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The effect of resistance training on hepatic FTO gene expression, fasting glucose and insulin resistance in Wistar rats with type 2 diabetes

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ABSTRACT

Introduction: Clinical studies have supported the effective role of gluconeogenic in hepatic glucose release, especially in type 2 diabetics (T2D). This study was aimed to investigate the effect of resistance training on hepatocyte fat mass and obesity associated (FTO) gene expression as well as fasting glucose and insulin resistance in obese rats with type 2 diabetes.

Methods: For this purpose, T2D induced by 6 weeks high-fat diet (HFD) and intraperitoneal STZ injection in 14 male Wistar rats (220 ± 10 grams) and were then randomly divided into exercise (n = 7, resistance training) and control (n = 7) groups. The rat in exercise group were completed 8 weeks resistance training (5 times/weekly) and control rats remained non-training. 48 hours after exercise intervention, fasting blood glucose, serum insulin, insulin resistance and hepatocyte FTO expression were compared by independent t test between groups.

Results: Compared to control subjects, significant decrease were observed in fasting glucose (P = 0.001), insulin resistance (P = 0.001) and FTO expression (P = 0.024) and increase in serum insulin (P = 0.005) by exercise intervention in resistance group.

Conclusion: Resistance training is accompanied by improving fasting glucose in T2D rats. This improvement may be attributed to the reduction of hepatocytes FTO expression and insulin resistance in response to resistance training.

Keywords:

Type 2 diabetes, FTO gene expression. Resistance training, Hepatic glucose release.

INTRODUCTION

From a health and wellness perspective, type 2 diabetes (T2D) has been recognized as a global epidemic. Scientific evidence has clearly supported obesity as the most important factor in the occurrence of T2D. Obesity also accelerates the incidence of T2D by increasing insulin resistance and increasing blood glucose (1). On the other hand, in people with insulin resistance, if there is sufficient insulin secretion capacity to compensate for insulin resistance, the severity of diabetes does not increase (2). However, chronic hyperglycemia is associated with impaired insulin secretion and function (3). Most studies have sought to understand how hormonal or metabolic factors affect insulin function and its synthesis or release from beta cells, but the processes of glucose production by some body tissues such as the liver, which leads to hyperglycemia, especially in diabetic patients, have received less attention.

Thus, increased endogenous glucose, often resulting from increased hepatic glucose output, is a major feature of T2D (4,5). In this regard, insulin resistance is associated with increased hepatic glucose production, which plays an important role in fasting hyperglycemia and

severe postprandial hyperglycemia. In this context, increased phosphatase activity plays an important role in the disruption of the gluconeogenesis process (6).

On the other hand, genome-wide studies have indicated the association of fat mass and obesity associated (FTO) variants with obesity and T2D (7, 8). These studies have revealed that FTO variants have an important role in both body weight and glucose metabolism (9, 10). The biological properties of FTO point to its possible role in regulating the expression of genes involved in the regulation of whole-body metabolism and the metabolic activities of multiple tissues. Hepatic FTO plays an important role in regulating metabolism, and its expression in liver cells changes in response to changes in other metabolic factors. FTO expression increases during fasting without any change in its protein levels (11).

Starvation and fasting lead not only to changes in glucose but also to changes in other metabolites and hormones such as insulin, which play an important role in regulating the expression of gluconeogenic genes. On the one hand, FTO plays an important role in the expression of gluconeogenic genes, and on the other hand, its expression in liver cells is inversely regulated by insulin, that is, increased insulin levels in fasting conditions lead to a decrease in FTO expression in liver cells (12). In general, FTO expression is inversely regulated by glucose and insulin, and its hepatic levels regulate the expression of gluconeogenic genes by changing the effect of metabolic signals such as hormonal and nutritional components.

Although some studies have reported no changes in hepatic FTO protein levels and expression in mice fed a high-fat diet for 14 to 17 weeks, increased hepatic FTO protein levels and expression have been reported in other studies following 6 to 12 weeks of high-fat diet in laboratory mice (12,13,14). However, the responses of protein levels or its expression in hepatic hepatocytes following exercise training have not been reported so far. Some studies have indicated the inhibitory effect of hepatic insulin on FTO expression (12). Based on this evidence, there are also limitations in studies that follow the effect of exercise training on FTO expression in the liver tissue of diabetic rats. The present study was conducted to determine the effect of resistance training, as one of the common training methods, on hepatic FTO expression as well as fasting glucose levels, serum insulin, and insulin resistance in T2D rats.

MATERIALS AND METHODS

In the present quasi-experimental study, T2D induced by 6 weeks high-fat diet (HFD) and intraperitoneal injection of STZ (30 mg/dL) in 14 male Wistar rats (10-week-old, 220 ± 10 grams) and were then randomly divided into resistance (n = 7, resistance training) and control (n = 7) groups. To prepare the high-fat diet, 1% cholesterol powder and 1% pure corn oil were added to the standard diet (15, 16). Then, to induce T2D, 30 ml/kg of freshly prepared STZ solution was injected intraperitoneally. Fasting blood glucose between 150 and 400 mg/dL was considered a criterion for ensuring that rats developed T2D (16, 17).

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 HFD.

Resistance training protocol: After the induction of diabetes, the rats were divided into resistance and control groups. The resistance group participated in a resistance training for 8 weeks (5 sessions/weekly) 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. Applying resistance in the form of tying weights to the rats' tails is equivalent to different percentages of body weight during the training program (17). 48 hours after the last training session, the rats were killed and dissected to extract the hepatocytes to measure FTO and PEPCK expression.

Table 1: Resistance training based on applying resistance as a percentage of body weight

Applying resistance	Exercise session (Week)				
	1	2	3-4	5-6	7-8
Percentage of body weight	40	50	60	80	100

Blood sampling and Tissue extraction: 48 hours after the last training session, the overnight fasted 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. Glucose concentration was measured by glucose oxidase method using glucose kit from Pars Azmoun Company-Tehran. Serum insulin was measured using ELISA method according to the standards of a commercial kit (Demeditec Diagnostic insulin ELIZA) made in Germany. The insulin resistance index was measured using fasting insulin and glucose values by the HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) formula as below (18):

$$\text{HOMA-R} = \frac{\text{Fasting Insulin (}\mu\text{U/ml) X Fasting Glucose (mmol/l)}}{22.5}$$

The liver tissue 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 (17). RNA Polymerasell was used as the control gene. The sequence pattern of the primers are shown in table 2.

Statistical analysis: Independent t-test was used in the SPSS/Win version 22 software to compare dependent variables.

RESULTS

Body weight changes in the control and resistance groups are shown in Table 3. No significant difference was observed in body weight between groups at baseline (p = 0.423). Compared to pre-training, a significant increase were observed in body weight after intervention in both groups (p = 0.001). However, after the exercise intervention, body weight in the resistance group was significantly higher than the control group (p = 0.19).

Table 2: The sequence pattern of the primers

Genes	Primer sequence	Product size	T m	Gene Bank
FTO	For: TACACAGAGGCCGAGATTGC Rev: AAGGTCCACTTCATCATCGCAG	159 bp	60	NM_001191052.1
RNA PolymraseII	For: ACTTTGATGACGTGGAGGAGGAC Rev: GTTGGCCTGCGGTCGTTC	164 bp	60	XM_008759265.1

Table 3: Pre and post-training of body weight in studied groups.

Group	Pre-training	Post-tainting
Control diabetic	321 ± 8	398 ± 12***
Resistance diabetic	313 ± 9	420 ± 11*** #
P-value (independent t test)	0.423	0.019

*** shows p<0.001 compared to pre-training group.
# shows p<0.05 compared to control diabetic group.

Based on the findings of the statistical test, a significant difference was observed in each of the variables of fasting glucose (p = 0.001, Fig 1), serum insulin (p = 0.005, Fig 2), and insulin resistance (p = 0.001, Fig 3) between the study groups. In other words, resistance training in the resistance group led to a significant increase in serum insulin and a significant decrease in fasting glucose and insulin resistance compared to the control group.

In addition, the results of the independent t-test showed a significant difference in hepatocytes FTO expression between groups (P = 0.024, Fig 4). In other words, 8 weeks of resistance training resulted in a significant decrease in hepatocytes FTO expression compared to the control group.

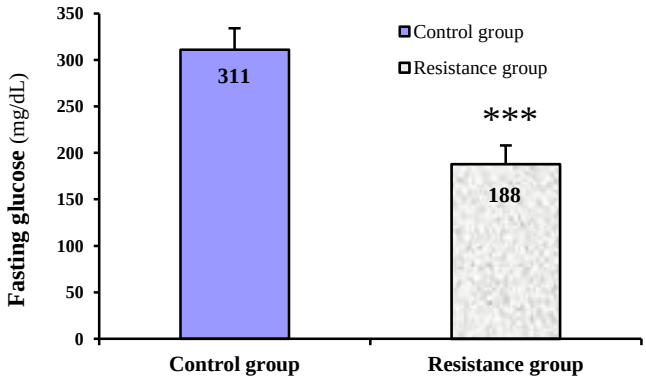

Fig 1: Fasting glucose after exercise training in resistance compared with control groups.

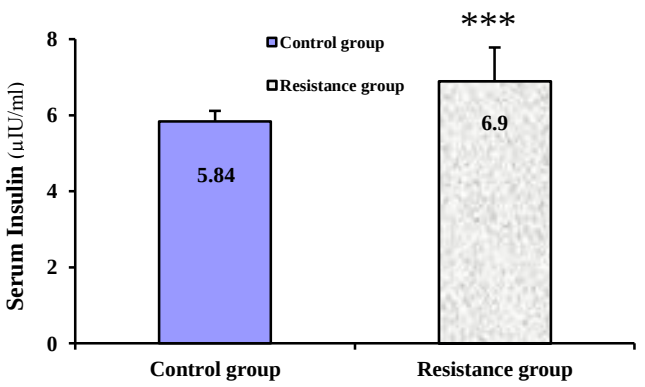

Fig 2: Serum insulin after exercise training in resistance compared with control groups.

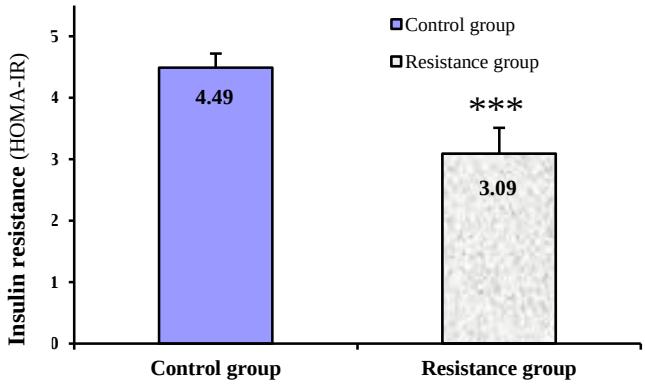

Fig 3: Insulin resistance index after exercise training in resistance compared with control groups.

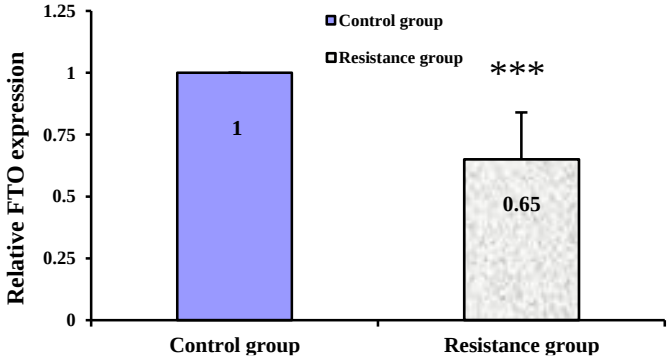

Fig 4: Relative FTO expression after exercise training in resistance compared with control

DISCUSSION

The reduction in hepatocytes FTO gene expression in response to resistance training is the main finding of the present study. In other words, 8 weeks of resistance training, 5 sessions per week, resulted in a reduction in FTO expression in hepatocytes of T2D obese rats. These changes were also associated with increased serum insulin and decreased fasting glucose in the resistance group compared to control rats that did not participate in training. These findings are consistent with some of the existing studies and contradictory with others.

In this regard, Maltais et al (2016) reported that no changes in insulin and glucose levels were observed after 4 months of resistance training (19). Another study found that 20 weeks of exercise training, 3 to 5 sessions per week at an intensity of 70% of maximal oxygen consumption, did not lead to changes in glycosylated hemoglobin (20). Also, according to a study conducted by Leggate et al (2012), six sessions of high-intensity interval training over two weeks had no effect on insulin sensitivity in overweight men (21). However, consistent with our findings, a study by Lopes et al (2016) showed that 12 weeks of combined training including resistance and aerobic training led to a significant decrease in blood glucose levels and an increase in insulin sensitivity (22). Also, Glans et al (2009) reported a significant decrease in glucose levels after 6 months of aerobic and resistance training in diabetic patients (23).

Based on clinical evidence, the decrease in fasting glucose levels after exercise or other stimuli is often due to insulin resistance or factors affecting it. Therefore, when examining the mechanisms affecting glucose level changes after treatment, the first hypothesis that is put forward is insulin resistance. In this regard, the results of the present study indicate a significant decrease in insulin resistance in response to resistance training. As 8 weeks of resistance training led to a significant decrease in insulin resistance in T2D mice compared to the control group that did not participate in the training programs. Based on these findings, the decrease in glucose levels may be attributed to the decrease in insulin resistance in response to resistance training. In this regard,

Lopez et al (2016) observed that 12 weeks of combined training in overweight girls led to a significant decrease in insulin resistance (24). Also, in the same year, Stegling et al, reported improvements in glucose and HbA1C levels after 12 weeks of high-intensity interval training (HIIT) performed three times a week at an intensity of 70–90% of maximum heart rate, which could be attributed to reduce insulin resistance or improved inflammatory profiles (25). Abdaqader et al. also showed in 2013 that 12 weeks of moderate-intensity aerobic exercise reduced insulin resistance in T2D patients and led to improved HbA1C (26).

Although glucose changes or glycemic profile, especially in T2D patients, are strongly dependent on insulin action in target tissues such as adipose and muscle tissue, blood glucose levels are also partly rooted in the rate of hepatic glucose release, which is a function of the rate of hepatic gluconeogenesis. Apart from intermediary enzymes in inhibiting or stimulating gluconeogenesis, the role of transcriptional or genetic factors in it has been repeatedly proposed and studied. In a way, some genetic components, called gluconeogenic genes, regulate and control the rate of gluconeogenesis, and their expression or protein levels are disrupted in the presence of diabetes (27, 28).

Based on this evidence, the improvement in fasting glucose in response to resistance training in the present study may be attributed to changes in FTO expression in hepatic hepatocytes. It should be noted that resistance training in exercise resulted in a decrease in hepatic FTO expression compared to the control group. In this context, some studies have pointed to a key role of FTO in gluconeogenesis-dependent hepatic glucose release (29, 30). These researchers have attributed the role of FTO on hepatic glucose release to its effect on the activity or expression of the hepatic enzymes G6Pase and PCK1 (31).

In normal individuals, increased glucose or insulin in healthy rats leads to inhibition of FTO expression in hepatic hepatocytes. On the other hand, decreased hepatic FTO expression in turn leads to decreased G6Pase and PCK1, which inhibits the rate of gluconeogenesis, or in other words, reduced hepatic glucose release into the bloodstream (31). In other words, in healthy individuals, increased glucose itself, under a compensatory mechanism, leads to decreased activity or expression of FTO-dependent G6Pase and PCK1. In contrast, increased blood glucose in type 2 diabetics leads to increased FTO expression, which results in increased hepatic G6Pase and PCK1 expression. FTO also positively regulates another transcription factor, ATF4. In other words, increased FTO expression leads to accelerated hepatic glucose uptake by stimulating increased ATF4 (31). This evidence supports the idea that hepatic ATF4 mediates the effect of FTO on glucose metabolism via hepatic gluconeogenesis (29, 32). Based on this evidence, it seems that the inhibitory effect of FTO on the activity or expression of gluconeogenic enzymes is mediated by ATF4 inhibition in response to resistance exercise, which results in reduced hepatic glucose output, especially in diabetic patients.

CONCLUSION

Resistance training reduces fasting glucose in T2D rats. Given the role of FTO inhibition on the activity and expression of gluconeogenic enzymes, the improvement in glucose may be attributed to the reduction in FTO expression in response to this training regimen. Despite this evidence, further studies are needed to understand the underlying mechanisms responsible for the inhibition of hepatic gluconeogenesis by resistance training. Although measuring FTO gene expression in response to resistance training is considered a strength of this study, measuring other genetic components affecting gluconeogenesis is considered a limitation of the study, and it is recommended to measure them in response to different training methods.

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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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