Research Article

The long-term effect of moderate-intensity exercise on the expression of the genes irisin and sirtuin-1 in the skeletal muscle of diabetic rats with streptozotocin

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Abstract

Disorders of glucose metabolism in various tissues, including skeletal muscle tissue and adipose tissue are features of diabetes. The aim of the present study was to evaluate the long-term effect of moderate-intensity continuous exercise on the expression of irisin and sirtuin-1 genes in the skeletal muscle of streptozotocin-nicotinamide-diabetic rats. Thirty-six 8-week-old males were randomly divided into three groups: healthy control (n = 12), diabetic (n = 12), and moderate-persistent diabetes (n = 12). Diabetic groups developed diabetes by intraperitoneal injection of nicotinamide and STZ solution at a doses of 95 and 55 mg/kg. The diabetic-moderate-intensity continuous exercise group performed their training protocol by running on a treadmill for 12 weeks, 5 sessions per week. Forty-eight hours after the last training session, the subjects were anesthetized and their horseshoe muscle tissue was removed and the expression of the genes Irisin and Sirtuin-1 was measured. 12 weeks of moderate-intensity continuous exercise in diabetic mice resulted in a significant increase in the expression of the genes Irisin and Sirtuin-1 ($p < 0.05$). Performing 12 weeks of continuous exercise with moderate intensity in diabetic rats increased the expression of Irisin and sirtuin-1. Thus, changes in the expression of irisin and serotonin-1 may improve the symptoms of metabolic syndrome and can be a compensatory mechanism for reducing oxidative stress in diabetics.

Key Words: **Continuous training, Diabetes, Irisin, Sirtuin-1**

Introduction

One of the main metabolic diseases in the world is type 2 diabetes, which is a state with health problem and is highly associated with overweight and obesity (Rotondi et al., 2011). According to the World Health Organization, about 422 million people were affected with diabetes in 2014 and it increases from 4.7 percent in 1980 to 8.5 percent in 2014 among adults, who live in middle-income society (Jayakrishnapillai et al., 2017). The growing trend of obesity and the promotion of related diseases such as cardiovascular disease, hypertension and type 2 diabetes in today's society is a problem that needs special attention (Ogden et al., 2014). Adipose tissue plays an important role in fatty acid metabolism and glucose homeostasis and also secretes a large number of hormones (Prenzler et al., 2007). In addition to metabolic activity, skeletal muscle is known as an endocrine tissue that has biological activity through the secretion of hormones called myokines. Myokines are involved in various processes such as tissue repair, cell signaling, and cell differentiation and are usually secreted in response to physiological factors and in conditions such as exercise and nutritional changes (Agh et al., 2017). In recent years, adipose tissue and skeletal muscle have been shown to be closely associated with metabolic diseases, including insulin resistance and diabetes. Diabetes mellitus is characterized by elevated blood sugar levels due to impaired insulin secretion or the effect of insulin due to insulin resistance, and can have devastating effects on the heart, kidneys, and peripheral and central nervous system (Alipour et al., 2012).

Adipose tissue is found in the body in the form of white and brown adipose tissues. The most important feature of white adipose tissue is energy storage, and brown adipose tissue is involved in the expression of the Ucp1 gene, and in exchange for a certain production of ATP, it consumes more fatty acids that produce heat. In other words, the role of white adipose tissue is to separate fatty acids when receiving high energy, and brown adipose tissue is responsible for creating non-vibrational exotherm to help maintain energy balance and is strongly affected by the sympathetic nerves to raise body temperature

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(Tsiloulis & Watt, 2015). The conversion of white adipose tissue to brown adipose tissue is considered as a therapeutic target for obesity and metabolic disorders. Studies have shown that the conversion of white adipose tissue to brown and the increase in calorific value resulting in weight loss is mediated by myokine secreted by skeletal muscle called irisin, which was identified by Bostrom et al in n 2012. They showed that after exercise, the expression of PGC1-α (a key regulator of gluconeogenesis and fatty acid metabolism) increased, followed by an increase in muscle FNDC5 expression and the breakdown of the FNDC5 protein and secreted a hormone or myokine irisin. Through the bloodstream, irisin increases the expression of the UCP1 gene in white adipose tissue, which converts white adipose tissue to brown adipose tissue. Therefore, it has been suggested that browning of adipose tissue reduces overweight and obesity (Wu et al., 2012). Research in this area considers irisin to be a potential bridge for skeletal muscle to interact with other tissues involved in metabolism, including fat. According to studies, the level of irisin has a significant negative relationship with insulin resistance and obesity (Huh et al., 2014). In another study, irisin levels were significantly reduced in patients with type 2 diabetes, suggesting that irisin may play an important role in glucose intolerance and type 2 diabetes (Choi et al., 2013).

Another protein that plays an important role in controlling obesity and metabolic disorders is sirtuin-1, which counteracts oxidative stress. Sirtuin-1 distills and inhibits the PGC-1α inhibitor, thereby maintaining PGC-1α activity (Nie et al., 2009). PGC-1β is also stimulated by the distillation of sirtuin-1 and the expression of the GLUT4 glucose transporter in skeletal muscle. It should be noted that sirtuin-1 affects insulin signaling, which it does by reducing PTP1B (a dephosphorylation and inactivating insulin receptor) and increasing insulin sensitivity. Sirtuin-1 is also involved in stimulating insulin secretion. Prediction of these proteins in pancreatic islet beta cells increases ATP production by inhibiting mitochondrial UCP2 expression, leading to the closure of ATP-sensitive K-channels and ultimately insulin secretion (Sun et al., 2007). Suppression of sirtuin-1 causes systemic inflammation, increased oxidative stress, and decreased aerobic fuel, which is involved in the production and control of ROS through the FOXO pathway (Menzies et al., 2013).

Exercise affects the levels of irisn and sirtuin-1, which are involved in diabetes and fat metabolism. Khodadadi et al. (2014) observed a significant increase in irisn after one session of high-intensity interval training in mice (Khodadadi et al., 2014). In another study, after 6 weeks of endurance training with an intensity of 75% of maximal oxygen consumption, no significant changes were observed in irisn levels (Timmons et al., 2012). Also, in a study with 26 weeks of aerobic exercise with 60% of maximal heart rate in young people, no significant change was observed in the amount of irisn (Hoffman, 2014). Regarding the effect of exercise on the expression of sirtuin-1 gene, it was shown that 8 weeks of moderate intensity endurance training (5 days a week and one hour a day) in diabetic rats increased the stimulation of sirtuin-1 expression and thus decreased Inflammation and metabolic disorders. In another study, 8 weeks of water training (3 sessions per week with a training intensity of 80-60% of maximum heart rate) in obese men significantly increased PGC-1α and sirtuin-1 levels (Soltani et al., 2018). Previous studies have shown conflicting results regarding the effect of exercise on irisn. Also, according to our study, there were few studies on the effect of chronic exercise on sirtuin-1 in diabetics and obese people, and most studies have examined the acute effect of exercise on changes in this type of protein or other types of sirtuins. In general, according to the positive results, the effect of exercise on irisn and sirtuin-1 may be a new therapeutic target for the prevention, control and treatment of type 2 diabetes. Therefore, despite the importance of the physiological role of sirtuin-1 and irisn in diabetics, the response of these proteins, especially sirtuin-1, to exercise in these patients is not entirely clear. Due to this, in the present study, the long-term effect of moderate-intensity continuous training was investigated on the expression of irisin and sirtuin-1 genes in the skeletal muscle of diabetic rats.

Materials and Methods

Animals

In the present study, thirty-six 8-week-old male Wistar rats (with a mean weight of 250 ± 20 g) were purchased from Baqiyatallah University Research Institute. After being transferred to the laboratory, the animals were randomly divided into 3 groups: healthy control (n = 12), diabetic (n = 12) and diabetic-exercise (n = 12). Rats were kept in rearing cages (3 rats in each cage) with ambient conditions of 20-23 ° C, relative humidity of 50% and dark light cycle for 12 hours. The animals were fed with food made by Behparvar Company in the form of free access pellets as well as drinking water.

Exercise training protocol (HIIT)

After transferring the animals to the laboratory, 2 weeks were considered to get acquainted with the environment and how to work on the treadmill (at a speed of 8 meters per minute, 3 days a week). The training program of the diabetic-exercise group included continuous exercises with moderate intensity for 12 weeks and 5 sessions per week according to Table 1 (Eizadi et al., 2017).

Induction of diabetes

Nicotine amide and streptozotocin (STZ) were injected to induce

Table 1. Exercise training protochol

Week	Time (min)	Speed(m/min)
1	10	18
2,3	20	20
4,5	30	22
6,7	40	22
8,9	50	24
10-12	50	26

type 2 diabetes. At first, nicotinamide solution at a dose of 95 mg / kg of rat weight was injected intraperitoneally and after 15 minutes, STZ solution was prepared in citrate 1 buffer. 0.5 M (pH = 4.5) was injected intraperitoneally at a dose of 55 mg / kg. One week after the injection, rat blood intravenous blood glucose was measured and the criterion for diabetes was blood sugar above 200 mg / dl (Eizadi et al., 2017).

Biochemical assay

Forty-eight hours after the last training session of the rats in all groups using ketamine-xylazine solution, by injecting 3 units of ketamine solution (30-50 mg / kg) and xylazine (3-5 mg / kg), anesthetized and their soleus muscle tissue were collected. Irisin mRNA and Sirtuin-1 mRNA in soleus muscle tissue were evaluated and analyzed using RNA purification method. For this purpose, the soleus muscle was first homogenized in buffer solution and centrifuged at 153 g for 15 minutes. The resulting solution was used to measure the levels of Irisin mRNA and Sirtuin-1 mRNA. To analyze the qPCR results data, $2^{\Delta\Delta CT}$ method was used to evaluate the relative quantitative expression of P53 gene as follows. All the analyses were performed separately for the three sample groups. The primers used in the present study can be seen in Table 2.

Relative fold change in gene expression = $2^{-\Delta\Delta CT}$

ΔCT = CT target gene – CT reference gene

$\Delta\Delta CT$ = ΔCT test sample – ΔCT Control

Table 2. Real-time PCR Primer Pequences

	Gene name	Primer sequence
1	Irisin	Forward: CAGCTAGCCACAGGTTCTCC
		Reverse: CTCTCTCCCAGGGCTTTGTG
2	Sirtuin-1	Forvard: AAGGCCACGGATAGGTCCATA
		Reverse: CCAACTCAGGTGGAGGAATTGT
5	GAPDH	Forward: ATCACTGCCACTCAGAAGAC
		Reverse: ACATTGGGGGTAGGAACAC

Statistical analysis

SPSS software version 21 was used to analyze the data. Kolmogorov-Smirnov test was used to show the normal distribution of data and after determining the normal distribution of data, one-way analysis of variance and Tukey post hoc test were used to investigate the differences between each of the indicators. In these analyses, the value of $P \leq 0.05$ was considered as rejecting the null hypothesis.

Results

As can be seen in Figure 1-a, the results of one-way analysis of variance (F = 52.96) indicate a significant difference in the expression of irisin between the groups (p <0.001). According to Tukey post hoc test, irisin expression was significantly different in the diabetic and healthy control groups (p = 0.005), so that irisin expression decreased to 53.8% compared to healthy control group in diabetic group. Also, irisin expression was significantly different in the diabetic and exercise-diabetic groups (p <0.001), which showed an increase of 387.9% in the diabetic and exercise groups compared to the diabetic group.

One-way analysis of variance (F = 25.245) showed that there was a significant difference between the expression of sirtuin-1 in different groups (p <0.001). Significant difference between the two groups of diabetics and healthy expression was not observed in sirtuin-1 (p = 0.371). The expression of sirtuin-1 in the diabetic and exercise groups was significantly higher than that in the diabetic group (p <0.001), which increased by 349.3%. (Figure 1-b).

Discussion

According to the present study, we observed that the expression of irisin in the diabetic and exercise groups had a significant increase of 387.9% compared to the diabetic group. Therefore, moderate-intensity continuous exercise had an effect on the expression of the irisin gene in the skeletal muscle of streptozotocin-treated nicotinamide diabetic rats. Accordingly, moderate-intensity continuous exercise had an effect on the expression of the sirtuin-1 gene in the skeletal muscle of diabetic rats.

Obesity is caused by an increase in body fat stores and is associated with a variety of diseases, including cardiovascular, metabolic, and type 2 diabetes. The aim of this study was to evaluate the effect of 12 weeks of moderate-intensity continuous training on the expression of irisin and sirtuin-1 genes in the skeletal muscle of diabetic rats. The first finding of this study shows that the exercise + diabetes group has significant differences in changes in irisin gene expression from the control and diabetes groups. Consistent with the results of the present

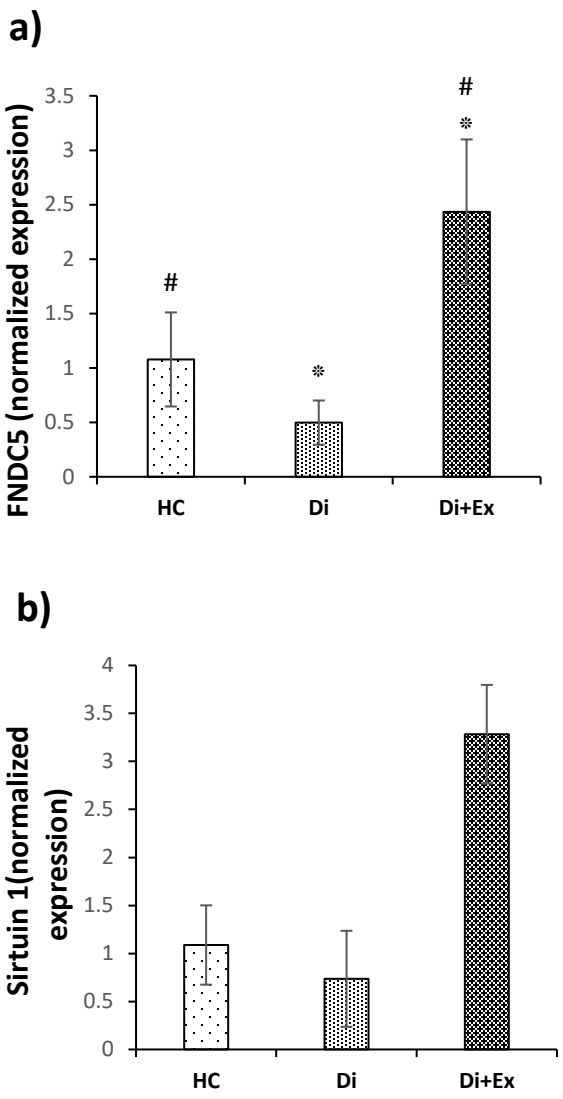

Figure 1. a: The expression values of irisin gene (FNDC5) of soleus muscle tissue based on mean and standard deviation. b: Values of sirtuin-1 gene expression in soleus muscle based on mean and standard deviation. * = Significant difference compared to the HC group and # = Significant difference compared to the Di group. significant value is considered at the level (P≤0.05). HC: Healthy Control, Di: Diabetes, Ex: Exercise.

study, Bostrom et al. (2012) reported a 65-fold increase in irisin levels in mice after 3 weeks of endurance training on a treadmill (Boström et al., 2012). Another study shows the effects of 10 weeks of high-intensity interval training (10 weeks, 3 sessions per week with an intensity of 85-85% of maximum heart rate) with and without green tea consumption on irisin levels and insulin resistance in women with diabetes. Significant increase and decrease of irisin and insulin resistance occurred in both groups, respectively (Taghian, 2018). Contrary to the results of our study, studies showed that 6 weeks of aerobic exercise (5 sessions per week) on diabetic male mice (blood glucose above 300 mg / dl) increased serum levels of irisin which were not significant. Also, in another study, the effect of 6 weeks of endurance training on a ergometer with an intensity of 75% of maximum oxygen consumption did not show significant changes in irisin levels (Timmons et al., 2012). Huo et al. (2014) did not report a significant change in circulating irisin after a period of whole-body vibration training in healthy women. In this regard, they stated that due to the secretion of irisin to other organs of the body, there may be no significant changes in circulating irisin (Huh et al., 2014). Moreover, in studies performed with 26 weeks of aerobic exercise with 60% of maxillary heart rate by young people, no significant change was observed in the amount of irisin, which was reported to be a long time effect for freezing of serum samples as a factor in the degradation of irisin (Hoffman, 2014). In another study by Pakala et al. (2013), 21 weeks of endurance training did not significantly alter irisin levels (Pekkala et al., 2013). These inconsistent results may be due to differences in training protocols, intensity or duration of training, or the type of subjects participating in terms of gender and body fat percentage, and in particular whether the subject is healthy or ill or, as stated, the duration. In relation to the mechanisms involved, according to the findings of Bostrom et al. (2012), one of the possible mechanisms for increasing irisin levels could be increased PGC1-α expression after exercise, followed by increased muscle FNDC5 expression (Boström et al., 2012). Also, in the study of Eaton et al. (2018), considering the effect of intense and intermittent high-intensity interval exercise on skeletal muscle FNDC5 changes, it was shown that 3 hours after recovery, FNDC5 gene expression and PGC1-α expression increased. Also, a transcriptional activating molecule and stimulator of FNDC5 membrane protein expression increased in muscle cells (Eaton et al., 2018). After increased expression, FNDC5 is broken down and a hormone or myokine called irisin enters the bloodstream and binds to PPAR-α receptors on the surface of white adipose tissue, causing tissue conversion by increasing the expression of these receptors and white fat turns brown into fat tissue. On the other hand, the irisin molecule can increase the mitochondrial content of white adipose tissue and increases its conversion to brown adipose tissue by increasing the expression of UCP-1 molecule on the surface of white adipose tissue. This action of irisin is associated with energy consumption in the body and may be considered in the treatment of metabolic diseases (Zong et al., 2002). In connection with the mechanisms mentioned, a study investigated the effect of 8 weeks of endurance training and high-fat diet on the expression of PGC1-α and UCP-1 genes in subcutaneous white adipose tissue and brown adipose tissue in obese and normal weight male rats. The overall results of this study showed that in obese mice the levels of PGC1-α and UCP-1 in subcutaneous white and brown adipose tissue decreased, which indicates metabolic disorders due to ob-

-esity, while endurance training led to the expression of PGC1- α and UCP-1 were subcutaneous in adipose tissue (Shirkhani et al., 2019). It has also been reported that sympathetic stimulation increases after endurance training. This increase causes the release of more catecholamines, which ultimately activate the adrenergic pathways in the cells and create cascades of metabolic changes. Therefore, sympathetic stimulation and secretion of catecholamines is considered as an important part in the conversion of white adipose tissue to brown adipose tissue (Wu et al., 2014). Therefore, in the present study, pathways from previous evidence may play a role in increasing the expression of irisin in the exercise group.

Another important finding of our study was the significant difference in changes in the expression of sirtuin-1 gene in the exercise-diabetes group compared with the diabetes and control groups. Previous studies have shown that sirtuin-1 plays an important role in controlling glucose homeostasis. This means that under diabetic conditions, the activity and expression of sirtuin-1 protein decrease in different tissues (Choi & Mostoslavsky, 2014). In particular, despite the important metabolic role of sirtuin-1 in the prevention and treatment of diabetes, the response of sirtuin-1 to exercise has been studied in very few diabetics. Consistent with our findings in one study, 8 weeks of moderate-intensity endurance training (5 days per week) in diabetic rats showed increased stimulation of sirtuin-1 expression and thus reduced inflammation and metabolic disorders. In another study, two months of aerobic exercise program (3 sessions per week and 50 to 60 minutes per session) in diabetics significantly increased the level of sirtuin-1 in the intervention group compared to the control group (Saremi et al., 2016). Little et al. (2010) also reported that high-intensity interval training for two weeks in young men increased the expression of PGC1-α nuclei in muscle cells and increased sirtuin-1 levels (Little et al., 2010). The results of our study are also consistent with the studies that have reported that calorie restriction is a strong stimulant to increase the expression of sirtuin-1. Therefore, exercise can act as a calorie restriction and increase the likelihood of sirtuin-1 expression. In fact, this evidence suggests that at least some of the positive effects of exercise on diabetic metabolic problems may be due to this protein associated with mitochondrial biogenesis and glucose regulation (Satoh et al., 2011). Related to this, it has been shown that the improvement of aerobic capacity after a period of aerobic exercise has been associated with increased sirtuin-1 activity, reduced inflammation and oxidative stress. Caso et al. (2015) also showed that after 6 weeks of exercise, levels of sirtuin-1 and mitochondrial biogenesis increased in healthy mice (Casuso et al., 2015). In a heterogeneous study, Guard et al. (2010) did not observe a change in sirtuin-1 levels after 6 weeks of intermittent exercise with a peak intensity of 90% oxygen consumption (Gurd et al., 2010). It has been reported that the reasons for discrepancies in the results of the studies include the type of training, the intensity of training, the type of subjects and the level of readiness of individuals. The results of previous evidence showed that after aerobic exercise, phosphate-dependent pathways, calcium, nicotine-amidophosphoribosol transferase (NAMPT) activity (and AMP-dependent protein kinase (AMPK)) increased NF-KB and ultimately stimulated sirtuin. Sirtuin-1 induces lipid oxidation and mitochondrial biogenesis through alpha-receptor alpha and PGC-1α receptor depletion (Oliveira et al., 2014). Calmodulin kinase, calcineurin and stimulation of sirtuin-1 and PGC-1α gene expression and activation of PPAR in various tissues lead to lipid oxidation and available mitochondrial fatty acids and increase capillary network and mitochondrial density which finally increases maximal oxygen consumption and decreases fat weight. It has also been reported that sirtuin-1 is associated with ROS and inflammation, so that with increasing inflammation and ROS, the amount of sirtuin-1 decreases and as a result, its activity is impaired. In fact, sirtuin-1 increases FOXO activity and thus may affect ROS (Menzies et al., 2013). The present study was also associated with a reduction in ROS and inflammation, as previous evidence has shown that exercise increases the body's antioxidant capacity, which in turn reduces inflammation and ROS (Menzies et al., 2013). Despite the contradictory results with the present study, it is believed that regular exercise leads to an increase in sirtuin-1 may have some of the positive effects of physical exercise on the body's metabolic conditions through this factors.

Conclusion

The results of the present study showed that twelve weeks of continuous exercise with moderate intensity has a significant positive effect on increasing the expression of irisin and sirtuin-1 genes in diabetic mice. Therefore, it can be stated that twelve weeks of moderate-intensity aerobic exercise may improve the symptoms of metabolic syndrome, and changes in the expression of irisin and sirtuin-1 may also be a compensatory mechanism for reducing oxidative stress in diabetics.

What is already known on this subject?

It has been confirmed that irisin increases in circulation through regular exercise training and is involved in the conversion of white to brown adipose tissue.

What this study adds?

Twelve weeks of moderate-intensity aerobic exercise may improve the symptoms of metabolic syndrome, and changes in the expression of irisin and sirtuin-1 may also be a compensatory mechanism for reducing oxidative stress in diabetics.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All experimental protocols were approved by the Ethics Committee of the Baqiyatallah University of Medical Science.

Informed consent All authors consent to this manuscript submission.

Author contributions

Conceptualization: M.R., A.M.; Methodology: M.R., A.M.; Software: M.R., A.M.; Validation: A.M.; Formal analysis: M.R.; Investigation: M.R., A.M.; Resources: M.R.; Data curation: A.M.; Writing - original draft: M.R., A.M.; Writing - review & editing: M.R.; Visualization: A.M.; Supervision: M.R.; Project administration: A.M.; Funding acquisition: M.R.

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