Roles of Ferulic acid on muscle atrophy and grip strength in diabetic mice

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Abstract: Diabetes is an inducement of muscle atrophy to cause motor disturbance. Ferulic Acid (FA) possessed various pharmacological effects in diabetes and muscle function. This research aimed to appraise potential role of FA on muscle atrophy in high glucose environment. Our result displayed FA promoted myofiber size and grip strength to reduce high glucose-mediated muscle function discord. In serum, FA exhibited its anti-oxidative and anti-inflammatory capabilities via enhancing T-AOC, SOD and CAT levels and reducing MDA, TNF-α and IL-6 levels. In skeletal muscle, FA suppressed FBXO-32 and MURF-1 expressions to improve ubiquitin-proteasome system. Moreover, GA restrained DDIT-3 and GRP-78 expressions to relieve high glucose-mediated endoplasmic reticulum stress. Lastly, GA increased BAX expressions and decreased BCL-2 expressions to attenuate high glucose-mediated apoptosis. In conclusion, FA preserve against muscle atrophy by meliorating ubiquitin-proteasome system, endoplasmic reticulum stress and apoptosis in high glucose environment.

Keywords: Diabetes, ferulic acid, muscle atrophy, grip strength.

INTRODUCTION

Diabetes mellitus has become a fastest growing health problem and its prevalence in adult population was over 10.5% in 2021. Diabetes is harm for life expectancy in human (Heald et al. 2020). The expenditure on diabetes-associated health is about 783 million in 2045 (Sun et al. 2022). Diabetes is closely related to many complications, such as cardiomyopathy, nephropathy and myopathy (Bassi-Dibai et al. 2022, Murtaza et al. 2019, Refardt et al. 2020). Skeletal muscle is a locomotive organ and metabolic organ, which can utilize and uptake glucose in our body. The main characteristic of diabetes is hyperglycemia. Hyperglycemia is a cause of skeletal muscle injury and has vital influence on skeletal muscle atrophy (Richter-Stretton et al. 2022). Skeletal muscle atrophy easily results in dyskinesia and metabolic disorders, which is strongly linked with disabling and life threatening diseases. Hence, treatment of diabetic muscle atrophy has become a research focus.

The pathogenesis of muscle atrophy is multifarious. Hyperglycemia causes injury to multiple organs, especially muscle. Clinically, hyperglycemia results in weakness and wasting in skeletal muscle (Perry et al. 2016). Hyperglycemia can also induce fibrosis and affect cells differentiation in skeletal muscle (Khromova et al. 2021). In diabetes, skeletal muscle is a common target organ attacked by hyperglycemia, which cause different types of cell damage. The mechanism of muscle loss associated with diabetes is complex. Endoplasmic reticulum stress is triggered by hyperglycemia to aggravate muscle atrophy (Afroze and Kumar 2019, Mustapha et al., 2021). Ubiquitin-proteasome system is abnormal to induce protein degradation and proteolysis during the development of skeletal muscle atrophy (Singh et al. 2021). In addition, hyperglycemia-induced endoplasmic reticulum stress is linked to cell death.

Ferulic Acid (FA) is a phenolic compound and has protective effects on management of diabetic complication (Li et al. 2022). In pancreatic tissues, FA prevented STZ-mediated β-cells damage to exert a hypoglycemic effect (Roy et al. 2013). In liver, FA was proved to regulate glycogenesis to ameliorate glucose homeostasis (Son et al. 2011). Moreover, FA could improve lipid metabolism characterized by reducing free fatty acid in STZ-mediated diabetic animal (Ohnishi et al. 2004, Sri et al. 2003). In skeletal muscle, FA also possesses several pharmacological effects. In weaned piglets, FA was proved to convert muscle fiber type to enhance the quality of pork (Wang et al. 2021). In zebrafish, FA accelerated muscle growth by promoting protein synthesis capacity (Wen and Ushio 2017). In C2C12, FA was involved in fiber type formation (Chen et al. 2019). However, the defensive function of FA on hyperglycemia-related skeletal muscle atrophy was not clarified.

MATERIALS AND METHODS

Experimental design
C57BL/6 mice were obtained from Hunan SJA Laboratory animal (Changsha, China). The age of mice were about 8 weeks. Mice were kept at SPF conditions. Ferulic Acid was obtained from Sangon Biotech. Diabetic mice were established by streptozotocin (STZ). Then, blood was gained from tail to evaluate hyperglycemia. Diabetic mice were administration of FA (50 mg/kg/day)
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for 8 weeks. Experimental animal included three different groups: control (CON), mice intervened by streptozotocin (STZ) and diabetes treated by Ferulic Acid (STZ + FA).

**Grip strength**
The grip strength meter was purchased from Anhui Zhenghua Bioinstrumentation (Huaibei, China). Mice were allowed to grasped and pull backward plate. Grip strength was obtained by 3 repeated detections.

**Gastrocnemius harvest**
Experimental animal was executed via pentobarbital sodium. Gastrocnemius were weighed. Parts of gastrocnemius was stored at -80°C for western blotting. Other gastrocnemius was placed in 4% paraformaldehyde for histological analysis.

**Biochemical determination**
The blood was centrifuged and utilized to test biological indicators. T-AOC, SOD, CAT and MDA contents were analyzed by spectrophotometer. TNF-α and IL-6 concentrations were measured by ELISA. Biochemical indices were determined with corresponding commercial kits, which were reported previously (Liu et al. 2021, Xia 2010).

**Histological analysis**
Gastrocnemius histopathology was observed by Haematoxylin and Eosin (HE) test. Gastrocnemius was treated with different concentrations of alcohol and xylems. The image was magnified up to 200 times to analyze gastrocnemius histopathology.

**Western blotting**
Gastrocnemius was ground with RIPA for protein extraction. The homogenate was centrifuged to collect supernatant. The protein contents were measured by BCA method. The proteins were separated by SDS-PAGE. The antibodies were reported previously (Liu et al. 2021). Protein signal was visualization by ECL system. The expression was analyzed after standardizing band intensity quantification.

**Ethical approval**
The experiments were approved by the Ethics Committee of the Hunan University of Arts and Science (No. HUAS-2021-TY-231).

**STATISTICAL ANALYSIS**
The results were analyzed by SPSS 16.0. Statistical difference was demonstrated by one-way ANOVA test. p <0.05 was deemed statistically significant.

**RESULTS**

**Roles of FA on skeletal muscle atrophy**
In fig. 1A-B, hyperglycemia markedly decreased muscle fiber size (p<0.01), while FA remarkably augmented myocyte cross-sectional area via Haematoxylin and Eosin test (p<0.01). In fig. 1C, hyperglycemia markedly reduced grip strength, while FA remarkably increased muscle function (p<0.01).

**Roles of FA on oxidation damage**
In fig. 2, hyperglycemia markedly reduced T-AOC, SOD and CAT contents (p<0.01), while MDA content was markedly enhanced in serum of STZ group (p<0.01). However, FA remarkably alleviated oxidative damage by reversing these adverse changes (p<0.01).

**Fig. 1**: Roles of FA on (A, B) muscle fiber size and (C) grip strength. ** p<0.01 vs CON. ## p<0.01 vs STZ.

**Fig. 2**: Roles of FA on (A) T-AOC, (B) SOD, (C) CAT and (D) MDA in serum. ** p<0.01 vs CON. ## p<0.01 vs STZ.

**Fig. 3**: Roles of FA on (A) TNF-a and (B) IL-6 in serum. ** p<0.01 vs CON. ## p<0.01 vs STZ.
Roles of FA on inflammatory response
In fig. 3, hyperglycemia markedly elevated TNF-α and IL-6 concentrations in serum (p<0.01). However, FA remarkably showed its anti-inflammatory function by alleviating diabetic-evoked increase of inflammatory cytokine (p<0.01).

Roles of FA on ubiquitin-proteasome system
In fig. 4, hyperglycemia markedly strengthened FBXO-32 and MURF-1 expressions in skeletal muscle (p<0.01). However, FA remarkably suppressed FBXO-32 and MuRF-1 expressions to improve ubiquitin-proteasome system (p<0.01).

Roles of FA on endoplasmic reticulum stress
In fig. 5, hyperglycemia markedly heightened DDIT-3 and GRP-78 expressions in skeletal muscle (p<0.01). However, GA remarkably restrained DDIT-3 andGRP-78 expressions to relieve high glucose-mediated endoplasmic reticulum stress (p<0.01).

DISCUSSION
FA possesses multiple biological effects both on muscle and diabetes. In zebrafish, FA regulated muscle development as represented by decreasing fat deposition (Yin et al. 2022). In insulin resistance, FA might enhance glycogen synthesis to modulate glucose metabolism of skeletal muscle (Kang and Chiang 2020). Moreover, diabetic muscle atrophy impair fiber size and grip strength in skeletal muscle. We proposed that FA maintained structure and function of muscle in diabetes. In this study, FA relieved pathological damage of skeletal muscle tissues in STZ-mediated diabetic mice, as represented by increasing cross-sectional muscle area and grip strength.

Oxidative stress is a precipitating factor of muscle injury (Aoi and Sakuma 2011). In diabetes, hyperglycemia disturbs oxidative imbalance in internal environment homeostasis (de Carvalho Vidigal et al. 2012, Giacco and Brownlee 2010). In diabetic nephropathy, FA decreased MDA level in renal to attenuate oxidative stress (Qi et al. 2020). In diabetic wound healing, FA exhibited firm antioxidant activity by increasing SOD level (Ghaisas et al. 2014). In addition, oxidative stress was increase in skeletal muscle of diabetic mice. Previous study showed promotion of oxidation resistance can availably mitigate high glucose-induced muscle injury (Al-Shwaheen et al. 2022). In this study, FA increased T-AOC, SOD, CAT and decreased MDA to relieve skeletal muscle injury.

Inflammatory response is related to muscle function (Schiaffino et al. 2013). Hyperglycemia aggravates the
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progression of inflammatory reaction. In diabetic nephropathy, FA restrained sucrose-induced inflammation to improve renal structural (Choi et al. 2011). In diabetic neuropathy, FA mitigated STZ-mediated pro-inflammatory mediators in sciatic nerve tissue (Dhaliwal et al. 2020). In addition, inflammatory response was increased in skeletal muscle of diabetic mice. Previous study showed alleviation of inflammatory response can effectively mitigate high glucose-induced dysfunction in skeletal muscle (Malardé et al. 2015). In this study, FA decreased TNF-α and IL-6 levels to relieve skeletal muscle function in STZ-mediated diabetic mice.

Hyperglycemia intensifies ubiquitin E3 ligase production which results in the alters of protein degradation in skeletal muscle (Rom and Reznick 2016, Svikle et al. 2021). Muscle atrophy is characterized by significantly increased in content of Fbxo-32 and MuRF-1. Fbxo-32 and MuRF-1 are important ubiquitin-proteasome system components. Previous study showed that reduction of Fbxo-32 and MuRF-1 is an effective approach for mitigating muscle atrophy in diabetes (Yin et al. 2021). In this study, FA alleviated high glucose-induced ubiquitin-proteasome system discords by decreasing Fbxo-32 and MuRF-1 expressions to relieve skeletal muscle atrophy, which showed that FA possessed protective effects on muscle structure and function.

Endoplasmic reticulum stress was involved in development of muscle atrophy (Hunter et al. 2021, Kny and Fielitz 2022). In diabetes, endoplasmic reticulum stress was activated to cause organ damage (Bohnert et al. 2018). Many investigations implied that FA suppressed endoplasmic reticulum stress. In diabetic retinopathy, FA might be involved in altering hyperglycemic environment to DDIT-3 expression (Zhang et al. 2018). In diabetic cardiomyopathy, FA exhibited hypoglycemic effects to mitigate GRP78 level (Chowdhury et al. 2016). In addition, amelioration of endoplasmic reticulum stress is beneficial to diabetic muscle atrophy (Reddy et al. 2019). In this study, FA decreased DDIT-3 and GRP78 expressions in STZ-associated diabetic mice. These results indicated that FA was involved in inhibition of high glucose-induced endoplasmic reticulum stress to improve muscle atrophy.

Apoptosis dysfunction is harm for muscle mass (Dirks-Naylor and Lennon-Edwards 2011). Hyperglycemia was proved to perturb apoptosis dysfunction (Fiorentino et al. 2013). In many disease models, FA boosted anti-apoptosis defenses (Li et al. 2021). In gestational diabetes mellitus, FA enhanced BCL-2 expression to protect β cells of female rats (Zhao et al. 2020). In db/db mice, FA relieved hyperglycemia-induced apoptosis mediators in retina tissues (Zhu et al. 2022). In NRK-52E cell, FA restrained high glucose-induced apoptotic death of cells (Chowdhury et al. 2019). Moreover, amelioration of apoptosis-related markers is conducive to improve muscle-cross sectional area in diabetes (Reddy et al. 2019). In this study, FA decreased BAX expression and increased BCL-2 expression in STZ-mediated diabetic mice. These results indicated that FA improved diabetic-associated muscle atrophy via alleviation of apoptosis.

CONCLUSION

Our result displayed FA promoted myofiber size and grip strength to reduce muscle atrophy in diabetic mice. In serum, FA enhanced T-AOC, SOD and CAT levels and reduced MDA, TNF-α and IL-6 levels in high glucose environment. Moreover, FA relieved FBOXO-32, MURF-1, DDIT-3, GRP-78 and BAX expressions and elevated BCL-2 expressions in high glucose-mediated skeletal muscle injury. The protective action of FA on muscle atrophy attributed to its biological effect on improvement of ubiquitin-proteasome system, endoplasmic reticulum stress and apoptosis in high glucose environment.

REFERENCES


