Original Article

The effect of six-week high-intensity interval training on muscle expression of FTO and PPAR-γ in obese diabetic rats

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Abstract

Background and purpose: Recent research suggests that obese people are up to 80 times more likely to develop type 2 diabetes than those with a normal BMI. Besides, obesity, inadequate physical activity, and unhealthy diets are the main causes of this metabolic disease. The purpose of this study was to investigate the effect of six weeks of high-intensity interval training on muscle expression of Fat mass and obesity-associated protein (FTO) and Peroxisome proliferator activator receptor gamma (PPAR-γ) in obese diabetic rats.

Material and methods: This experimental study was carried out on 12 male Wistar rats (220±20 g bodyweight and 10 weeks old). Animals received a high-fat diet within six weeks, and then in order to induce type 2 diabetes, an intraperitoneal injection of a single dose of 30 mg/kg freshly prepared streptozotocin (STZ) (Sigma, USA) solved in citrate buffer (pH 4.5) was performed. Diabetic rats were divided into two (High-Intensity Interval Training and control) groups randomly. HIIT program included five sessions of 30 minutes per week. 48 hours after the last training session, the outcomes were measured. The muscle expression of FTO and PPAR-γ was measured using the real-time PCR method. Independent samples t-test and Analysis of covariance (ANCOVA) were applied to compare the means.

Results: The expression of FTO ($P<0.01$), fasting blood sugar ($P<0.001$), weight ($P<0.001$) and HOMA-IR ($P<0.004$) significantly decreased after six weeks of high-intensity interval training, whilst PPAR-γ expression ($P<0.007$) significantly increased.

Conclusion: Regarding the results of this study, it can be stated that a six-week HIIT program can improve glucose metabolism and insulin sensitivity. It can also increase the expression of diabetes- and obesity-associated genes (*e.g.*, PPAR-γ and FTO), and thereby plays a prominent role in the control and treatment of type 2 diabetes in obese patients.

Keywords: Aerobic; Exercise; Insulin; Type 2 diabetes; Glucose

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1. *Introduction*

Overweight and obesity are defined as abnormal or excessive accumulation of fat, which are accompanied with decreased health and quality of life. Approximately, more than 4 million people die each year worldwide as a result of overweight or obesity. In 2016, more than 1.9 billion adults (*i.e.*, ≥18 years of age) were overweight and more than 650 million adults were obese (1). Extensive studies have shown that obesity increases the risk of chronic diseases, such as cancer, cardiovascular disease, stroke (the leading cause of death worldwide), diabetes and its complications, and musculoskeletal disorders (*e.g.* osteoarthritis) (2–5). Type 2 diabetes (T2D) is a metabolic disease caused by a gradual decrease in insulin secretion or the inability of the body to respond to insulin. Obesity, inadequate physical activity, and unhealthy diets are the main causes of this disease (6). According to WHO, the prevalence of people with T2D increased from 108 million in 1980 to 422 million in 2014 and 463 million in 2019. T2D was also responsible for 1.5 million direct deaths in 2019 (7). T2D is associated with various complications and disorders, such as vision problems (retinopathy), atherosclerosis, neurological diseases, kidney diseases (nephropathy), heart diseases, stroke, and finally death (5,8,9).

The fat mass and obesity-associated (FTO) gene is associated with the development of obesity and related diseases. It also plays an important role in controlling energy balance in adipose tissue, hypothalamus, and pituitary gland (10). Increased expression of the FTO gene in adipose tissue leads to diabetes in obese people. Reports also reveal high expression of the FTO protein in people with diabetes. On the other hand, decreased expression of FTO is associated with decreased blood glucose levels and insulin resistance (11,12). Unfortunately, there is insufficient evidence on FTO expression and its mechanisms, especially in human muscle. However, the results of a study indicated that the age-dependent decrease in FTO expression leads to peripheral defects of glucose and fat metabolism in adipose tissue and skeletal muscle (13). In another study, it was found that the increased expression of FTO in the muscle of T2D patients may alter oxidative metabolism and increase oxidative stress, as the main characteristic of muscles in these patients (14).

Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a major regulator of fatty acids and glucose metabolism, adipocyte differentiation, and inflammatory processes (15). Evidence suggests that PPAR-γ agonists directly activate glucose-sensing genes in the liver and pancreatic β-cells, contributing to improvements in glucose homeostasis and insulin sensitivity in people with T2D. In addition, PPAR-γ expression plays a prominent role in the incidence of diseases, such as obesity, diabetes, cancer, etc. (16,17).

PPAR-γ is significantly expressed in endothelial and vascular smooth muscle cells, where it regulates vascular pressure and blood pressure. Moreover, activation of PPAR-γ by rosiglitazone increases the absorption of glucose by muscle cells resulting in decreased plasma glucose levels. This is due to the higher expression and translocation of glucose transporter 1 (GLUT1) and glucose transporter 4 (GLUT4) (18).

Studies reveal the effectiveness of physical activity and regular exercise in controlling weight, balancing blood glucose level, and reducing cardiovascular risk factors (3,4,19–27). High-intensity interval training (HIIT) improves various health parameters by increasing skeletal muscle oxidative capacity, insulin sensitivity, and glucose metabolism, and thus controlling blood sugar level in people with T2D (28,29). Major advantages of HIIT over continuous exercise include positive effects on the cardiovascular system, enhanced endothelial function, sensitivity to insulin and blood pressure, improved body composition, aerobic fitness, and glucose control assessed by continuous glucose monitoring (CGM), reduced pain, fatigue, overtraining, and burnout, and increased performance and tolerance for performing high-intensity exercise over a long period of time (28). Accordingly, in a study on metabolic syndrome patients, HIIT was twice more effective than moderate intensity continuous training (MICT) in increasing cardiorespiratory fitness (30). In this regard, Cristian *et al.* (2017) carried out a study on 40 sedentary adult women at the risk of T2D. The women underwent a HIIT program while being closely monitored for cardiovascular health issues. The participants were divided into two groups based on their insulin resistance levels, and the two groups were compared in terms of their responses to the intervention. The researchers concluded that the HIIT program is associated with improvements in cardiometabolic health metrics, blood pressure, blood glucose, and insulin levels, as well as reduction in weight and body fat levels (31).

Regarding the mentioned evidence, lack of study in the field of FTO gene and exercise, as well as greater benefits of high intensity exercise compared with moderate intensity exercise in diabetic subjects, we assumed that this exercise can modify the expression of FTO and PPAR-γ in muscle tissue of obese diabetic rats. Therefore, the purpose of this study was to investigate the effects of 6 weeks high intensity interval training on muscle expression of FTO and PPAR-γ in obese diabetic rats.

2. *Materials and Methods*

This experimental study was performed on 12 male (the nominal power of 0.85 will give an effective power of only about 0.75) Wistar rats (220±20 g bodyweight and 10 weeks old) that were purchased from Iran Pasteur Institute. Before starting the study, all rats were adapted to the living conditions (room with dimensions of 1.60×2.20 meters, 22±3 °C temperature, 30–60 % relative humidity, and a half day light/ half day dark cycle) in the animal house of the Islamic Azad University, Alborz Province within one week. Animals had open access to standard high-fat food (32) and drinkable water. All processes were conducted in accordance with the Guide of the Care and Use of Laboratory Animals of Islamic Azad University, Alborz Province, and were confirmed by the ethics committee (96-8-3788).

To induce diabetes 2 type, animals received high-fat diet within 6 weeks, and then were performed an intraperitoneal injection of a single dose of 30 mg/kg freshly prepared streptozotocin (STZ) (Sigma, USA) solved in citrate buffer (pH 4.5). In order to prepare the high-fat food to meet the standards of food purchased from Pars Dam Company, 1% cholesterol powder and 1% pure corn oil were

added. It should be noted that the high-fat diet was continued for two groups until the end of the study (32). One week after the establishment of diabetes, fasting blood glucose was measured, and the blood sugar between 150 to 400 mg/dl was considered as a criterion for ensuring that rats develop type 2 diabetes (33). Then the diabetic rats were distributed randomly into two groups: (1) diabetic+ control group (n = 6), and, (2) diabetic+ high intensity interval training group (HIIT) (n = 6).

Animals of this group were familiarized with how to run on a treadmill within 2 weeks, then, they underwent a treadmill exercise within 6 weeks. HIIT program included 5 sessions of 30 minutes per week, with repetitions of 40 seconds (8 repetitions in the first week and 10 repetitions in the next weeks), and different speeds and slopes (Table 1), in addition to active rest of 2 minutes at the rate of 10 m/min between each repetition.

Table 1. High intensity interval training (HIIT)

Week	Repetition	Time of exercise	Intensity of exercise	Time of active rest	Intensity of active rest	Gradient
1	8	40 sec	25 m/min	12. sec	10 m/min	5%
2	10	40 sec	25 m/min	12. sec	10 m/min	10%
3	10	40 sec	28 m/min	12. sec	10 m/min	10%
4	10	40 sec	32 m/min	12. sec	10 m/min	10%
5	10	40 sec	35 m/min	12. sec	10 m/min	10%
6	10	40 sec	35 m/min	12. sec	10 m/min	10%

* Sec; second, m/min; meter/minute, %; percent

48 hours after the last training session, rats of two groups were anaesthetized with ketamine/xylazine mixture and sacrificed by cervical dislocation, then the thorax cavity was opened and blood sampling was directly done from the heart. Gastrocnemius muscles of rats were sampled and after washing in saline solution, they were immersed in microtubes containing RNA later ™ (RNA Stabilization reagent 50mL) with a ratio of 20%, and then transferred to -70 °C for further genetic testing in Tehran Pasteur Institute, Iran.

Blood samples were centrifuged at 1000×g within 2 min to separate serum and kept at -80 °C to measure serum glucose and insulin. Glucose level was evaluated by enzymatic colorimetric procedure with glucose oxidase technology using the glucose kit of Pars Azmoon Company (Tehran, Iran). The changes coefficients of intra-test and extra-test of the glucose were 1.74 and 1.19 percent, respectively, and the sensitivity of the measurement was 5 mg/dl. To measure serum insulin, a Demeditec laboratory kit (Germany) was used by ELISA Method. The changes coefficients of intra-test and extra-test and the sensitivity of insulin measuring were 2.6, 2.88 percent and 1.76 unit, respectively. Then, insulin resistance was obtained by the following formula:

Insulin resistance = (glucose (mmol/l) × insulin (μU/ml)) / 22.5 (34).

The sequence of primers was designed by a geneticist and its make order was given

to the Pishgam Biotech Company (Tehran, Iran). Meanwhile, RNA- polymers II gene was applied as the internal control to evaluate the relative quantitation of the mRNA expression. The designed primers are summarized in Table 2.

Table2. The primer sequences of FTO and PPAR-γ in this study

Genes	Primer sequence	Product size	Tm	Gene Bank
FTO	**For**: TACACAGAGGCCGAGATTGC **Rev**: AAGGTCCACTTCATCATCGCAG	159 bp	60	NM_001191052.1
PPAR-y	**For**: ACAACAGGCCACATGAAGAGC **Rev**: AAGCTTCAATCGGATGGTTCTTCG	159 bp	60	NM_001191052.1
RNA polymers II	**For**: ACTTTGATGACGTGGAGGAGGAC **Rev**: GTTGGCCTGCGGTCGTTC	164 bp	60	XM_008759265.1

RNA was extracted by applying Rneasy protect mini kit (QIAGEN, Germany) from gastrocnemius muscle and visceral adipose tissues according to the manufacturer's guidelines. To ensure the RNA concentration for the cDNA preparation, its OD was checked by a NanoDrop (2000, USA). Then, cDNA synthesis from RNA was carried out by cDNA synthesis kit (QIAGEN, Germany), and the obtained product was maintained at -20 °C. Determination of FTO mRNA and PPARy mRNA by Real-time PCR was done by Rotorgen 6000 system using One Step SYBR® Green kit (TaKaRa, Japan) according to the company's instructions. Melting curve analysis was also performed at the end of the PCR cycle to determine the validity of the PCR product. The used thermal cycle protocol in Real-time PCR included one cycle with 42 °C on 20 min, 95 °C on 2 min to actuate the enzymes, and 40 cycles with 94 °C on 10 sec and 60 °C on 40 sec. Finally, the extracted CTs of reactions were recorded by device software.

SPSS Software (version 16) was used to perform statistical analyses. Shapiro-Wilk Test was used to investigate the normality of distributions among the groups. Independent t-test and Analysis of covariance (ANCOVA) was applied to compare the means. The test's significance level was considered to be less than 0.05.

3. Result

Statistical analysis showed that FTO gene expression (p<0.01), fasting blood sugar level (P<0.001), weight (p<0.001) and HOMA-IR (p<0.004) significantly decreased after 6 weeks high intensity interval training in the exercise group compared to control group. Also, a significant increase was observed in PPAR-γ gene expression (p<0.007) in exercise group compared to control group after 6 weeks high intensity interval training.

Table 3. The results of independent t-test between HIIT and control groups

Variables	HIIT group	Control group	P value
Insulin (µIU/ml)	6.59±5	5.21±0.37	**0.000****
Glucose (mg/dL)	194±12.09	293±11.07	**0.000****
Homa-IR	56.92±6.99	67.89±5.74	**0.004****
Gastrocnemius FTO	0.71±0.26	1	**0.016****
Gastrocnemius PPARy	1.69±0.52	1	**0.007****

**: Correlation is significant at the 0.01 level (2-tailed).

Table 4. The results of weight changes between pre and post exercises

Variable	SS	df	MS	F	P
Weight	6768.658	1	6768.658	58.149	**0.000****

**: Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

Reducing the prevalence of metabolic diseases (*e.g.*, T2D) and their complications has always been among the main objectives of health planners. The results of the present study has shown a significant decrease of FTO (P<0.01), fasting blood sugar (P<0.001), weight (P<0.001) and HOMA-IR (P<0.004), and a significant increase of PPAR-y after 6 weeks of high-intensity interval training among diabetic rats.

The mean of the PPAR-y expression showed a significant increase in HIIT group compared to the control group after 6 weeks of intervention (P<0.007). A limited number of studies have investigated the effects of aerobic exercises, especially HIIT, on the expression of PPAR-γ in the muscle tissue of diabetic subjects. Increased insulin sensitivity and improved glucose metabolism are among the major mechanisms that increase the PPAR-γ expression and facilitate adaptation to exercise in diabetic individuals (35). Further, it has been reported that intensive exercise has a positive effect on the expression of PGC-1α and PPAR-γ genes and insulin resistance in diabetics (36). Furthermore, Kim *et al.* reported increased expression of PPAR-γ, PGC-1α, GLUT4, insulin sensitivity, and muscle glucose uptake in the soleus muscle of diabetic rats after 6 weeks of swimming exercise (37). The results of another study showed that the PPAR-γ gene expression in the liver and muscle tissue decreased in response to severe acute stress *e.g.*

exercise, but the continuation of this stress for six weeks leading to a binding to thiazolidinedione (TZD) seemed to significantly improve the whole-body insulin sensitivity, which in turn resulted in reduced insulin and glucose levels (38). The positive effects of increased PPAR-γ expression are mainly due to a combination of improved insulin sensitivity and the direct effect of PPAR-γ on genes involved in glucose transport and glycolysis (36). At the cellular level, high expression and activation of PPAR-γ increases insulin sensitivity and decreases blood glucose levels through activating insulin signaling cascades and influencing the expression of specific genes and molecules (36). These signaling pathways mainly involve a series of intracellular phosphorylation events including tyrosine phosphorylation of insulin receptor substrate (IRS) proteins and activation of phosphatidylinositol-3-kinase (PI3-kinase) and other downstream kinases. These mechanisms ultimately led to an increase in glucose uptake into muscle cells, improved lipid metabolism, and increased expression and transcription of genes associated with high insulin sensitivity (36,39). On the other hand, studies have confirmed increased PI3K pathway activation in adaptation to HIIT (36). Increased GLUT4 expression is also known as a potential mechanism involved in increasing PPAR-γ expression. Therefore, an increase in the expression of PPAR-γ generally increases the expression of GLUT4, IRS-1, and P85 (the regulatory subunit of PI3K), as well as the expression of proteins related to glucose metabolism and insulin sensitivity (37,40), and thereby plays a major role in preventing and controlling T2D. An increase in the expression of other transcription factors involved in insulin signaling can also be associated with increased expression of PPAR-γ, which can synergistically lower blood glucose levels in diabetic patients. Therefore, increasing muscle-specific expression of PPAR-γ can improve the health of diabetic people by lowering blood glucose levels and increasing insulin sensitivity.

After the intervention, a significant decrease was found in the FTO gene expression in the exercise group compared with that of the control group ($P<0.01$). Few studies have investigated the effects of FTO gene expression and exercise on people with T2D. Danaher *et al.* (2020) reported a significant decrease in FTO mRNA expression of healthy men and women following a high intensity exercise program (41). Accordingly, Sailer *et al.* (2016) observed a decrease in FTO expression of healthy men following a 9-month moderate intensity aerobic training (42). In addition, Zlatohlavek *et al.* reported a significant decrease in FTO gene expression of obese and overweight children after 4 weeks of aerobic exercise (43). The exact effect of exercise`s mechanisms on decreasing FTO expression have not yet been clearly identified. High expression of the FTO gene, also known as alpha-ketoglutarate-dependent dioxygenase gene, has been reported in adipose and muscle tissues. Moreover, FTO gene serves various functions in the nervous and cardiovascular systems (44). Studies have shown that high FTO expression is strongly associated with both body mass index (BMI) and obesity (45–47). The results of several studies indicated that this gene responds to insulin when influenced by environmental factors, such as hunger and nutrition; hence, this gene is

associated with insulin sensitivity (48,49). Furthermore, high FTO expression is directly related to defects in glucose metabolism in adipose and muscle tissues (13). Therefore, weight loss, increased insulin sensitivity, and improved glucose metabolism were found to be the possible mechanisms of reducing muscle FTO expression in adaptation to HIIT programs.

After the intervention, a significant decrease was observed in the weight (g) in the exercise group compared with that of the control group ($P<0.000$) and decrease blood glucose ($P<0.000$) in the exercise group compared with the control group; besides, Homa-IR in the exercise group significantly decreased compared with the control group ($P<0.004$). Positive effects of various exercises (*e.g.* HIIT) on weight loss improved glucose metabolism, and increased insulin sensitivity of diabetic patients, all of which have been confirmed (50). Chronic exercise also helped diabetic individuals lose weight by creating a negative energy balance and boosting their metabolism. Weight loss in turn played an important role in improving blood glucose levels. In addition, some studies have confirmed that increased expression of GLUT4 and PGC-1α, increased mitochondrial biogenesis, increased beta-oxidation, and decreased body fat percentage in adaptation to HIIT exercises all lead to improved glucose metabolism and increased insulin sensitivity (51–53). Finally, studies have reported that high-intensity exercises, such as HIIT, increase activation of AMPK and Ras-related C3 botulinum toxin substrate 1 (Rac1), which in turn result in increased expression of GLUT4 and PGC-1 α. This can somewhat explain the positive effects of HIIT on glucose metabolism and insulin sensitivity (54,55).

5. Conclusion

Regarding the results of this study, it can be stated that six-week HIIT program can improve glucose metabolism and insulin sensitivity, and also increase the expression of diabetes- and obesity-associated genes (*e.g.*, PPAR-γ and FTO), and thereby, this program plays a prominent role in the control and treatment of type 2 diabetes in obese patients.

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Conflicts of interest

None declare.

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