent repeats.

### *Effect of MI on lipid metabolism in db/db mice*

Compared with WT mice, the db/db mice displayed significantly elevated plasma triglyceride (2.73 ± 0.29 vs. 1.16 ± 0.17 mmol/l, Fig. 3a) and total cholesterol (6.35 ± 0.40 vs. 2.52 ± 0.34 mmol/l, Fig. 3b). However, after being treated with MI or metformin for eight weeks, db/db mice showed approximately 19%~23% and 23%~27% decreases in plasma triglyceride and total cholesterol, respectively (Fig. 3).

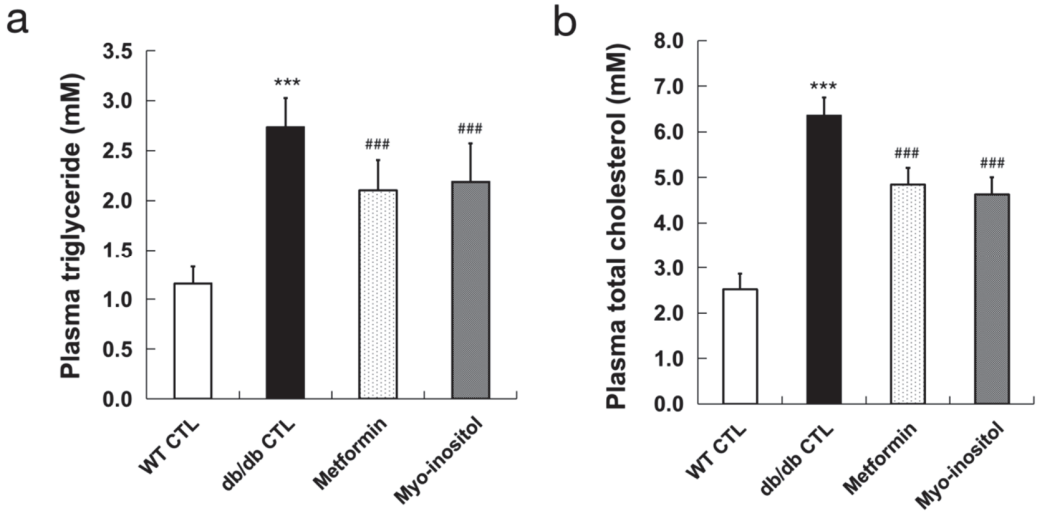


**Fig. 3.** Effects of MI on lipid metabolism in db/db mice. The plasma triglyceride levels (**a**) and plasma total cholesterol levels (**b**) were detected in 12-week-old mice after being treated with Myo-inositol (1% in drinking water), metformin (0.25% in drinking water), or a normal diet for eight weeks. *** $p < 0.001$ vs. WT CTL; ### $p < 0.001$ vs. db/db CTL. Data are presented as mean ± SD ($n = 6$).

### *Effects of MI on differentiation of MSCs into adipocytes*

Fig. 4a showed a larger red staining area of lipid droplets in db/db CTL than that in WT CTL, indicating a higher proportion of differentiation into adipocytes in MSCs isolated from db/db mice than that in MSCs from WT mice under the same inducing conditions. Nevertheless, after being treated with MI for 28 days, the lipid droplet formation of MSCs of db/db mice was significantly suppressed, as shown by obviously decreased red staining. The difference in the area of Oil Red O staining between the WT CTL, db/db CTL, and MI group is statistically significant (Fig. 4b). It suggested that MI could effectively inhibit the differentiation of db/db mouse MSCs into adipocytes, which may be the reason for the attenuated hyperlipidemia after the MI supplement in db/db mice.

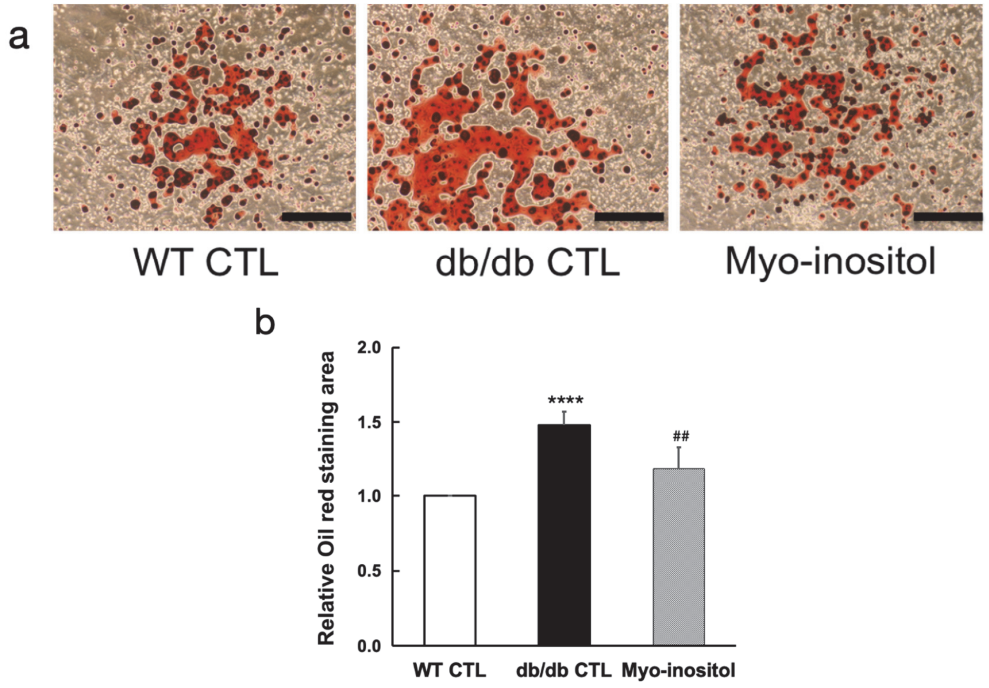


**Fig. 4.** Effect of MI on adipocytic differentiation of MSCs. MSCs were cultured in an adipogenic medium without (CTL) or with 1 mmol/l MI. Adipocytes containing lipid droplets were stained with Oil Red O solution as red (bars = 100 μm). (**a**) Images were captured at ×40 magnification. (**b**) Relative Oil Red O staining area was quantified by Image J analysis and assessed by the ratio to the WT CTL group. **** $p < 0.0001$ vs. WT CTL; ## $p < 0.01$ vs. db/db CTL. Data are presented as mean ± SD ($n = 6$).

### *Effects of MI on the obesity of db/db mice*

Compared with WT mice, all the db/db mice groups showed obvious obesity with continuous and substantial weight gain (Fig. 5a, the mean body weight of 47.0 g vs. 27.3 g at 12 weeks). Although MI and metformin could successfully ameliorate the disturbed glucose and lipid metabolism in db/db mice, they hardly rescued db/db mice's obesity. The db/db mice without MI or metformin treatment had lower body weight than those treated with MI or metformin since the age of 10 weeks, which was statistically significant at the age of 12 weeks between the metformin and db/db CTL group. This may be because long-term poor glycemic control led to accelerated lipolysis and proteolysis, negative nitrogen balance, and pathological weight loss. In addition, when the body length was investigated, we found no difference between the WT and db/db mice with or without MI/metformin treatment (Fig. 5b).

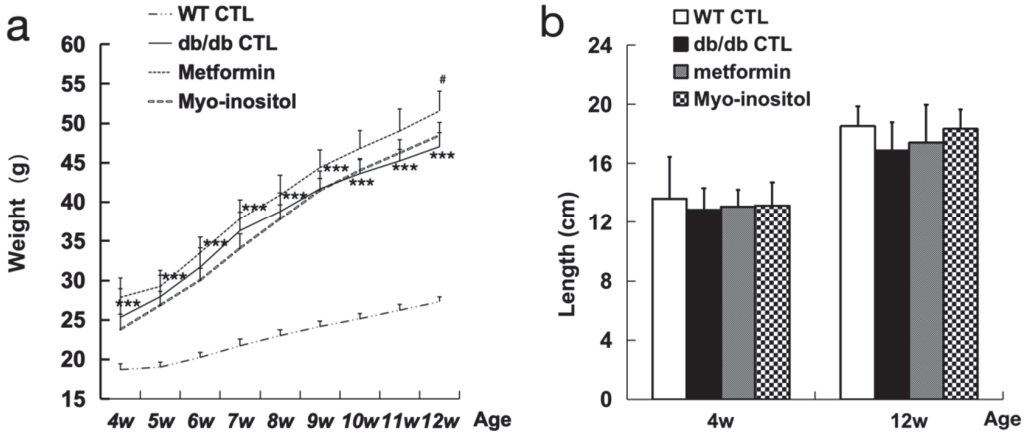


**Fig. 5.** Effect of MI on Body weight (**a**) and body length (**b**) of mice of different groups. Mice were treated with Myo-inositol (1% in drinking water), metformin (0.25% in drinking water), or a normal diet from the age of 4 to 12 weeks. *** $p < 0.001$ vs. WT CTL and # $p < 0.05$ vs. db/db CTL at the respective time point. Data are presented as mean ± SD ($n = 6$).

## Discussion

T2DM, a disease characterized by high levels of blood glucose, is the most common subtype of diabetes. Currently, it is becoming one of the largest threats to global health. As estimated by the 10th International Diabetes Federation Diabetes Atlas, approximately 537 million adults around the world have been diagnosed with diabetes, and 541 million have prediabetes or impaired glucose tolerance. The pathogenesis of T2DM involves two basic links: insulin resistance and a relative insulin deficiency. Thus, the treatment of T2DM has changed from simple anti-hyperglycemia to rescuing insulin resistance, which can comprehensively prevent the risk factors, reduce the incidence of diabetic vascular complications, and improve the patient's quality of life. It is important to prevent or delay diabetes complications by maintaining blood glucose and cholesterol levels and blood pressure as close to normal as possible (Zoungas et al., 2017). In this work, we explored the role of MI in modulating insulin resistance and dyslipidemia in db/db mice with T2DM.

MI and its isomers or derivatives play potential roles in metabolic diseases and present therapeutic interests. In a study of obese insulin-resistant monkeys, Ortmeyer (1996) first found that both MI and D-chiroinositol supplementation lowered monkeys' postprandial plasma glucose levels without promoting insulin secretion, and MI supplementation also decreased urine glucose levels. In mice, Dang et al. (2010) demonstrated that MI and its derivatives lowered the plasma glucose and insulin levels by increasing the translocation of glucose transporter 4 (GLUT4) and stimulating glucose uptake in the skeletal muscle.

In our study, the MI supplement effectively suppressed hyperglycemia in db/db mice, comparable to the effect of the traditional anti-hyperglycemic agent metformin. Like metformin, MI did not show an insulin-like act or stimulate insulin secretion. Conversely, the serum insulin levels were downregulated in db/db mice after the MI supplement. We further compared the cell proliferation and insulin secretion stimulated by low- and high-concentration glucose in MIN6 beta-cells. MI supplement did not influence cell proliferation of MIN6 beta-cells stimulated either by low- or high-concentration glucose. However, it restrained the upsurge of insulin secretion of MIN6 beta-cells stimulated by high-concentration glucose, which is different from the effects of metformin on MIN6 beta-cells. Jiang et al. (2014) found that metformin suppressed MIN6 β cell proliferation and promoted apoptosis, indicating that an overdose of metformin may lead to potential β cell toxicity.

These different effects of MI and metformin on islet beta cells may have greater significance in the late stage of diabetes with islet dysfunction. The calculated HOMA-IR further demonstrated the relieved insulin resistance in MI-treated db/db mice. The above data showed that the potential of MI in T2DM treatment is to lower blood sugar and protect islet function by relieving insulin resistance, rather than promoting islet cell proliferation or increasing insulin secretion.

MI is also suggested to significantly affect dyslipidemia and has therapeutic potential in impaired lipids metabolism by animal and clinical studies. In Triton WR-1339-induced hyperlipidemic rats, oral administration of MI remarkably reduced the levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c), and enhanced the level of high-density lipoprotein cholesterol (HDL-c) (Antony et al., 2017). Monotherapy with MI also enhances serum plasmalogens and reduces small dense LDL, particularly in hyperlipidemic subjects with metabolic syndrome (Maeba et al., 2008). However, Okazaki and Katayama (2008) suggested that it was dietary inositol hexakisphosphate (IP6) and not MI, which had significant influences on elevated serum cholesterol and phospholipids levels in rats with fatty liver induced by a

casein-based diet containing 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane.

Meanwhile, MI was reported to improve the metabolic profile of polycystic ovary syndrome (PCOS) women. The combined therapy of MI and D-chiro-inositol improved LDL, HDL, and triglyceride levels in PCOS patients (Minozzi et al., 2013). Monotherapy with MI decreased TG and very low-density lipoprotein cholesterol (VLDL-c) levels while not affecting the TC level in patients with PCOS (Shokrpour et al., 2019).

Similarly, Zhang et al. (2020) demonstrated that MI supplementation significantly reduced TG but not TC in rat PCOS models.

Our study proved that MI supplementation could improve dyslipidemia in diabetic mice. After 8 weeks of MI treatment, db/db mice showed approximately 19%–23% and 23%–27% decreases in plasma TG and TC, respectively, similar to those with metformin treatment. We speculate that MI may affect dyslipidemia differently in special pathological statuses. Croze et al. (2013) found that mice with MI treatment displayed reduced white adipose tissue accretion compared with controls.

The reduction in white adipose tissue deposition was because of the decreased adipocyte volume but not adipocyte number. In this study, we observed the adipocytic differentiation of MSCs in db/db mice. The increased lipid droplet formation of MSCs in db/db mice was significantly diminished by the MI supplement, suggesting that MI effectively inhibited the differentiation of db/db mouse MSCs into adipocytes. Several studies reported that MI could significantly reduce the incidence of fetal macrosomia in pregnant women with GDM or glucose intolerance (D'Anna et al., 2013; Santamaria et al., 2018). We presume that MI supplements may also attenuate the differentiation of stem cells into adipocytes during embryonic development in pregnant women with insulin resistance or GDM, resulting in the incidence reduction of fetal macrosomia. Since fetal macrosomia is a risk factor for obesity and diabetes in adulthood, it is fairly important for the prevention of obesity and diabetes.

Although MI, similar to metformin, showed satisfactory effects on the disturbed glucose and lipid metabolism in db/db mice, the two agents did not improve their obesity status. After the treatment of metformin or MI, db/db mice showed more weight gain at 11 and 12 weeks compared with untreated db/db mice. Usually, metformin is considered to contribute to ideal weight control in T2DM patients. Previous studies also showed that MI supplementation could effectively reduce the body mass index (BMI) of patients with PCOS (Genazzani et al., 2012). However, in some studies, the low metformin dose does not affect the body mass index and weight gain in pregnant obese women (Dienstmann et al., 2020). Our study did not observe the benefit of metformin and MI on body weight. The pathological weight loss caused by long-term poor glycemic control in db/db mice might be one of the reasons. In addition, various factors such as genetic defects, special physiological conditions, and dietary intake may also lead to unsatisfactory improvement of obesity in db/db mice with metformin and MI treatments. Meanwhile, as expected, no difference in body length was observed between the WT and db/db mice with or without MI/metformin treatment.

## Conclusion

In conclusion, MI can lower blood glucose, alleviate hyperinsulinemia, improve insulin resistance, and reduce plasma triglyceride and total cholesterol in db/db mice. These findings present a potential for myo-inositol as an effective adjunctive treatment for hyperglycemia and dyslipidemia in T2DM patients.

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The study's funding sources had no role in the study design; the collection, analysis, and interpretation of data; the writing of the report; or in the decision to submit.

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***Ethical aspects and conflict of interest***

The authors have no conflict of interest to declare.

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