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Effect of Resistance Training on Soleus Muscle Mass and Gene Expression of AMPK and PI3K in Rats with Type 2 Diabetes

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ABSTRACT

Introduction: Clinical studies have pointed to muscle atrophy in diabetic patients. This study was conducted with the aim of determining the effect of resistance training on the expression of some transcription factors effective on muscle hypertrophy in type 2 diabetic rats.

Methods: For this purpose, type 2 diabetes was induced by intraperitoneal injection of nicotinamide-STZ in fourteen male Wistar rats (220 ± 10 g). Then diabetic rats were randomly selected in resistance and control groups. Rats in the resistance group participated in a 10-week resistance training (5 times weekly) in the form of climbing a step ladder with resistance (tying a weight to the tail based on the percentage of body weight), and the control group did not participate in the exercises. 48 hours after the last training session, the expression of PI3K and AMPK genes in the soleus muscle, as well as the weight of the soleus muscle and the ratio of soleus muscle weight to body weight (soleus/body weight ratio) were measured and compared by independent t test between groups.

Results: Compared to control group, resistance training resulted in significant increase in PI3K expression, soleus muscle weight and soleus/body weight ratio ($P < 0.05$).

Conclusion: Resistance training leads to soleus muscle hypertrophy in diabetic rats, and this improvement can probably be attributed to the increased PI3K expression.

Keywords:

Type 2 diabetes, Gene expression. Resistance training, Muscle hypertrophy, PI3K and AMPK genes.

Introduction

Complications caused by type 2 diabetes are not only related to hyperglycemia or increased blood sugar and insulin resistance, but several disorders in the target tissues, such as heart and blood vessels, fat tissue, muscle and liver are also consequences of this disease, and sometimes these disorders themselves affect the severity of the disease (1). In this regard, disturbances in protein levels or the expression of some transcription factors affect the processes of glycemic profile, hypertrophy or muscle atrophy. In this regard, AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme composed of three subunits, γ, β, and α, which is activated by the phosphorylation of threonine 172 in the α subunit (2,3). As a cell energy sensor, AMPK is effective in the activity of catabolic (glucose absorption and glycolysis, lipolysis, autophagy, mitophagy) and anabolic (lipogenesis, glycogenolysis, gluconeogenesis, protein synthesis) mechanisms (4,5).

On the other hand, based on the available evidence, Phosphoinositide 3-kinase (PI3-K)-dependent signaling pathways are involved in metabolic or genetic processes leading to diabetes, and disruption of this pathway leads to obesity and type 2 diabetes. The role of this signaling pathway is focused on skeletal muscle, adipose tissue, heart, liver, brain and pancreas. Thus, genetic disorders of PI3K downstream pathways are associated with insulin resistance in obese or diabetic people and in turn affect the processes of muscle synthesis and hypertrophy (6).

PI3K, also known as phosphatidyl-inositol 3-kinase, phosphorylates inositol lipids that directly activate AKT Serine/Threonine Kinase 1 (AKT-1) and translocates AKT1 to the cell plasma membrane. AKT activation is regulated by insulin and nutritional status as well as exercise

training (7). In fact, it can be said that AKT1 is one of the target genes of PI3K (8) which plays a role in the regulation of exercise-induced hypertrophy (9). Although the main mechanisms responsible for hypertrophy are still not fully understood. However, it has been found that the presence of type 2 diabetes leads to the reduction or inhibition of hypertrophy due to the reduction of PI3K expression (10,11).

Exercise training have been introduced as a protective factor to prevent or treat complications or abnormalities related to diabetes. Some studies have introduced the increase in AKT/mTOR pathway activity in response to exercise as a stimulus for skeletal muscle hypertrophy, which is regulated by the GH/IGF axis signaling pathway through PI3k/AKT and finally AKT/mTORc1 (12). Regarding AMPK, research results are different, for example, some researchers reported an increase in AMPK following aerobic exercise, while Keith et al (2008) reported no change in AMPK following exercise (13). However, the role of exercise training on muscle hypertrophy dependent on the PI3K/AMPK signaling pathway has not been studied. Therefore, in the present study, in addition to determining the effect of resistance training on the expression of PI3K and AMPK in the soleus muscle of diabetic rats, its effect on muscle hypertrophy is also measured in the form of changes in soleus muscle weight and the ratio of soleus muscle weight to body weight.

Materials and Methods

In this experimental-applied study, 14 male Wistar rats (10-week-old, 220 ± 10 grams) were randomly divided into resistance (7 weeks of resistance training, n = 7) and control (n = 7) groups after induction of type 2 diabetes. The studied rats were kept under controlled light conditions (12 hours of light and 12 hours of darkness, lighting starts at 6 in the evening and turns off at 6 in the morning) with temperature (22 ± 3 C) and humidity in the range of 30 to 60. For this purpose, plexiglass cages with a mesh door and dimensions of 25 x 27 x 43 cm were prepared so that the mice could have free access to water and standard food. To ensure proper environmental conditions and maintain proper humidity, temperature and ventilation (to adjust the level of pollution in the place and reduce the bad smell of the environment caused by the accumulation of ammonia from animal urine and reduce the possibility of respiratory diseases in animals) from the air conditioner and from Thermometer and hygrometer were used to monitor daily temperature and humidity changes. Also, the animal cages were washed daily with water and detergent. Pushal (wood chip) was used to keep the cages clean and to collect animal urine and feces. At the end of the research period, the rats were moved and handled by one person.

Induction of type 2 diabetes: Type 2 diabetes was induced by intraperitoneal injection of nicotinamide and STZ. So that after an overnight fast, nicotinamide solution with a dose of 110 mg per kilogram of mouse weight was injected intraperitoneally; After 15 minutes, freshly prepared solution of STZ in citrate buffer with pH=4.5 was also injected intraperitoneally with a dose of 60 mg/kg (14). One week after the induction of diabetes, fasting blood glucose was measured and high blood sugar between 150-400 mg/dL was considered as a measure to ensure that the mice had type 2 diabetes (14).

Resistance training protocol: After the induction of diabetes, the rats were divided into two resistance and control groups. The resistance group participated in a resistance training for 10 weeks, 5 sessions per week in the form of 3 sets with 6 repetitions in each sets (table 1). The rest time between sets is 3 minutes and the rest time between repetitions in each set are 45 seconds (15). At the end, 48 hours after the last training session, the rats were killed and dissected to extract the soleus muscle to measure AMPK and PI3K expression. Applying resistance in the form of tying weights to the rats' tails is equivalent to different percentages of body weight during the training period.

Table 1: Resistance training protocol based on body weight percentage

Exercise session (Week)	1-2	3-4	5-6	7-8	9-10
Resistance (body weight %)	20	40	60	80	100

Tissue extraction: 48 hours after the last training session (10 to 12 hours of fasting), the studied rats in both groups were injected intraperitoneally with a mixture of 10% ketamine at a dose of 50 mg/kg and 2% xylosin at a dose of 10 mg/kg were anesthetized. Next, the soleus muscle was extracted and after washing in physiological serum, it was immersed in 1.8 microtubes containing 20% RNAlater liquid to

perform genetic tests. RNA extraction was performed using the commercial kit RNeasy mini kit of QIAGEN Company. mRNA gene determination was done by RT-Real time PCR by Rotorgen 6000 system using One Step SYBR TAKARA kit from Takara company according to the company's instructions. The thermal cycle protocol used by the device, RNA Polymerasell was used as the control gene. The sequence pattern of the primers are shown in table 2.

Table 2: The sequence pattern of the primers

Genes	Primer sequence	Product size	T m	Gene Bank
PI3K	For: ACTGAGATGGAGACACGGAAC Rev: GCATCCAAGGGTCCAGTTAGTG	159 bp	60	NM_001191052.1
AMPK	For: CCCCTTGAAGCGAGCAACTATC Rev: GCATCATAGGAGGGGTCTTCAG	159 bp	60	NM_001191052.1
RNA PolymraseII	For: ACTTTGATGACGTGGAGGAGGAC Rev: GTTGGCCTGCGGTCGTTC	164 bp	60	XM_008759265.1

Statistical analysis: Shaperovic's test was used to ensure the normal distribution of the data. Comparison of variables between two groups was done using independent t-test. All statistical studies were done using SPSS/Win version 22 software. Changes of less than 5% were considered significant.

Results

Body weight changes in the control and resistance groups are shown in Table 3. At the beginning of the study, the results of the one-way ANOVA test showed that there was no significant difference with regard to body weight between groups (P = 0.864). On the other hand, although at the end of the study, the body weight in both groups increased significantly compared to before the study, but the body weight in the resistance group after the intervention was significantly higher than the control group (P < 0.05).

Table 3: Pre and post-intervention of body weight (g) of studied groups.

Group	Pre-training	Post-tainting	p-value (paired t test)
Control	229 ± 3	278 ± 8	0.001
Resistance	229 ± 4	303 ± 13	0.001
P-value (independent t test)	0.864	0.001	-------

The results of the independent t-test showed no significant difference in AMPK expression in the soleus muscle between groups (P = 0.332). In other words, 10 weeks of resistance training did not lead to a significant change in the expression of AMPK in the soleus muscle compared to the control group (fig 1). However, a significant difference in PI3K expression was observed between the two groups (P < 0.05). In other words, resistance training resulted in significant increase in PI3K expression compared to the control group (fig 2).

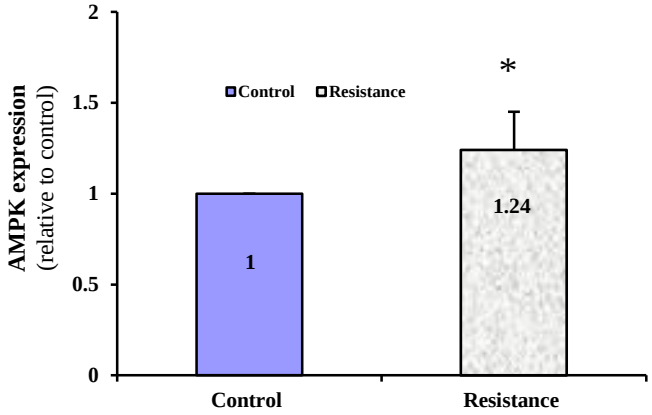

Fig 1: AMPK gene expression in soleus muscle in resistance compared to control group.

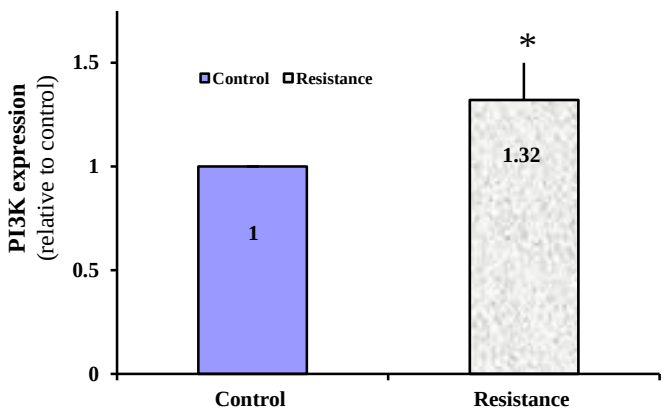

Fig 2: PI3K gene expression in soleus muscle in resistance compared to control group.

On the other hand, a significant difference in the weight of the soleus muscle and the soleus/body weight ratio was observed between groups. In other words, 10 weeks of resistance training led to a significant decrease in soleus muscle ($P < 0.05$, fig 3) and soleus/body weight ratio ($P < 0.05$, fig 4) compared to the control group.

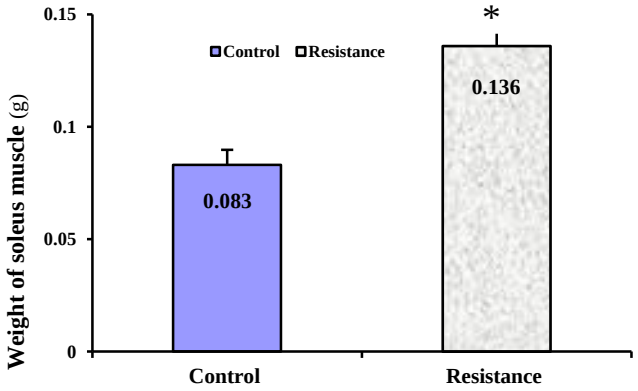

Fig 3: The weight of soleus muscle in resistance compared to control group.

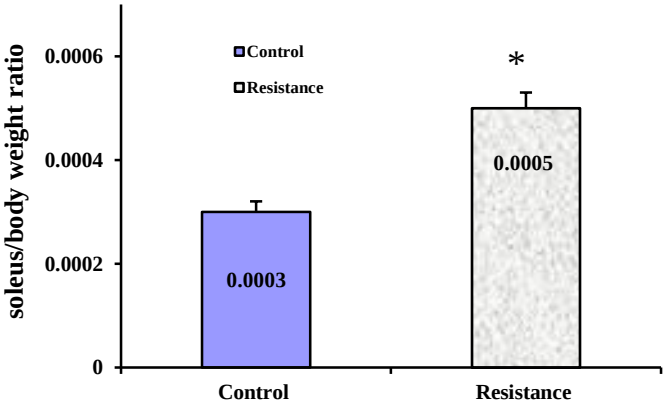

Fig 4: The soleus/body weight ratio in resistance compared to control group.

Discussion

The findings of the present study refer to muscle hypertrophy in response to resistance training in type 2 diabetic rats. So this training method in the form of 10 weeks, 5 sessions per week, led to an increase in the weight of the soleus muscle compared to the groups that did not train. On the other hand, resistance training also resulted in an increase in the weight ratio of the soleus muscle compared to body weight. Based on experimental and cellular molecular evidence, this hypertrophy can be attributed to hormonal, metabolic and genetic changes related to exercise. In this regard, aside from the effective role of hormonal changes in response to resistance training that has been mentioned in other studies, the change in protein levels or the expression of some transcription factors effective in protein synthesis should not be ignored. Thus, in the present study, although the increase in the expression of AMPK in the soleus muscle of rats in the resistance group was not statistically significant compared to the control group, this training method led to a significant increase in the expression of PI3K compared to the control group.

The lack of change in AMPK expression is reported while Cao et al, (2012) have pointed out the increase in phosphorylation and AMPK signaling and downstream components to PI3K in response to both acute and chronic resistance training (16). Takekoshi et al, (2006) also cited their findings and indicated that long-term aerobic training leads to an increase in AMPK activity in laboratory rats (17). Despite the aforementioned evidence, the increase in AMPK expression in the soleus muscle of trained diabetic rats in the present study was insignificant compared to the control group, which may be attributed to the small number of studied samples or the dispersion of changes. Some researchers have also concluded based on their findings that obese diabetic rats need to perform exercises at higher levels of exercise intensity to stimulate AMPK activity (18). de Souza et al, (2013) who in their study evaluated the effect of aerobic, resistance and combined resistance training on the expression of transcription factors AKT/MTOR and AMPK in the skeletal muscles of laboratory rats concluded that only combined training (resistance + aerobic) lead to significant changes in the adaptation of muscle hypertrophy signaling pathways (19).

Despite the aforementioned evidence, it is still not well known that AMPK affects PI3K-dependent signaling pathways by affecting the activity of which PI3K isoform (20). In the present study, despite the fact that the increase in AMPK to resistance training was not statistically significant, this increase of 24% compared to the control group is significant from a clinical point of view. On the other hand, the expression of PI3K in the soleus muscle increased significantly in response to resistance training. Meanwhile, clinical studies have pointed to a decrease in the activity of insulin and PI3K signaling pathways in the skeletal muscles of insulin-resistant

individuals and type 2 diabetes patients, while the improvement of insulin-induced glucose absorption after exercise is rooted in the increase of insulin receptor substrates (IRS-1, IRS-2) and PI3K in skeletal muscles (21). In this context, it has been stated that exercise training at higher intensities effectively increases the expression of AMPK in the skeletal muscles of type 2 diabetic rats (18). On the other hand, some studies have pointed out that the effect of resistance training on insulin sensitivity usually lasts longer than aerobic training, and researchers have attributed this stability to some effects caused by increasing muscle mass (22).

Regarding the effect of exercise training on PI3K expression, despite the fact that some studies have not reported any effect of acute exercise training on insulin-dependent signal changes such as changes in 1IRS phosphorylation or PI3K activity (23,24), some studies have indicated that continuous endurance training Acutely, during 45 to 60 minutes with 65 to 75% of the maximum oxygen consumption, it leads to an increase in the expression of PI3K in the muscles of healthy people who have exercised as well as people with insulin resistance (25,26). Zhichao et al, (2013) also reported a 36% increase in PI3K protein levels following 8 weeks of endurance swimming training 5 sessions per week compared to the control group, which was associated with an increase in the activity of the PI3K/AKT1 signaling pathway. swimming exercise in order to activate the signaling pathway of proteins that cause hypertrophy of myocytes also has a reducing and inhibitory effect on known proteins that cause pathological hypertrophy (such as PTEN) (27). On the other hand, some researchers have reported an increase in the activity of cardiac PI3K/P110α) in rats in response to exercise training (28).

Conclusion

Long-term resistance exercises are associated with hypertrophy of soleus muscle in type 2 diabetic rats. This improvement is probably rooted in the increase in PI3K expression in skeletal muscles in response to this training method. Despite these changes, knowing the main mechanisms responsible for muscle hypertrophy in diabetic patients requires more studies.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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