doi.org/10.36721/PJPS.2023.36.1.REG.217-221.1

Rutin improves diabetes-induced muscle atrophy in mice

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Abstract: Muscle atrophy is a common complication in diabetes mellitus. Rutin has multiple biologic and therapeutic effects both in diabetic complications and muscle functions. However, no researches have implied prevention and treatment of rutin on muscle atrophy in diabetes mellitus. Our data demonstrated that rutin increased myocyte area and weight of gastrocnemius to promote muscular strength ($p<0.01$). Moreover, rutin attenuated Atrogin-1 and MuRF1 expressions to improve atrophy ($p<0.01$). The mechanism of rutin against diabetes-associated muscle atrophy is closely linked to its regulations on decreasing Bax expression ($p<0.01$) and increasing Pgc-1α, Nrf-1 and Bcl-2 expression ($p<0.01$). In conclusion, rutin protected against diabetic myopathy through its modulation of mitochondria bioactivity and apoptosis. These data suggested rutin could be a therapeutic agent on the improvement of diabetic muscle atrophy.

Keywords: Apoptosis, diabetes, mitochondria, muscle atrophy, rutin

INTRODUCTION

Diabetes mellitus is a metabolic disturbance and affects normal daily activities in current world (Zheng *et al*., 2021). Its prevalence has grown because of poorly controlled hyperglycemia. Long-term hyperglycemia can cause multi-organic complications, such as myopathy, nephropathy, retinopathy and neuropathy (Gan *et al*., 2020). Muscle atrophy is one of major pathological phenomenon of diabetic myopathy (Zickri *et al.,* 2020). Its outstanding characteristic is impairment of muscle mass and myofiber size which is harm for muscle growth and development (He & Ye, 2020). Nowadays, more and more people sufferfrom muscle atrophy as a result of a growing number of diabetic patients. However, the etiology and therapy of diabetes-associated muscle atrophy have not been completely clarified.

In diabetes mellitus, skeletal muscle was involved in modulating glucose homeostasis (Krause *et al.,* 2011). Hence, skeletal muscle inevitably becomes the primary target tissue under lack of insulin conditions. More seriously, hyperglycemia-induced skeletal muscle injury affects insulin reaction to disturb glucose homeostasis (Kua *et al.,* 2019). Clinic pathologically, hyperglycemia is verifiability related to abnormities of size and strength in skeletal muscles. The pathogenesis diabetic-associated muscle injury is multifarious and complex. In general, muscle physical function is tightly associated with muscle mass, which is regulated by protein metabolism (Hirata *et al.,* 2019). Ubiquitin-proteasome system is a key pathway of protein degradation, and Atrogin-1 and MuRF1 as its marker proteins are increased in diabetic muscle atrophy process (Yin *et al.,* 2021).

Rutin is a kind of flavonoid and possesses multiple pharmacological and biologic activities. In physical fatigue, rutin mitigated MDA level in muscle tissues to enhance swimming time (Su *et al*., 2014). In diabetes, rutin has the ability to alleviate plasma glucose and enhance insulin levels via ameliorating glycolytic and gluconeogenic enzymes (Stanley & Kamalakkannan, 2021). In db/db mice, rutin was involved in regulation of PPARγ in skeletal muscles (Cai *et al.,* 2021). However, no research has implied improvement function of rutin on hyperglycemia-induced skeletal muscle atrophy. Previous results showed type 1 diabetes as an autoimmune disease leads to atrophy in skeletal muscles (Sala & Zorzano, 2015). Moreover, STZ destructes pancreatic β cells to induce diabetes in many experiments (Furman, 2015). Hence, the aim of our research was to illuminate muscle protection and its mechanism of rutin in diabetic.

MATERIALS AND METHODS

Animals

C57BL/6 mice (male, 20±2g) were purchased from Hunan SJA Laboratory animal Co., Ltd (Changsha, China) and housed at SPF conditions. Total number of mice is 60. All mice were allowed to drink water freely and eat standard chow diet. Animal experiments were inspected by the Ethics Committee of Hunan University of Arts and Science (No. HUAS-2021-TY-237).

Chemicals and reagents

Rutin (purity: ≥95%) and streptozotocin were obtained from Sangon Biotech (Shanghai, China). BAX and Bcl-2 antibodies were obtained from proteintech (Wuhan, China). Atrogin-1, MuRF1, Pgc-1a and Nrf-1 antibodies were obtained from Sangon Biotech (Shanghai, China).

Experimental design

Mice were divided into three groups: control (CON), diabetes mellitus (DM), and diabetes treated with rutin (DM + RUT). The number of mice is 20 in each group.

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Mice were intraperitoneally injected with STZ. Blood was acquired from tail to detect glucose concentration. Blood glucose level was over 16.7 mmol/L, which was deemed as experiment mice. Rutin was orally fed at 50 mg/kg/day for 8 weeks.

Grip Strength

The grip strength meter was obtained from Anhui Zhenghua Bioinstrumentation (Huaibei, China). Mouse grasped the grip to pull backward plate. The peak grip strength was observed until mouse released plate. Then, the average value was calculated through three repeated tests.

Skeletal muscle tissue harvest

After 8 weeks of treatment, mice were euthanized deeply by pentobarbital and gastrocnemius samples were isolated and collected immediately. A part of skeletal muscle was fixed in 4% paraformaldehyde for histological analysis. Other skeletal muscle samples were stored at -80ºC for protein detection.

Histological analysis

Gastrocnemius histology was analyzed according to Haematoxylin and Eosin staining. Muscle samples were dehydrated by alcohol. Xylene was used as transparent reagent. Rotary microtome was performed to make pathological section. The thickness of slices was 5 um. The histomorphology was captured by light microscope (200X magnification).

Western blot

The proteins of gastrocnemius were transferred by wet electroblot system. Nonspecific binding site was blocked by 5% milk sealant (25ºC, 1h). The membrane was incubated with primary antibodies (4ºC, overnight) and secondary antibodies (25ºC, 2h). Protein signal was captured according to ECL system.

STATISTICAL ANALYSIS

The data were analyzed with SPSS 16.0 software. Statistical difference was demonstrated by one-way ANOVA test. $p<0.05$ was deemed statistically significant.

RESULTS

Properties of rutin on skeletal muscle atrophy

The histopathology of skeletal muscle was measured to evaluate regulation of rutin against diabetic myopathy. In Haematoxylin and Eosin tests, the myocyte area was observably reduced in gastrocnemius of diabetic mice, while rutin dramatically enhanced muscle fiber size (fig. 1A and B). Moreover, the gastrocnemius weight and grip strength was remarkably decreased in DM group, while rutin dramatically augmented these alters (fig. 1 C and D).

Properties of rutin on ubiquitin E3 ligase

To evaluate amelioration of rutin on STZ-evoked protein degradation, Atrogin-1 and MuRF1 were examined. The results showed that Atrogin-1 and MuRF1 expressions were observably elevated in gastrocnemius of diabetic mice. However, rutin remarkably abrogated STZ-evoked specific E3 ubiquitin ligases (fig. 2).

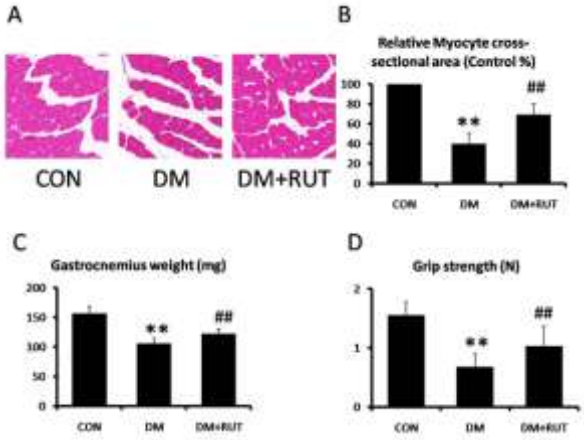

Fig. 1: Properties of rutin on (A, B) gastrocnemius histopathology, (C) gastrocnemius weight and (D) grip strength. ** $p<0.01$ VS CON. ## $p<0.01$ VS DM.

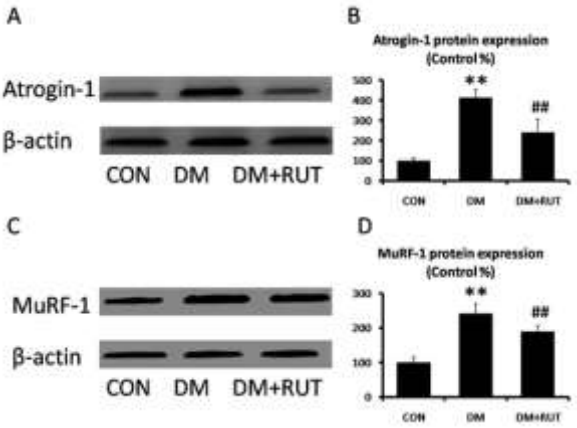

Fig. 2: Properties of rutin on (A, B) Atrogin-1 and (C, D) MuRF-1 expressions. ** $p<0.01$ VS CON. ## $p<0.01$ VS DM.

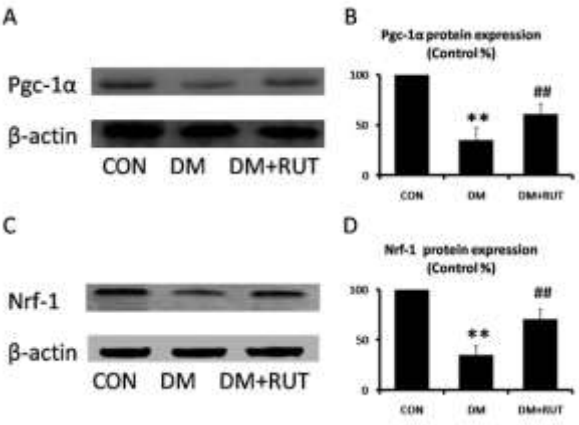

Fig. 3: Properties of rutin on (A, B) Pgc-1α and (C, D) Nrf-1 expressions. **$p<0.01$ VS CON. ##$p<0.01$ VS DM.

Properties of rutin on mitochondrial biogenesis

To evaluate alleviation of rutin on STZ-evoked mitochondria damage, Pgc-1α and Nrf-1 were examined. Pgc-1α and Nrf-1 expressions were observably reduced in gastrocnemius of diabetic mice. However, rutin remarkably facilitated STZ-evoked reduction of Pgc-1α and Nrf-1, suggesting that rutin protected gastrocnemius against diabetes-associated mitochondrial dysfunction (fig. 3).

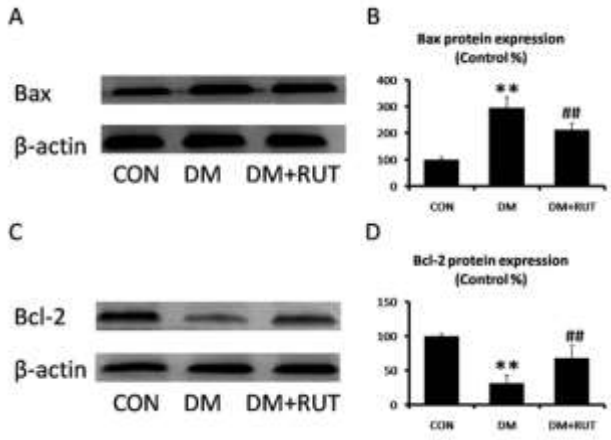

Fig. 4: Properties of rutin on (A, B) Bax and (C, D) Bcl-2 expressions. ** p<0.01 VS CON. ## p<0.01 VS DM.

Properties of rutin on anti-apoptosis effect

To investigate modulation of rutin on diabetic-evoked apoptosis, Bax and Bcl-2 were tested. Bax level was observably elevated in gastrocnemius of diabetic mice, while rutin remarkably abrogated STZ-evoked increase of Bax (fig. 4A and B). In contrast, Bcl-2 level was observably weakened in gastrocnemius of diabetic mice, while rutin remarkably promoted STZ-evoked reduction of Bcl-2 (fig. 4C and D).

DISCUSSION

This study showed rutin increased representative myocyte area and weight of gastrocnemius, and promoted grip strength to improve diabetic myopathy. Further studies have suggested that rutin ameliorate muscle atrophy, as represented by restraining Atrogin-1 and MuRF1 expressions. Rutin was also showed to augment Pgc-1α and Nrf-1 expressions to prevent mitochondrial dysfunction. Moreover, rutin reduced Bax level and elevated Bcl-2 level to mitigate apoptosis. These results showed that rutin attenuated muscle atrophy which was related to mitochondrial function and apoptosis.

Diabetic muscle atrophy is a metabolic disease induced by long-term hyperglycemia, which causes multiple negative influences on skeletal muscle function (O'Neill *et al.*, 2019). The cross-sectional area of gastrocnemius was also declined in diabetes animal model (Huang *et al.*, 2020). In addition, the grip strength of skeletal muscle was reduced in diabetic myopathy (Tseng *et al.*, 2019). Rutin was reported to have various protective effects on both diabetes and myopathy because of its widely biological function and pharmacological action. In diabetes, rutin was proved to regulate carbohydrate metabolism by attenuating plasma glucose and increasing insulin levels to improve glucose disorder (Stanley & Kamalakkannan, 2021). Rutin was also involved in glucose uptake in tertiary butyl hydrogen peroxide-induced myotube injury (Dhanya *et al.*, 2014). In db/db mice, rutin could mitigated glucose and lipids in plasma and increased PPARγ in skeletal muscle (Cai *et al.*, 2012). Moreover, rutin protected against trichlorfon-induced muscle injury by improving bioenergetics homeostasis and fatty acid profile (Baldissera *et al.*, 2020). In this study, rutin ameliorated muscle atrophy by increasing gastrocnemius weight and muscular strength.

Rutin with muscle protection was involved in preventing myopathy to ameliorate muscular function. Previous research showed that rutin regulated glucose uptake via activating synthesis of GLUT-4 in soleus muscle (Bonaldo & Sandri, 2013; Kappel *et al.*, 2013). Rutin also potentiated glucose and calcium uptake associated with regulation of GLUT-4 translocation (Kappel *et al.*, 2013). The pathogenesis of diabetic myopathy was attributed to alterations of multiple ubiquitination system, which trigger myocyte degradation by a disturbance of protein metabolism (Ono *et al.*, 2015). Atrogin-1 and MuRF1belong to ubiquitin E3 ligases, which are increased in atrophy process of muscular tissue. In glucocorticoid-induced atrophy, dexamethasone enhanced Atrogin-1 level in myotubes (Castillero *et al.*, 2013). In immobilization-induced atrophy, MuRF1 level was increased in experimental mice (Kim *et al.*, 2020). In endotoxin-induced atrophy, lipopolysaccharide aggrandized MuRF1 level in skeletal muscle (Martín *et al.*, 2014).DM, especially type 1 diabetes, is confirmed as a precipitating factor of muscle atrophy (Frier *et al.*, 2008). Hyperglycemia is an activity factor of ubiquitin ligases. Our results demonstrated rutin alleviated Atrogin-1 and MuRF1 expressions. It is reasonable to infer that rutin can improve muscle atrophy in diabetic mice.

Mitochondrial biological action is involved in maintaining muscle function (Schrauwen-Hinderling *et al.*, 2016). The dysfunction of mitochondria leads to pathogenesis of muscle atrophy. In addition, high glucose is harm for mitochondrial function and cause abnormal expression of mitochondrial related genes (Kusminski *et al.*, 2020). In diabetes, rutin attenuated mitochondrial ROS production against STZ-induced neuropathy (Mittal *et al.*, 2018). In pancreatic beta cells, rutin restrained glucotoxicity to maintain insulin signaling and mitochondrial function (Cai & Lin., 2009). Previousresearch showed that rutin potentiated muscle mitochondrial biogenesis by accommodating Pgc-1α, Nrf-

1, Tfam and Sirt-1 expressions to improve metabolic disorders in obese rats (Seo *et al.*, 2105). In weight-loaded forced swim test, rutin enhanced expressions of Pgc-1α and Sirt-1 in soleus muscle to increase maximal endurance capacity (Su *et al.*, 2014). Our results demonstrated rutin promoted mitochondrial biological action against diabetic atrophy by elevating Pgc-1α and Nrf-1 expressions.

Chronic hyperglycemia leads to excessive apoptosis in skeletal muscle. Besides, inflammatory stimulation and mitochondrial dysfunction are adverse adjustment factors that cause cell damage. In diabetic myopathy, apoptosis-related proteins, such as Bax, were increased in skeletal muscle of diabetic animal model (Reddy *et al.*, 2019). Moreover, rutin protected muscle from aeromonas hydrophila-induced apoptosis and promoted cell survival by ameliorating ratio of Bax/Bcl-2 (da Rosa *et al*., 2019). In STZ-induced hyperglycemia, rutin mediated blood glucose and apoptosis signaling to improve hepatic function (Parmar *et al.*, 2015). In diabetes cardiomyopathy, rutin reduced Bax and enhanced Bcl-2 to inhibit diabetes-associated apoptosis (Wang *et al.*, 2015). In order to explore anti-apoptosis effect of rutin in muscle atrophy, we examined Bax and Bcl-2 expressions in skeletal muscle. Our results showed hyperglycemia elevated Bax level and decreased Bcl-2 level, while treatment with rutin reversed these changes to restrain apoptosis against diabetic atrophy.

CONCLUSION

Our research indicated that rutin attenuated diabetes-associated muscle atrophy which was related to its improvement of mitochondria function and prevention of apoptosis. These data showed rutin could be used as a potential drug for mitigating muscle atrophy in diabetes.

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