Research Article

Resistance training reduces FTO gene expression in subcutaneous adipose tissue and improves glycemic control in diabetic rats

Davood Khorshidi[1], Porya Mohammadiyan[1], Kamal Azizbeigi[2*]

Abstract

Fat mass and obesity-associated gene (FTO) is directly associated with increased risk of obesity and type 2 diabetes mellitus (T2DM). The purpose of current study was to investigate the effect of 12 weeks of resistance training (RT) on FTO expression in subcutaneous adipose tissue, glucose, and insulin levels in T2DM rats. Sixteen males Wistar rats (220±10 gr) with T2DM induced by streptozotocin-nicotinamide injection were randomly assigned into resistance training (RT; n=8) and control (Con; n=8) groups. RT was performed for 12 weeks, 5 days per week. FTO expression in subcutaneous adipose tissue, fasting blood glucose (FBS), insulin and insulin resistance (HOMA-IR) were measured 48 hours after the last exercise training session. After the exercise training intervention, the FTO expression ($p=0.004$) and FBS ($p=0.001$) were significantly lower in the RT compared to the Con group while the insulin in the RT was significantly higher than that in the Con group ($p=0.001$). There was no significant difference in the insulin resistance between the two groups ($p>0.05$). According to findings, it seems that RT can decrease FBS and FTO expression in subcutaneous adipose tissue of T2DM rats. Improved blood glucose in diabetic rats might be partially attributed to reduced FTO expression in response to RT.

Key Words: **FTO gene, Glucose, Resistance training, Type 2 diabetes**

Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial disorder leading to impaired glucose homeostasis. T2DM is the consequence of the activation of multiple pathways and factors involved in insulin resistance (IR) and beta cell dysfunction (Borse et al., 2021). However, many factors affecting the diabetes prevalence are still unknown. The risk factors of T2DM include genetic, metabolic, and environmental factors that interact with each other, and play a role in its spread (Galicia-Garcia et al., 2020). In the recent years, many studies have been conducted to identify the genetic factors involved in the pathophysiology of T2DM. Based on this, several genetic factors have been identified that play a role in IR or impaired insulin secretion (Kwak & Park, 2018; Mambiya et al., 2019). Among these factors, the fat mass and obesity-associated gene (FTO) has been identified by genome-wide association studies (GWAS) as the first gene predisposing to obesity (Frayling et al., 2007; Scuteri et al., 2007).

The FTO gene is expressed in many tissues, including adipose tissue, skeletal muscles, liver, pancreas, and especially the hypothalamus, and plays an important role in regulating energy homeostasis (Kang et al., 2017; Lan et al., 2020). According to studies, FTO is associated with increased obesity risk and related diseases such as T2DM (Shill et al., 2021; Yang et al., 2017). FTO expression is also positively correlated with body mass index (BMI), blood glucose, Hemoglobin A1C (HbA1c) and IR, and it has been suggested as a clinical index for the diagnosis and T2DM treatment (Wang et al., 2018). The molecular mechanisms related to the effect of FTO on T2DM are not well understood, however, studies have shown that FTO is associated with T2DM through increased obesity, mainly by affecting IR (Kwak & Park, 2018). Therefore, it seems that the reduction of body fat percentage (BF %) has an effect on the changes FTO.

Although resistance training (RT) was found more effective than aerobic and combined training program in some T2DM syndrome such as peripheral vascular disease. It has been rep-

1. Exercise Physiology, Department of Physical Education, Saveh Branch, Islamic Azad University, Saveh, Iran. 2. Associate Professor of Exercise Physiology, Department of Physical Education, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.

*Author for correspondence: kazizbeigi@gmail.com

D KH: 0000-0002-4740-9159; P M: 0000-0002-6763-0264; K A:0000-0002-5963-2323

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-orted that short-term resistance training does not change the expression of the FTO gene in the subcutaneous fat tissue of type 2 diabetic rats (Yazdanpazhooh et al., 2018). Based on this, it seems that there is still no comprehensive study on the effectiveness of RT on the FTO gene expression changes or its polymorphisms in the adipose tissue of diabetic animal models. Therefore, the present study was conducted to investigate the effect of 12 weeks of resistance training on FTO gene expression in subcutaneous adipose tissue, glucose, and insulin levels in type 2 diabetic rats.

Materials and Methods

Animals

Sixteen male Wistar rats (10 weeks age; 220±10 gr weight) were prepared, and kept in the animal laboratory for one week to familiarize with the lab environment. All animals were maintained under controlled conditions (temperature 23±3° C, humidity 45% - 55% and 12-h light-darkness cycle). This study has been approved by the Ethics Committee of the Research Institute of Physical Education and Sports Sciences with code IR.SSRI.REC.1400.1028.

Induction of diabetes

First, the nicotinamide solution (NAM) (110 mg /kg) was injected intraperitoneally; after 15 min, STZ solution (60 mg/kg) in citrate buffer with pH=4.5 was injected intraperitoneally. One week after the induction of diabetes, fasting blood glucose (FBS) level was measured and FBS between 150 -400 mg/dl was considered as a criterion for type 2 diabetes (Kalhor et al., 2018). Then, the diabetic rats were randomly assigned into two resistance training (RT; n=8) and control (Con; n=8) groups.

Resistance training

Resistance training (RT) was performed for 12 weeks, 5 sessions per week, by climbing a 16-step ladder with a height of 1 meter and a slope of 80% with a weight attached to the tail. Each session consisted of 3 sets and 6 repetitions in each set. The rest interval between repetitions was 45 sec and between sets was 3 min. The loads were 10% of weight in the first week, 20% of weight in the second and third weeks, 40% of weight in the fourth and fifth weeks, 60% of weight in the sixth and seventh weeks, 80% of weight in the eighth and ninth weeks, and 100% of weight in the tenth, eleventh and twelfth weeks (Eizadi et al., 2016). The rats in the control group did not participate in the exercise training, but after the exercise intervention, they were dissected at the same time as the rats in the training group.

Table 1. The pattern of primers used in the research

Genes	Primer sequence	Product size	Gene Bank
FTO	F: TACACAGAGGCCGAGATTGC R: AAGGTCCACTTCATCATCGCAG	159 bp	NM_001191052.1
RNA Polymrase II	F: ACTTTGATGACGTGGAGGAGGAC R: GTTGGCCTGCGGTCGTTC	164 bp	XM_008759265.1

Sampling and biochemical analyses

48 hours after the last RT session (following a12-h fasting), rats in both groups were anesthetized by intraperitoneal injection of 10% ketamine (50 mg/kg) and 2% xylosin (10 mg/kg). Then, blood samples were collected from their heart. FBS was measured by enzymatic colorimetric method with glucose oxidase (Pars Azmoon Kit, Iran). Inter- and intra-assay coefficients of variation (CVs) for FBS were 1.19% and 1.74%, respectively. Serum insulin was assessed by insulin ELISA kit (Demeditec, Germany). Inter- and intra-assay CVs were 2.6% and 2.88% for insulin respectively. After measuring fasting insulin and glucose, insulin resistance was calculated through homeostasis model assessment of insulin resistance (HOMA-IR).

In addition, the subcutaneous adipose tissue was sampled and immersed in microtubes containing RNA later at a ratio of 20% and transferred to the laboratory for genetic tests. RNA was extracted from subcutaneous adipose tissue using RNeasy Protect mini kit (QIAGEN, Germany). FTO mRNA was determined by Real-time PCR by Rotor-gen 6000 system using one-step SYBR Green kit (Takara, Japan) (Table 1).

Statistical analysis

Komogrove-Smirnov (K-S) test was used to test the data normal distribution. The comparison of the RT and control groups on the

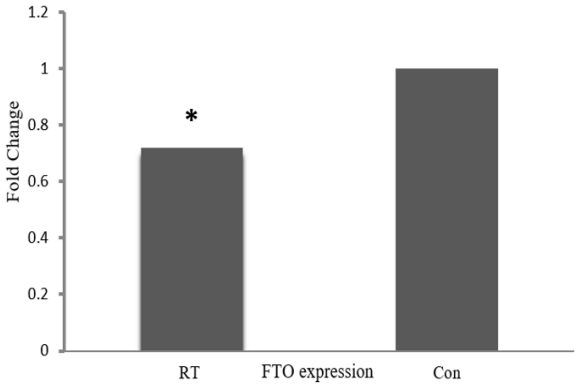

Figure 1. Comparison of FTO expression after resistance training. RT: Resistance training; Con: Control; FTO: Fat mass and obesity-associated gene. *: Sign of significant compare to control group.

variables was done using the independent t-test. p≤0.05 was also considered significant level. Statistical analysis was done using SPSS/Win version 16 software.

Results

There was a significant difference in the FTO gene expression in the subcutaneous adipose tissue between two groups. The FTO gene expression in the rats of the RT group was lower than the control group (Figure 1). Therefore, RT led to a significant decrease in FTO gene expression in the subcutaneous adipose tissue of the RT group.

After RT intervention, significant differences were found in FBS and insulin level between RT and control. In the RT, the FBS was lower, and the insulin was higher than the control. However, there was no significant difference in the HOMA-IR between the two. Changes in FBS, insulin and insulin resistance in the RT and control are presented in Table 2.

Discussion

In present study, the resistance training effects were studied on FTO gene expression in subcutaneous fat tissue, glucose, and insulin of type 2 diabetic rats. It was found that 12 weeks of resistance training could reduce FTO gene expression in subcutaneous adipose tissue of type 2 diabetic rats. Several factors such as FTO gene, play a significant role in the pathophysiology of T2DM (Kwak & Park, 2018). Wang et al. (2018) showed that FTO gene expression is higher in type 2 diabetic patients than in healthy individuals, and increases with disease progression. They also reported that 12 weeks of treatment reduces FTO protein expression, and blood glucose in severe type 2 diabetic patients (Wang et al., 2018). Similarly, in another study, it was shown that the treatment of diabetes with Rosiglitazone decreases FTO mRNA in subcutaneous adipose tissue, and improves insulin sensitivity in T2DM patients (Bravard et al., 2013). Based on this, our findings indicate that RT can be used as an important non-pharmacological intervention to reduce the genetic effect of FTO on the T2DM.

There is a few evidence available about the effect of exercise training on the expression of FTO gene in adipose tissue in diabe-

Table 2. The changes of the blood parameters in the two groups

Variable	Control	RT	p-value
FBS (mg/dl)	309 ± 20	223 ± 12	0.001
Insulin (μIU/ml)	4.13 ± 0.29	5.56 ± 0.33	0.001
HOMA-IR	3.14 ± 0.06	3.10 ± 0.14	0.82

FBS: Fasting blood sugar; RT: resistance training

-tic. In this context, Yazdanpazhooh et al. (2018) showed that six weeks of RT does not change the FTO gene expression in the adipose tissue of diabetic rats (Yazdanpazhooh et al., 2018). Despite the same exercises training used in the study and the current study, the inconsistent results may be partially attributed to the difference in the training duration (12 weeks vs. six weeks). On the other hand, it seems that exercise training mode is an important factor that can affect FTO changes. In this regard, it has been reported that six weeks of high intensity interval training leads to a decrease in FTO gene expression in skeletal muscles in diabetic rats (Kushkestani et al., 2022). It seems that FTO responses to intensity exercise are more sensitive. Danaher et al. (2020) showed that acute bout of high intensity exercise (80% vo2peak) reduces the expression of FTO mRNA in the skeletal muscles of healthy people in the early stages of recovery while after low intensity exercise (40% vo2peak), this change did not occur (Danaher et al., 2020).

Although the mechanisms by which exercise intensity may alter FTO gene expression are still not clearly understood, there is evidence that exercise training can reduce the effect of FTO gene on diabetes by activating AMP-activated protein kinase (AMPK) signalling. (20). AMPK activation decreases FTO expression and its inhibition increases FTO expression (Wu et al., 2017). Furthermore, several studies have shown that exercise training play a role in regulating AMPK activity, and activates the AMPK signalling pathway (Jun-Ho, 2018; Vieira et al., 2020). These evidences may partially explain the mechanism by which exercise reduces FTO gene expression. However, further studies are needed to clearly understand the molecular mechanisms involved in exercise-induced changes in FTO expression.

In the present study, in line with the decrease in FTO expression, FBS decreased and insulin levels increased but there was no change in insulin resistance. These results are different from some previous studies reporting a decrease in insulin levels and insulin resistance by resistance exercise (Botezelli et al., 2016; Tavakol et al., 2019). However, Bronczek et al. (2021) indicated that RT can improve glucose homeostasis by improving pancreatic beta-cell function and insulin secretion (Bronczek et al., 2021). Furthermore, there is evidence that FTO gene may contribute to pancreatic beta-cell dysfunction and inhibit insulin secretion (Fan et al., 2015). Therefore, the FBS improvement in trained rats may be attributed to the changes caused by resistance training in insulin secretion, which may be at least partially associated with decreased FTO gene expression. This study focused on three parameters of subcutaneous fat tissue, glucose, and insulin of type 2 in diabetic rats. To better understand the effect of resistance training on diabetes, it is reco-

-mmended to study more variables. In addition, the body fat changes of the rats were not measured, thus, future studies may focus on this point.

Conclusion

Based on the results of this study, long-term resistance training reduces blood glucose levels and FTO gene expression in subcutaneous fat tissue in type 2 diabetic rats. Improved glycemic control in the trained rats may be partially attributed to the reduction of FTO gene expression in subcutaneous adipose tissue. Further research is required to better understand the mechanisms underlying these changes.

What is already known on this subject?

Excessive body fat mass may cause diabetes and some metabolic disorders. Body fat percentage managements may improve diabetes.

What this study adds?

Resistance exercise training can decrease fasting blood glucose and FTO expression in subcutaneous adipose tissue of T2DM rats.

Organ Cross-Talk Tips:

- Cross-talk between skeletal muscle and subcutaneous adipose tissue is caused by fat mass and obesity-associated (FTO) gene expression with resistance exercise in diabetes.
- Cross-talk between skeletal muscle, adipose tissue and pancreas with resistance training leads to modulation of insulin resistance in diabetes.

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Compliance with ethical standards

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

Ethical approval This study has been approved by the Ethics Committee of the Research Institute of Physical Education and Sports Sciences with code IR.SSRI.REC.1400.1028.

Informed consent Animal study.

Author contributions

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

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