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## Protective Effects of Reduced Beta 2 Glycoprotein I on Liver Injury in Streptozotocin (STZ)-Diabetic Rats by Activation of AMP-Activated Protein Kinase

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Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
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**Background:** Protective effects of reduced beta 2 glycoprotein I (Rβ2GPI) against vascular injury of diabetes mellitus have been extensively investigated. However, the effects of Rβ2GPI on liver injury in diabetic animals have not been reported.


**Material/Methods:** A diabetic rat model was produced by systemic injection of streptozotocin (STZ). Rats were divided into a normal control group, a model group, and an Rβ2GPI treatment group (N=6 in each group). After treatments, blood serum and liver tissue were collected to test the protection of Rβ2GPI. AMP-activated protein kinase (AMPK) was detected by immunohistochemistry and Western blotting.

**Results:** Our results revealed that Rβ2GPI reduced blood glucose, serum creatinine, and urea nitrogen levels, as well as serum inflammation cytokines, including interleukin (IL)-6, tumor necrosis factor (TNF)-α and C-reactive protein in the diabetic rats. Importantly, Rβ2GPI prevented liver injury in the diabetic rats as confirmed by hematoxylin-eosin (H&E) staining, alanine transaminase, aspartate transaminase, and gamma-glutamyl transferase. Reactive oxygen species (ROS) were promoted by diabetic modeling and were attenuated by Rβ2GPI administration. Moreover, Rβ2GPI significantly reduced liver catalase, malondialdehyde, and superoxide dismutase levels in the diabetic rats. Rβ2GPI reduced liver glycolipid storage in STZ diabetic rats. Both immunohistochemistry and Western blotting demonstrated that Rβ2GPI promoted AMPK phosphorylation in the diabetic rats.

**Conclusions:** Our data proved that Rβ2GPI prevented liver injury in diabetic rats, likely through activating the AMPK signaling pathway.

**MeSH Keywords:** **AMP-Activated Protein Kinases • Diabetic Ketoacidosis • Pregnancy-Specific beta 1-Glycoproteins**

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## Background

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, affecting approximately 422 million people worldwide [1]. Liver injury is a major complication in DM patients, caused by activation of hepatic stellate cells and liver fibrosis [2]. Liver injury in diabetes mellitus has turned out to be a central issue for liver metabolic diseases. The mechanisms underlying liver injury are extremely interesting for disclosing the pathogenesis and finding therapeutic approaches for DM.

Beta 2 glycoprotein I ( $\beta$ 2GPI) is predominantly synthesized in hepatocytes and has now been identified as the most prominent antigen in antiphospholipid syndrome [3,4]. Interestingly, R $\beta$ 2GPI has been found to protect against vascular injury of DM. For example, R $\beta$ 2GPI protects endothelial cells from hyperglycemia-induced injury [5,6]. Our previous study has also demonstrated that R $\beta$ 2GPI inhibited vascular endothelial growth factor and basic fibroblast growth factor-induced angiogenesis [7]. Redox status of  $\beta$ 2GPI is an important disease marker for different stages of diabetic angiopathy [8]. However, whether R $\beta$ 2GPI has hepatoprotective effects in diabetic model is still unclear.

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase and acts as a sensor of cellular energy status. Accumulating evidence reveals that AMPK regulates a wide variety of gene expression and metabolic signaling pathways [9,10]. Moreover, AMPK plays a pathophysiological role in lipotrophic diabetes [11]. The present study investigated the effect of R $\beta$ 2GPI on liver injury in a streptozotocin (STZ)-induced diabetic model and assessed the underlying mechanisms.

## Material and Methods

### Purification of $\beta$ 2GPI and preparation of reduced $\beta$ 2GPI

$\beta$ 2GPI and R $\beta$ 2GPI were prepared following a method detailed in a previous publication [12]. Purified  $\beta$ 2GPI (2  $\mu$ M) was reduced using TRX-1 (3.5  $\mu$ M) activated with DTT (70  $\mu$ M). Reduced glutathione was used to protect the thiols of R $\beta$ 2GPI. The levels and structure of R $\beta$ 2GPI were confirmed, as previously described [13–15].

### Animal model and groups

Wistar rats (male, 6 weeks old, body weight 160–180 g) were kept in animal cages with free access to food and water with 12/12-h light/dark cycle and temperature (22 $\pm$ 2°C). Diabetes was induced by a single intraperitoneal injection of STZ at a dose of 50 mg/kg bw as previously described [16]. We randomly divided 18 rats into 3 groups (n=6 for each group): control rats, STZ-diabetic rats, and STZ-diabetic rats treated with R $\beta$ 2GPI.

Rats were administered R $\beta$ 2GPI once daily by an intravenous injection of 20  $\mu$ g R $\beta$ 2GPI once a day for 3 weeks starting on the third day after injection of STZ, as previously described [17]. A blood glucose level above 11 mmol/L was considered indicative of diabetes. All the protocols were approved by the Ethics Committee of Tianjin Medical University Metabolic Disease Hospital (Ethics protocol number: DXBYyhMEC2017-11).

Blood parameters were tested immediately after blood sampling from animals. The rats were decapitated under sodium pentobarbital anesthesia (60 mg/kg bw) 3 weeks after R $\beta$ 2GPI treatments. The livers were rapidly removed, rinsed with 0.9% NaCl, frozen in liquid nitrogen, and stored at –80°C for biochemical analysis and immunohistochemistry.

### Serum biochemical parameters

We collected 2-mL blood samples and placed them in pre-cooled tubes, centrifuged at 987 g  $\times$  for 10 min at 4°C. Serum levels of activities of glucose, creatinine, urea nitrogen, alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT) were measured using an automated biochemical analyzer (BioTek, USA). Serum IL-6 (RAB0311, SIGMA, USA) and TNF- $\alpha$  (RAB0479, SIGMA, USA) were detected followed the instructions of the assay kits.

### Histologic evaluation

Liver tissue was collected for hematoxylin-eosin (H&E) staining. The tissues were fixed in 4% paraformaldehyde for ~1 week at 4°C, cryoprotected in 30% sucrose for 1 h at 4°C, and sectioned into 5- $\mu$ m-thick sections with a frozen microtome. After that, the slides were stained by hematoxylin and eosin for 5 min. The images were taken under light microscopy.

### Liver reactive oxygen species (ROS)

Liver ROS level was measured by means of chemiluminescence (CL) in the Fenton reaction (2 mM Fe(II), 8.8 mM hydrogen peroxide, pH 7.4), as previously described [16]. Briefly, liver tissue was homogenized in Hanks' balanced salt solution (Sigma-Aldrich, USA) on ice. Then, CL was monitored for 30 s (Luminometer EA-1, Ukraine) at 37°C and continuous mixing. The sum of light signals at a sampling frequency of 0.25 Hz was calculated and is expressed as relative light units.

### Oxidative stress markers

The level of oxidative stress was determined in the liver by assessing the levels of malondialdehyde (MDA) (S0131, Beyotime, Shanghai, China), catalase (CAT100, SIGMA, USA), and superoxide dismutase (SOD) (ab65354, ABCAM, USA), as previously described [16].

**Table 1.** R $\beta$ 2GPI treatment attenuated signs of type 2 diabetes.

Groups	Control	Diabetic	R $\beta$ 2GPI
Body weight (g)	213.4 $\pm$ 12.9	207.0 $\pm$ 12.5	211.4 $\pm$ 14
Blood glucose (mM)	9.7 $\pm$ 1.1	21.9 $\pm$ 1.1**	13.4 $\pm$ 1.2 <sup>#</sup>
Serum creatinine ( $\mu$ M)	9.7 $\pm$ 0.8	22.9 $\pm$ 1.1**	13.2 $\pm$ 1.2 <sup>#</sup>
Blood urea nitrogen (mM)	9.4 $\pm$ 0.5	16.9 $\pm$ 0.7*	11.3 $\pm$ 1.1 <sup>#</sup>
IL-6 (pg/L)	96.4 $\pm$ 4.2	129.3 $\pm$ 2.3*	105.2 $\pm$ 5.3 <sup>#</sup>
TNF- $\alpha$ (pg/L)	0.78 $\pm$ 0.07	1.64 $\pm$ 0.07**	1.15 $\pm$ 0.06 <sup>#</sup>
CRP (mg/L)	4.6 $\pm$ 0.5	9.3 $\pm$ 0.4**	5.1 $\pm$ 0.3 <sup>#</sup>

\*  $p < 0.05$ , \*\*  $p < 0.01$  compared with control; <sup>#</sup>  $p < 0.05$  compared with diabetic.

### Liver glycolipid storage

The liver tissues were weighed, digested with 1 mol/L NaOH (1: 9 wt/vol) at 80°C for 10 min, and neutralized with 1 mol/L HCl. The resulting solution was incubated at 85°C for 2 h and neutralized again with 5 mol/L NaOH. After that, liver FFA was detected using a fatty acid kit (MAK044, Sigma, USA) following the manufacturer's instructions. The liver TG was determined using a triglyceride determination kit (T2449, Sigma, USA) following the manufacturer's instructions.

### Immunohistochemistry

After fixation in 4% paraformaldehyde for 1 h, liver tissues were cryoprotected in 30% sucrose for 1 h at 4°C and sectioned on a freezing microtome at 20  $\mu$ m. Sections were blocked in 0.1 M phosphate-buffered saline (PBS) containing 10% goat serum, but without Triton X-100, and then incubated with primary antibody (mouse anti-pAMPK, 1: 200, CST) in 0.1 M PBS containing 5% goat serum overnight at 4°C. Sections were washed 3 times (15 min each) in PBS and incubated with goat anti-mouse horseradish peroxidase (HRP) (IgG H&L) (Life Technologies, Carlsbad, CA, USA) for 2 h at room temperature. DAB was used to color the staining. The imaging and density analysis were following previous description [18] by using ImageJ software. At least 4 fields in each image were analyzed. The expression of p-AMPK was normalized to control.

### Western blotting

Liver tissue was used in Western blotting, and cytoplasmic protein was isolated using a Cytoplasmic Protein Extraction kit (Beyotime, Jiangsu, China). Protein concentration was measured using the bicinchoninic acid method (Beyotime, China), and an amount of protein (25  $\mu$ g) was loaded into 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels. The membranes were incubated with anti-AMPK, anti-p-AMPK, and anti-GAPDH at 4°C overnight. After washing, the membranes

were incubated with a secondary antibody (1: 5000; CST, USA). The signal was detected using an enhanced chemiluminescence detection kit (Amersham ECL RPN 2106 Kit, Amersham Pharmacia Biotech, QC, Canada).

### Statistical analysis

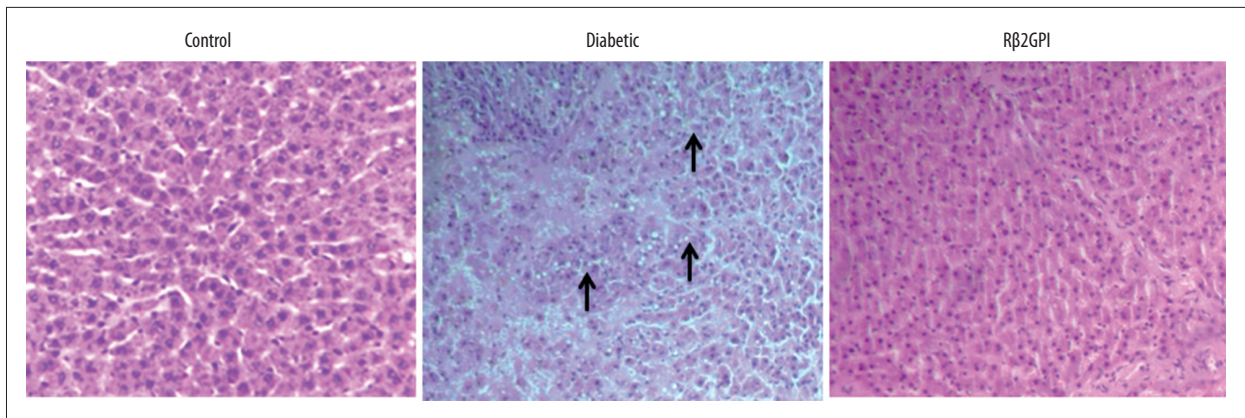
Results are presented as mean  $\pm$  standard deviation (mean  $\pm$ SD). Statistical analysis was performed using GraphPad Prism 5. Differences between groups were identified using one-way ANOVA, where  $p$ -values  $< 0.05$  were considered as statistically significant.

## Results

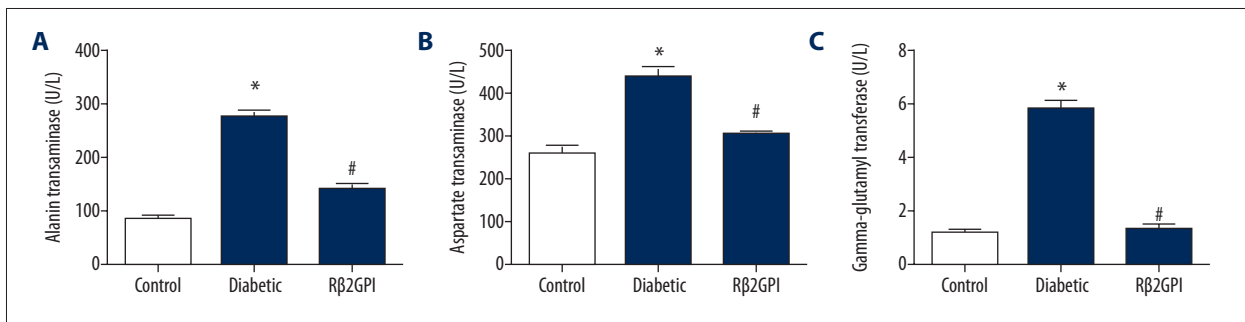
### R $\beta$ 2GPI reduces the liver injury in STZ diabetic rats

In the experiment, diabetic rats were confirmed by the increased blood glucose levels (more than 3 times compared to control) 3 days after the STZ injection. As shown in Table 1, STZ-diabetic rats showed signs of diabetes, such as hyperglycemia and higher levels of creatinine and urea nitrogen, but without bodyweight increase compared with control rats. By contrast, R $\beta$ 2GPI significantly reduced those signs compared with the STZ-diabetic group. We also detected the levels of inflammation cytokines. As shown in Table 1, diabetic modeling increased serum IL-6 and TNF- $\alpha$  levels, which were reduced by R $\beta$ 2GPI administration.

In this study, we focused on the liver injury in diabetic rats. Pathologically, liver histological structure was normal in the healthy control group, but diabetic rats showed fatty changes in centrilobular portions of the livers. R $\beta$ 2GPI prevented the pathologic changes and no fatty change was observed in the R $\beta$ 2GPI treatment group (Figure 1). The liver injury was also confirmed by biochemical indices. Alanine transaminase, aspartate transaminase, and gamma-glutamyl transferase levels were



**Figure 1.** H&E staining shows that Rβ2GPI reduces the liver injury in STZ diabetic rats (Magnification: 200×). Arrows indicate fatty changes.



**Figure 2.** Biochemical indices show that Rβ2GPI reduces the liver injury in STZ diabetic rats. (A) alanine transaminase; (B) aspartate transaminase; (C) gamma-glutamyl transferase. \*  $p < 0.05$  compared with control rats; #  $p < 0.05$  compared with diabetic rats.

significantly elevated in diabetic rats, which were down-regulated by Rβ2GPI administration (Figure 2). These data suggest that Rβ2GPI protects against liver injury in diabetic model rats.

### Rβ2GPI decreases oxidative stress in STZ diabetic rats

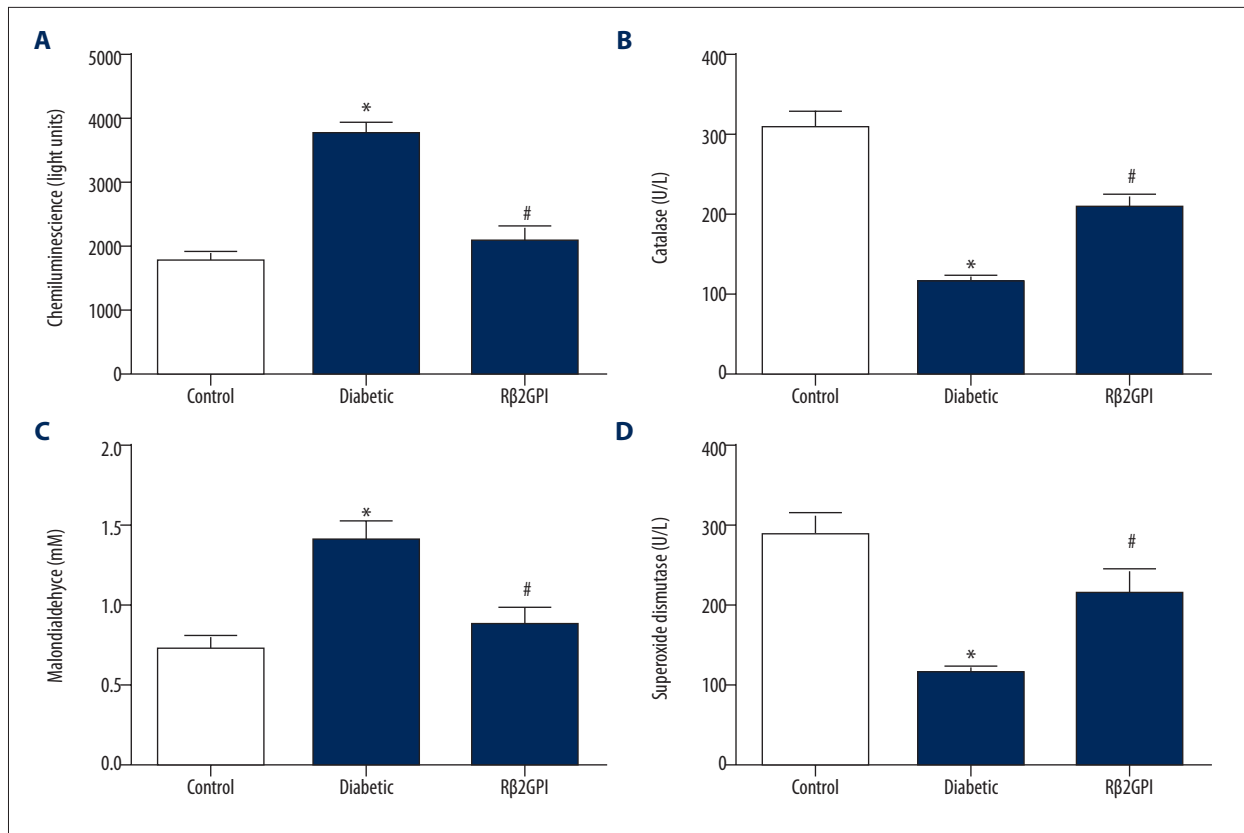
An intensification (by about 2-fold) of free radical oxidation was found in livers of diabetic rats compared to the control, as shown by chemiluminescence test (Figure 3A). ROS generation can lead to increased peroxide process and cause cellular oxidative stress. As shown in Figure 3C, the malondialdehyde level was significantly increased in the liver tissue in diabetic rats, which indicated an intensification of lipid peroxidation processes. Meanwhile, a depletion of the antioxidant system was shown in diabetic rats. The activities of catalase (Figure 3B) and SOD (Figure 3D) were reduced significantly in the livers of diabetic rats compared to the control rats. Critically, Rβ2GPI reduced the ROS and malondialdehyde levels, and significantly increased the activities of catalase and SOD compared with diabetic rats. These results suggest that Rβ2GPI prevents the ROS process in diabetic rats.

### Rβ2GPI reduces liver glycolipid storage in STZ diabetic rats

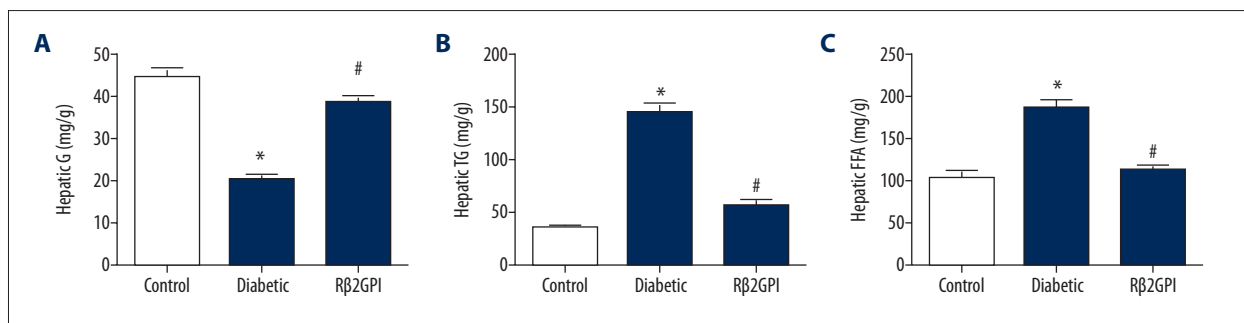
We also assessed glycolipid storage in liver tissue. As shown in Figure 4, liver glycolipid level was reduced, while TG and FFA were accumulated in liver tissue from diabetic rats. Liver glycolipid was elevated, while TG and FFA were reduced by Rβ2GPI administration.

### Rβ2GPI promotes AMPK phosphorylation in STZ diabetic rats

The signaling pathway was also investigated in the protection of Rβ2GPI. As shown in Figure 5, p-AMPK level was obviously down-regulated in liver tissue from diabetic rats, which was promoted by Rβ2GPI administration. Western blotting further confirmed that the phosphorylation of AMPK, but not total AMPK level, was reduced in liver tissue from diabetic rats (Figure 6). Rβ2GPI administration promoted the phosphorylation of AMPK.



**Figure 3.** R $\beta$ 2GPI decreases oxidative stress in STZ diabetic rats. (A) ROS level; (B) catalase level; (C) malondialdehyde level; (D) SOD level. \*  $p < 0.05$  compared with control rats; #  $p < 0.05$  compared with diabetic rats.

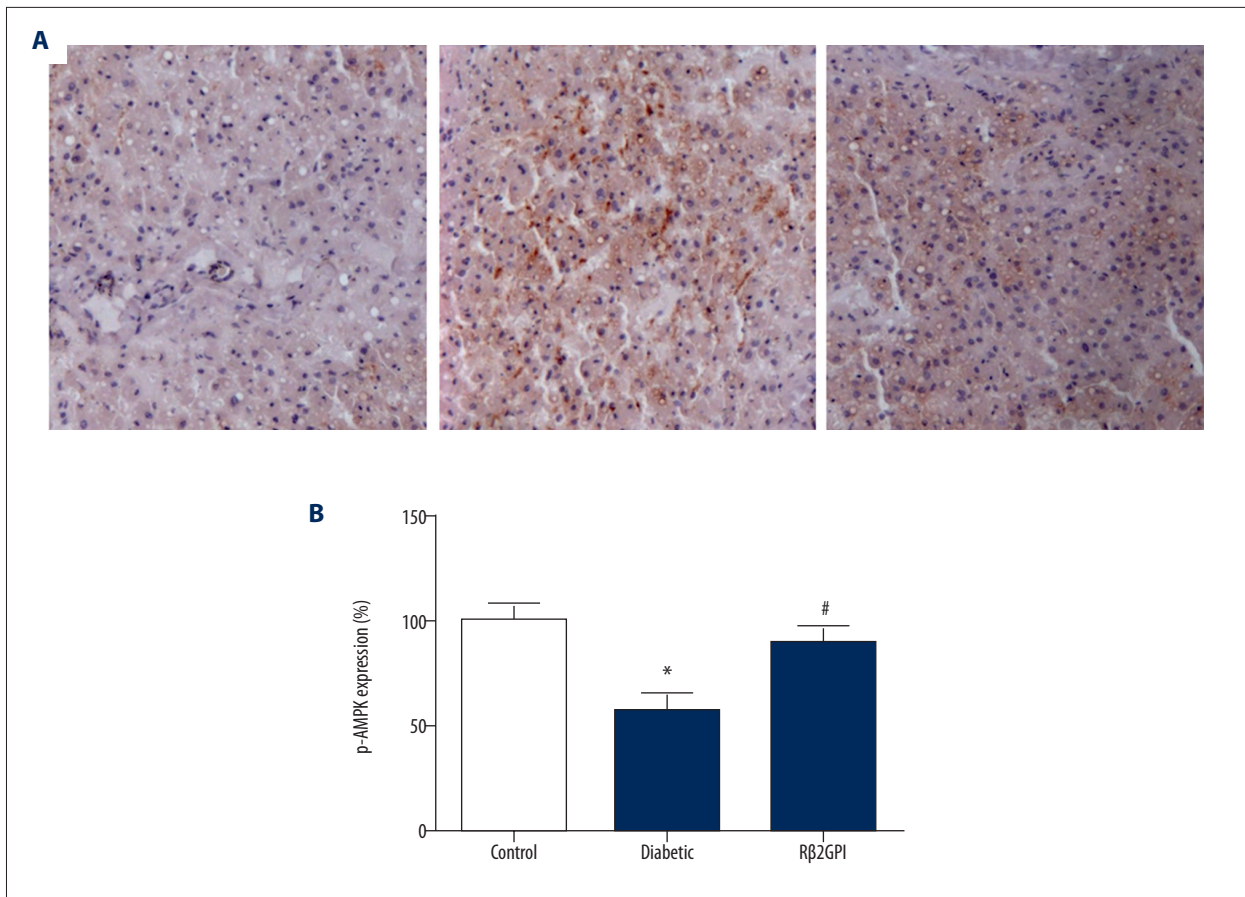


**Figure 4.** R $\beta$ 2GPI reduces liver glycolipid storage in STZ diabetic rats. (A) Hepatic G; (B) Hepatic TG; (C) Hepatic FFA. \*  $p < 0.05$  compared with control rats; #  $p < 0.05$  compared with diabetic rats.

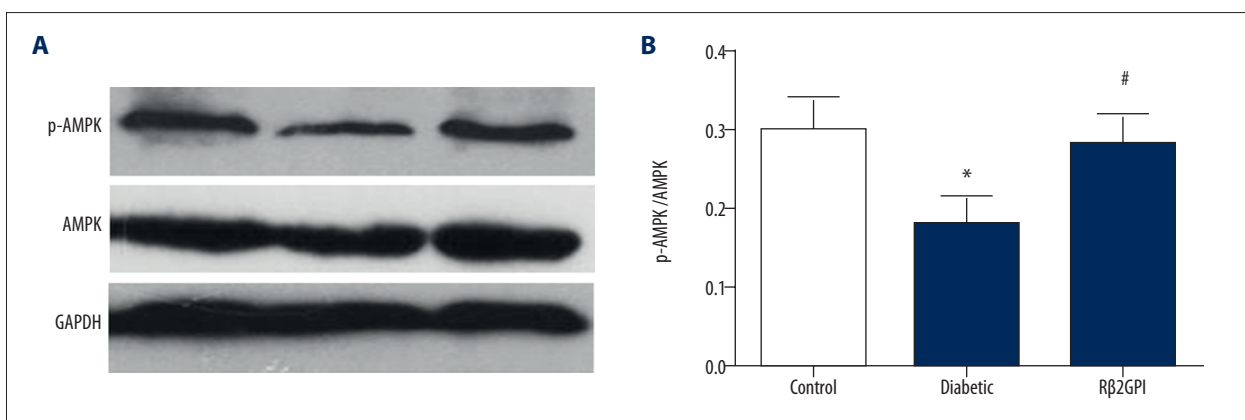
## Discussion

$\beta$ 2GPI is associated with antiphospholipid syndrome [19–21] as well as DM [22]. By contrast, we revealed that R $\beta$ 2GPI prevented liver injury in diabetic rats. Moreover, R $\beta$ 2GPI reduced liver oxidative stress and glycolipid storage in diabetic rats. As a mechanism, R $\beta$ 2GPI promoted AMPK phosphorylation in diabetic rats, which might explain the protection of R $\beta$ 2GPI against liver injury in diabetic model rats.

R $\beta$ 2GPI level is down-regulated in antiphospholipid syndrome [23,24]. Moreover, R $\beta$ 2GPI, but not non-R $\beta$ 2GPI, was reported to protect human umbilical vein cell line from oxidative stress-induced endothelial cell damage [14]. We have also reported that R $\beta$ 2GPI protects against high glucose-induced cell death in HUVECs through the miR-21/PTEN pathway [5,6]. R $\beta$ 2GPI is a candidate treatment for DM, especially the vascular complications. In our present study, we demonstrated that R $\beta$ 2GPI prevented liver injury in DM rats. On the one hand, DM signs were attenuated by R $\beta$ 2GPI treatment, including reduction of blood glucose, creatinine, and urea nitrogen levels.



**Figure 5.** Immunohistochemistry shows that R $\beta$ 2GPI promotes AMPK phosphorylation in STZ diabetic rats. **(A)** Representative images; **(B)** Quantification data. \*  $p < 0.05$  compared with control rats; #  $p < 0.05$  compared with diabetic rats.



**Figure 6.** Western blotting shows that R $\beta$ 2GPI promotes AMPK phosphorylation in STZ diabetic rats. **(A)** Representative images; **(B)** quantification data. \*  $p < 0.05$  compared with control rats; #  $p < 0.05$  compared with diabetic rats.

The increase of serum inflammation factors is an important indicator of DM [25]. We also found that diabetic modeling up-regulated IL-6 and TNF- $\alpha$  levels. On the other hand, liver injury, another feature of DM, was also prevented by R $\beta$ 2GPI injury. Both morphological observation and biochemical indices indicated that R $\beta$ 2GPI prevented liver injury in diabetic rats. Typical

fatty changes were observed in the diabetic model group but not in the control and R $\beta$ 2GPI treatment groups. Our data and results of others suggest that R $\beta$ 2GPI may have value in treatment of DM complications.

Reactive oxygen species (ROS) are reactive chemical species, including peroxides, superoxide, hydroxyl radical, and singlet oxygen. ROS are produced as a natural byproduct during normal metabolism of oxygen. As reported, ROS have important roles in cell signaling and homeostasis [26,27]. In DM, hepatic cells are widely recognized to be damaged by ROS [28]. In our study, we demonstrated that ROS level was elevated in the liver tissue of DM rats and  $\beta$ 2GPI obviously reduced ROS level. These data further confirmed the hepatic protection of  $\beta$ 2GPI in DM. Catalase and superoxide dismutase are effective regulators of hydrogen peroxide and superoxide, respectively, by converting these compounds into oxygen and hydrogen peroxide [29]. Therefore, we also detected liver catalase and superoxide dismutase. Our data showed that catalase and superoxide dismutase were reduced in DM rats and were promoted by  $\beta$ 2GPI treatment. Malondialdehyde (MDA) is also a marker for oxidative stress [30]. In our study, MDA levels in DM rats were elevated, which was reduced by  $\beta$ 2GPI treatment.

Hepatic glucose and lipid metabolic disorder are also features of liver injury in DM [31]. In our study, hepatic G was reduced, while TG and FFA were promoted in DM.  $\beta$ 2GPI treatment could promote hepatic G level and decrease TG and FFA levels. These data further show that  $\beta$ 2GPI can repair lipid metabolic disorder in DM.

AMPK is a key molecule in the regulation of bioenergy metabolism and is the core of the study of diabetes and other metabolic related diseases [32]. It is expressed in many metabolic-related organs, and can be activated by various stimuli, including cell

pressure, exercise, and many hormones and substances that can affect cell metabolism [33]. Genetic and pharmacological studies have shown that AMPK is essential for the body to maintain the balance of glucose [34]. In our study, we revealed that AMPK phosphorylation was down-regulated in liver tissue in DM rats. Consistent with the biochemical or morphological alterations,  $\beta$ 2GPI treatment also promoted p-AMPK level but did not affect total AMPK level. AMPK activation is elicited by phosphorylation at Thr172 catalyzed by liver kinase B1 in response to an increase of AMP-to-ATP ratio, or by the calmodulin-dependent protein kinase  $\beta$  (CaMKK $\beta$ ) in response to elevated  $\text{Ca}^{2+}$  levels [35]. Other possible mechanisms may involve liver kinase B1 or protein phosphatase-2C, a phosphatase that dephosphorylates and inactivates AMPK [35]. We plan to perform further research to elucidate the underlying mechanisms involved.

## Conclusions

We demonstrated the hepatoprotection of  $\beta$ 2GPI in diabetic rats for the first time. The potential mechanism is related to the regulation of ROS and the AMPK signaling pathway. Our study may provide important evidence for the protection of  $\beta$ 2GPI in liver complications of DM, although further experimental and clinical trials are needed.

## Conflict of interest

None.

## References:

1. Socodato R, Santiago FN, Portugal CC et al: Dopamine promotes NMDA receptor hypofunction in the retina through D1 receptor-mediated Csk activation, Src inhibition and decrease of GluN2B phosphorylation. *Sci Rep*, 2017; 7: 40912
2. Cade WT: Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. *Phys Ther*, 2008; 88(11): 1322–35
3. Rahgozar S: Revisiting beta 2 glycoprotein I, the major autoantigen in the antiphospholipid syndrome. *Iran J Immunol*, 2012; 9(2): 73–85
4. Blank M, Shoenfeld Y: Beta-2-glycoprotein-I, infections, antiphospholipid syndrome and therapeutic considerations. *Clin Immunol*, 2004; 112(2): 190–99
5. Zhang J-Y, Ma J, Yu P et al: Reduced beta 2 glycoprotein I prevents high glucose-induced cell death in HUVECs through miR-21/PTEN. *Am J Transl Res*, 2017; 9(9): 3935–49
6. Zhang JY, Ma J, Yu P et al: Effects of reduced beta2 glycoprotein I on high glucose-induced cell death in HUVECs. *Mol Med Rep*, 2017; 16(4): 4208–14
7. Yu P, Passam FH, Yu DM, Denyer G, Krilis SA: Beta-2-glycoprotein I inhibits vascular endothelial growth factor and basic fibroblast growth factor induced angiogenesis through its amino terminal domain. *J Thromb Haemost*, 2008; 6(7): 1215–23
8. Schmidt V, Schulz N, Yan X et al: SORLA facilitates insulin receptor signaling in adipocytes and exacerbates obesity. *J Clin Invest*, 2016; 126(7): 2706–20
9. Jung DY, Kim JH, Lee H, Jung MH: Antidiabetic effect of gomisin N via activation of AMP-activated protein kinase. *Biochem Biophys Res Commun*, 2017; 494(3–4): 587–93
10. Li X, Liu R, Zhang L, Jiang Z: The emerging role of AMP-activated protein kinase in cholestatic liver diseases. *Pharmacol Res*, 2017; 125(Pt B): 105–13
11. Viollet B, Guigas B, Leclerc J et al: AMP-activated protein kinase in the regulation of hepatic energy metabolism: From physiology to therapeutic perspectives. *Acta Physiol (Oxf)*, 2009; 196(1): 81–98
12. Zhang R, Zhou SJ, Li CJ et al: C-reactive protein/oxidised low-density lipoprotein/beta2-glycoprotein I complex promotes atherosclerosis in diabetic BALB/c mice via p38mitogen-activated protein kinase signal pathway. *Lipids Health Dis*, 2013; 12: 42
13. Wang T, Chen SS, Chen R et al: Reduced beta 2 glycoprotein I improves diabetic nephropathy via inhibiting TGF-beta1-p38 MAPK pathway. *Int J Clin Exp Pathol*, 2015; 8(3): 2321–33
14. Passam FH, Rahgozar S, Qi M et al: Redox control of beta2-glycoprotein I-von Willebrand factor interaction by thioredoxin-1. *J Thromb Haemost*, 2010; 8(8): 1754–62
15. Ioannou Y, Zhang JY, Passam FH et al: Naturally occurring free thiols within beta 2-glycoprotein I *in vivo*: Nitrosylation, redox modification by endothelial cells, and regulation of oxidative stress-induced cell injury. *Blood*, 2010; 116(11): 1961–70
16. Maksymchuk O, Shysh A, Rosohatska I, Chashchyn M: Quercetin prevents type 1 diabetic liver damage through inhibition of CYP2E1. *Pharmacol Rep*, 2017; 69(6): 1386–92
17. Wang T, Chen S-S, Chen R et al: Reduced beta 2 glycoprotein I improves diabetic nephropathy via inhibiting TGF-beta1-p38 MAPK pathway. *Int J Clin Exp Pathol*, 2015; 8(3): 2321–33

18. Wu S, Cao J, Zhang T et al: Electroacupuncture ameliorates the coronary occlusion related tachycardia and hypotension in acute rat myocardial ischemia model: potential role of hippocampus. *Evid Based Complement Alternat Med*, 2015; 2015: 925987
19. Petri M: Update on anti-phospholipid antibodies in SLE: The Hopkins' Lupus Cohort. *Lupus*, 2010; 19(4): 419–23
20. Agar C, de Groot PG, Marquart JA, Meijers JC: Evolutionary conservation of the lipopolysaccharide binding site of beta(2)-glycoprotein I. *Thromb Haemost*, 2011; 106(6): 1069–75
21. Jankowski M, Vreys I, Wittevrongel C et al: Thrombogenicity of beta 2-glycoprotein I-dependent antiphospholipid antibodies in a photochemically induced thrombosis model in the hamster. *Blood*, 2003; 101(1): 157–62
22. Wang C, Niu DM, Hu J et al: Elevated serum beta2-glycoprotein-I-lipoprotein(a) complexes levels are associated with the presence and complications in type 2 diabetes mellitus. *Diabetes Res Clin Pract*, 2013; 100(2): 250–56
23. Blank M, Baraam L, Eisenstein M et al: beta2-Glycoprotein-I based peptide regulate endothelial-cells tissue-factor expression via negative regulation of pGSK3beta expression and reduces experimental-antiphospholipid-syndrome. *J Autoimmun*, 2011; 37(1): 8–17
24. Rand JH, Wu XX, Quinn AS et al: Hydroxychloroquine directly reduces the binding of antiphospholipid antibody-beta2-glycoprotein I complexes to phospholipid bilayers. *Blood*, 2008; 112(5): 1687–95
25. Palem SP, Abraham P: A study on the level of oxidative stress and inflammatory markers in type 2 diabetes mellitus patients with different treatment modalities. *J Clin Diagn Res*, 2015; 9(9): BC04–7
26. Zhang J, Wang X, Vikash V et al: ROS and ROS-mediated cellular signaling. *Oxid Med Cell Longev*, 2016; 2016: 4350965
27. Ray PD, Huang B-W, Tsuji Y: Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signall*, 2012; 24(5): 981–90
28. Lu YL, Ye TT, Chen Y et al: Rosiglitazone protects diabetic rats from liver destruction. *J Endocrinol Invest*, 2011; 34(10): 775–80
29. Brioukhanov AL, Netrusov AI: Catalase and superoxide dismutase: Distribution, properties, and physiological role in cells of strict anaerobes. *Biochemistry (Moscow)*, 2004; 69(9): 949–62
30. Ho E, Karimi Galougahi K, Liu C-C et al: Biological markers of oxidative stress: Applications to cardiovascular research and practice. *Redox Biol*, 2013; 1(1): 483–91
31. Yi X, Cao S, Chang B et al: Effects of acute exercise and chronic exercise on the liver leptin-AMPK-ACC signaling pathway in rats with type 2 diabetes. *J Diabetes Res*, 2013; 2013: 946432
32. Ke R, Xu Q, Li C et al: Mechanisms of AMPK in the maintenance of ATP balance during energy metabolism. *Cell Biol Int*, 2018; 42(4): 384–92
33. Hughey CC, James FD, Bracy DP et al: Loss of hepatic AMP-activated protein kinase impedes the rate of glycogenolysis but not gluconeogenic fluxes in exercising mice. *J Biol Chem*, 2017; 292(49): 20125–40
34. Viollet B, Andreelli F: AMP-activated protein kinase and metabolic control. *Handb Exp Pharmacol*, 2011; (203): 303–30
35. Mihaylova MM, Shaw RJ: The AMP-activated protein kinase (AMPK) signaling pathway coordinates cell growth, autophagy, & metabolism. *Nat Cell Biol*, 2011; 13(9): 1016–23