Six Weeks Resistance Training Effect on FTO Expression in Type II Diabetes Rats

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Abstract

Objective: Fat mass and obesity associated gene (FTO) was identified as a candidate gene contributing to obesity, insulin resistance and type 2 diabetes mellitus (T2DM). This study aimed to determine the effect of resistance training on FTO expression in subcutaneous fat tissue, insulin resistance and glucose in T2DM rats.

Materials and Methods: For this purpose, sixteen obese males wistar rats with T2DM were randomly divided into exercise (n= 8) and control (n= 8) groups. Exercise group were completed six weeks resistance training included climbed on a stepladder (5 days per week) and control group did not participate in exercise intervention. Relative expression of FTO expression in subcutaneous fat tissue, fasting glucose and insulin resistance were measured 48 hours after last exercise session of two groups. Independent t-test used to compare data.

Results: In comparison with control group, resistance training induced significant decrease in fasting glucose (*P*-value: 0.001) and insulin resistance (*P*-value: 0.001). But FTO expression in subcutaneous fat tissue did not change by resistance training (*P*-value: 0.318).

Conclusion: Resistance training did not influence FTO expression in fat tissue of T2DM rats. Improved insulin resistance and glucose can be attributed to the change in other genetic or hormonal factors by resistance training.

Keywords: Diabetes mellitus, Gene expression, Glucose, Resistance training

Introduction

Over the years, researchers have come to the conclusion that type 2 diabetes mellitus (T2DM) is the result of complex interactions between hormonal, genetic and environmental factors. T2DM cause lipid and glucose metabolism disorder by insulin deficiency in liver and muscle, insulin secretion, fatty tissue metabolism, lipolysis of the total body, and possibly metabolic impairment in other organs (1). In recent decades, the role of genetic factors in both secretion of insulin from the pancreas and insulin resistance in target tissues has got particular importance. Some genetic factors such as TCF7L2 and its polymorphisms or GLP-1 severely influence the synthesis and

secretion of insulin from beta cells (2,3). On the other hand, some other genetic components, such as FOXO1, PPARγ, and Fat Mass and Obesity-associated Gene (FTO), also influence the energy homeostasis and metabolism of glucose and fat in target tissues such as skeletal muscles and fatty tissue (4,5). The relationship between protein levels and their expression with levels of obesity, lipid profile, and insulin resistance has been reported many times (4,5).

Among them, FTO have been introduced as an effective genetic candidate for development of obesity and its related diseases in both children and adults (4). There is a positive and significant correlation between FTO expression and visceral fat storage (6). The FTO is located on the long arm of chromosome 16 (7), and it is strongly expressed in the fatty tissue, hypothalamus, pituitary and adrenal glands that are involved in controlling energy homeostasis in the brain (7). An increase in protein levels and its expression in fatty tissue induce diabetes and increased insulin resistance in obese people (8,9). Several studies have supported the association of its polymorphisms with BMI and the risk of overweight at different ages (8,10).

The main mechanisms responsible for the effect of this gene on the risk of T2DM are not well defined, but clinical implications indicate that protein levels and its expression in muscle cells and fatty tissue increase in patients with T2DM compared to healthy participants (11). The available evidence supports its role in glucose homeostasis and insulin function in targeted tissues such as muscles and fatty tissue. It is hypothesized that if protein levels or expression of the protein can be reduced in target tissues by internal or external stimuli, it will reduce insulin resistance and consequently will improve blood glucose levels. In this regard, although some studies have supported the beneficial effects of exercise training on hormonal or inflammatory components effective on T2DM (12), studies on genetic factors or its polymorphisms are limited. The

following studies point out the response of genetic components to exercise trainings in diabetic patients or other obese populations.

According to Eizadi et al. (2016), 3 months of resistance training resulted in decreased expression of TCF7L2 in the pancreatic tissue along with increased beta cells function and decreased blood glucose levels in T2DM rats (13). Rashidi et al. (2016) found that aerobic exercises by diabetic rats led to an increase in the expression of the MTNR1B gene along with increased synthesis and secretion of insulin in T2DM rats (14). Nevertheless, Lee et al. (2014) revealed that 8 weeks low intensity exercise increased the expression of PPARγ in fatty tissue of obese rats, but glucose response and insulin resistance were not mentioned in this study (15). Despite the mentioned evidence, there is no study assessing the direct effect of short or long-term exercise training on FTO expression in subcutaneous fatty tissue. Therefore, in the present study, the effect of 6 weeks of resistance training on FTO expression in subcutaneous fatty tissue and also insulin resistance and blood glucose levels were measured in T2DM rats.

Materials and Methods

In this experimental study, eighteen 10 weeks old male wistar rats (220 ± 30 g), procured from the institutional animal house facility were used for all the experiments and after induction of T2DM were randomly divided into exercise (resistance training / 6 weeks, n = 8) and control (no training, n = 8) groups. Sample size and the number of rats in each groups designed according to the same previous studies (13-15) that assessed gene expression in T2DM rats in response to exercise training. Animals were maintained under standardized conditions (12-h light/dark cycle, 25 ± 2 °C & humidity 45-55 %). The rats were left for 1 week for adaptation prior to the start of the experiment. The study was approved by department of Exercise physiology of Islamic Azad University, South Tehran Branch, Iran and carried out in

accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animal) guidelines. (Ethic code: 14121407952009)

T2DM induced by 6 weeks high fat diet (HFD) followed by a single intraperitoneal injection of 25 mg/kg streptozotocin (dissolved in citrate buffer, pH 4.5) (16). Hyperglycemia was confirmed by elevated blood glucose levels on 7th day after diabetes induction and only animals with fasting blood glucose (FBG) level between 150-400 mg/dl were selected for were served as T2DM rats and used in the study (17).

After ensuring diabetes induction, the exercise group was climbed on a stepladder a 26-step, 1 meter vertical ladder with a gradient of 80% without any resistance for 6 times in 3 training sessions in order to learn how to exercise. Then they completed a resistance training lasted 6 weeks for 5 days in weeks. In order to warm and cool the rats before and after the workout, they were climbed and descended the ladder 2 times without any resistance.

Each session of resistance training was performed in the form of 5 courses with 4 repetitions on each course, and the resistance was increased through attaching a weight to rats' tails. Breaks were every 3 min for 45 sec, respectively. The resistance was increased gradually during training intervention. Finally, all rats were dissected 48 hours after the last training session following 10 to 12 hours overnight fasting. It should be noted that the diabetic control rats were not included in the training program during this period.

Finally, 48 hours after the lasted exercise session, the fasting rats in both groups (with 10-12 hours overnight fasting) were anesthetized through intraperitoneal injection of 10% ketamine at a dose of 50 mg/kg along with 2% xylocaine at a dose of 10 mg/kg, after which they were underwent dissection. After the rats were anesthetized, blood samples were collected through cardiac puncture. Then, subcutaneous fatty tissue was removed and immersed in RNA later until biochemical analysis was performed for determine FTO expression. The blood samples were used to analyze the blood glucose and serum insulin. The serum was separated by centrifugation (5 min, 3,000 rpm) and was analyzed for glucose by glucose using a Cobas 6000 Analyzer (Roche, Germany). Glucose was determined by the oxidase method (Pars Azmoon kit, Tehran). The remaining serum samples were then stored at −20 ∘C until the insulin determination was made by ELISA method (Demeditec, Germany) and the intra- assay and inter-assay coefficient of variation of the method were 2.6% and 2.88 respectively. Insulin resistance was assessed using the homeostasis model assessment for insulin resistance formula derived from fasting insulin and glucose levels (18).

The RNA was extracted by Rneasy protect mini kit (QIAGEN) from subcutaneous fatty tissue according to manufactures instructions (19). RT-Real time PCR quantification of FTO mRNA was performed with Rotor gene 6000 system using One Step SYBR PrimeScript RT PCR kit (Takara co.) according to manufactures instructions. Melting curve analysis was performed at the end of PCR cycles in order to validate the specificity of the expected PCR product. We used RNA polymerase as a normalizer.

Statistical analysis

Data were analyzed by computer using the Statistical Package for Social Sciences (SPSS) for Windows, version 16.0. Normal distribution of data was analyzed by the Kolmogorov-Smirnov normality test. Independent student T-test was used for comparison of variables between two groups. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Based on statistical data, no significant difference was found in body weight between 2 groups before (*P*-value: 0.318) and after training (*P*-value: 0.962). On the other hand, body weight increased at the end of study than before training in both exercise (*P*-value <

0.001) and control (*P*-value < 0.001) groups (Table 1). It should be noted that high fat diet was continued to the end of study for 2 groups. FBG levels were significantly lower in exercise group in comparison to control group (*P*-value< 0.001). In other words, 6 weeks resistance training induced significant decrease in glucose (table 2). In addition, a significant difference was observed between 2 groups with regard to insulin resistance (*P*-value< 0.001). In other words, 6 weeks resistance training induced significant decrease in insulin resistance compared with control subjects (table 2).

Despite remarkable decrease in FBG and insulin resistance in response to resistance training, but there was no statistically significant difference between the exercise (1.19 ± 0.5) and control rats with regard to FTO expression in subcutaneous fatty tissue (*P*-value: 0.318) (Figure 1).

Discussion

The lack of alteration of FTO expression in subcutaneous fatty tissue of the exercise group compared with the control group is the main finding of the current study. In other words, 6 weeks resistance training did not significantly change the expression of FTO in subcutaneous fatty tissue of T2DM obese rats. Meanwhile, training intervention significantly reduced insulin resistance in the experimental rats compared to the control group. With respect to the research background that points out the effective role of FTO in insulin resistance and function in target tissues such as muscles and fatty tissue (8.9), our findings are somewhat controversial.

In this regard, there are few studies about exercise training on FTO expression in muscle and fatty tissues, Graff et al. (2017) revealed that physical activity can reduce and regulate genetic disorders that are effective in the development of obesity and related diseases (20). In a study by Sailer et al. (2016), 9 months of moderate-intensity aerobic training led to reduction of FT08050136 in healthy men, but a reduction was reported in body weight independent of changes in FTO rs8050136 (21). Zlatohlavek et al. (2013) also pointed out that the change in FTO variants increases the effect of exercise activity (4 weeks of aerobic training) on BMI in obese or overweight children (22). However, Hubáček et al. (2011), estimating the effect of physical activity and daily calorie intake in the form of recording hours assigned to exercise, walking, and other light and heavy physical activities, found no significant relationship between FTO variants and physical activity level and total daily absorption of energy. This study revealed that the effect of FTO rs17817449 polymorphism on BMI in adults is independent of calorie intake or physical activity levels (23).

Despite the findings which are often restricted to the response of FTO polymorphisms to exercise trainings and levels of physical activity in human specimens, there are not adequate studies available on the effect of exercise training on FTO expression in fatty tissue that is considered to be the strongest genetic components effective on insulin resistance (4). However, some recent studies have reported the effects of different training methods on other genetic components that cause insulin resistance. As in Azad et al. (2016), both acute and long term eccentric

Table 1. Before and after training body weight of 2 studied groups (Mean ± SD)

Group	Before	After	*P*-value
Control	280 ± 13	370 ± 7	< 0.001
Resistance	279 ± 19	381 ± 8	< 0.001
***P*-value**	0.318	0.962	-----

Table 2. FBG and insulin resistance after training intervention in 2 studied groups (Mean ± SD)

Variable	Control group	Exercise group	*P*-value
FBG (mg/dl)	302 ± 15	198 ± 34	< 0.001
Insulin resistance (HOMA-IR)	4.49 ± 0.33	3.22 ± 0.58	< 0.001
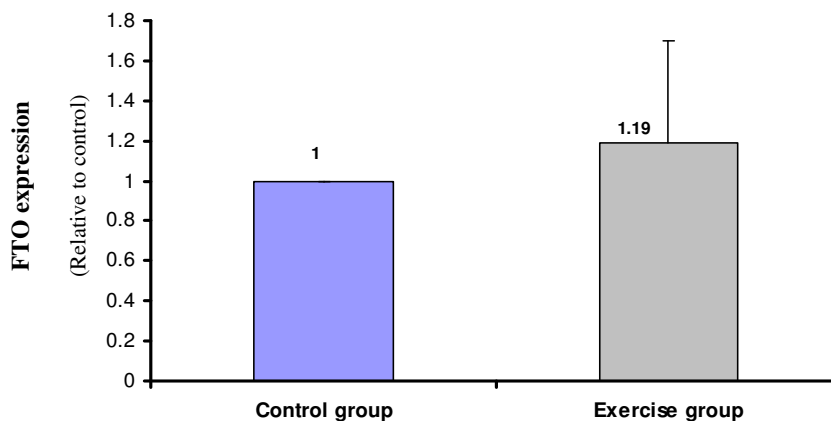
Figure 1: FTO expressions in subcutaneous fatty tissue in exercise rats compare to control group (*P*-value: 0.318).

trainings (9 weeks) in the form of running on treadmill with negative slope led to a significant change in FOXO1 in the external muscle of the experimental rats, while acute exercise increased FOXO1 expression, and long term trainings (9 weeks) decreased FOXO1 expression in comparison with the control group (24). In the study of Slopack et al. (2016), although a 60-minute aerobic training session led to an increase in FOXO1 expression in fatty tissue of 9-week-old rats, prolonged aerobic trainings decreased its protein levels. The researchers emphasized that long term exercise trainings can reduce the functional role of FOXO1 protein (25). It should be noted that FOXO1 is another genetic component that, in a mechanism similar to that of the FTO, leads to reduced insulin function or increased insulin resistance in fatty tissue or other target tissues, such as muscles (26). This mechanism has been numerously reported for obesity and T2DM (27).

Despite the lack of change in FTO expression in subcutaneous fatty tissue in the present study, resistance training intervention significantly reduced both insulin resistance and blood glucose levels compared to the control group rats that did not receive the training program. Accordingly, one might conclude that exercise trainings may likely improve glucose and insulin resistance independent of changes in genetic components. Therefore, improvement of glucose and insulin resistance can be attributed to the anti-inflammatory effects of exercise training. In this regard, Abd El-Kader et al. (2013), based on their findings, the reduction of FBG and glycosylated hemoglobin following 12 weeks of aerobic training in T2DM men and women were attributed to the reduction in TNF-α and IL-6 inflammatory mediators (12). Lucotti et al. (2011) reported a significant reduction in insulin resistance following combined resistance and aerobic trainings in T2DM patients due to a decrease in weight and an increase in serum adiponectin levels (28).

Apart from the change in the inflammatory components in response to exercise trainings, the decrease in insulin resistance and glucose may be attributed to the change in other genetic components such as the change in the signaling pathways of FOXO1, PPARγ, IRS1, or GLUT4. It should be noted that these genetic factors strongly influence the insulin signaling pathways in target tissues such as fatty tissue, skeletal muscle and liver (4,5). Hence, the lack of their measurement can be described as the main limitation of the present study. However, some scholars have also supported the relationship between changes in cardiovascular fitness and changes in genetic

components. Sailer et al. (2016) measured the effect of 9 months exercise training on below lactate threshold intensity of FTO and VO2max polymorphisms; the findings revealed that the change in FTO rs8050136 in response to aerobic exercises was only observed in those with increased VO2max (21).

Conclusions
Despite of FBG and insulin resistance improvement, 6 weeks of resistance training did not influence the expression of FTO in fatty tissue of T2DM male rats. Improvement in FBG and insulin resistance may be attributed to changes in other genetic or hormonal components. Hence, the implementation of new studies is recommended to measure other genetic variables in response to different training methods.

Conflict of Interest
Authors declare that they have no competing interests.

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