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Over Expression of FOXO1 in Subcutaneous Fatty Tissue and its Response to Resistance Training in High Fat Diet and Type 2 Diabetic Rat

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Abstract

Objective: Forkhead box proteins and Forkhead box transcription factor O1 (FOXO1) in particular, mediate insulin signaling pathways and glucose homeostasis. This study aimed to compare *FOXO1* expression in subcutaneous adipose (SA) tissue between obese rats with and without type 2 diabetes (T2D) and its response to resistance training in T2D.

Materials and Methods: 21 male wistar rats (220±20 g) were obese by 6 weeks high fat diet (HFD) and randomly assigned to either non-diabetes (n=7) or two T2D groups (control and exercise groups, n=7 in each case). Fasting glucose, insulin, insulin resistance and *FOXO1* expression were compared between non-diabetes and diabetes groups. All variables were also assigned after resistance training in the form of climbing a ladder (6 weeks/5 times weekly) in exercise compare with control groups. Data were compared by ANOVA, independent and paired t-test methods (P<0.05).

Results: Induction of diabetes resulted in significant increase in insulin resistance, glucose, and *FOXO* expression in SA tissue and a decrease in insulin compared to obese health rats (P< 0.0001). A significant decrease in fasting insulin (P< 0.0001), insulin resistance (P< 0.0001) and *FOXO1* expression in SA tissue (P< 0.0001) and increase in insulin (P: 0.002) were observed by resistance training compared to control diabetes rats.

Conclusion: Based on our results, improving insulin resistance and glucose in response to resistance training in obese diabetic rats may be rooted in decreased insulin expression following these exercises.

Keywords: Adipose tissue, Exercise, Insulin resistance, Diabetes

QR Code

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Introduction

Apart from the genetic factors influencing insulin secretion from pancreatic β-cells, some transcription factors also affect insulin function in target tissues. Disruption at protein levels or their expression due to the development of insulin resistance or decreased insulin sensitivity leads to improper insulin function. For instance, PPARγ and FTO affect energy hemostasis and metabolism of glucose and lipid in target tissues (1-3). Disruption at protein levels and their expression increases the tendency to obesity and in turn exacerbates diabetes in obese individuals (4).

Forkhead box transcription factor O1 (FOXO1), as a protein from the FOXOs family, is encoded in humans by the *FOXO1* gene, and affect insulin signaling pathways, gluconeogenesis and insulin resistance in the target tissue. So increased protein levels on gene expression in target tissues such as muscle or adipose tissue are associated with increased insulin resistance and glucose, especially in type 2 diabetes (T2D) (5). It also mediates the effects of growth factors or insulin on various physiological functions including metabolism, cell proliferation and apoptosis (5). Its role has also been previously reported in regulation of G6pase and PEPCK as gluconeogenesis enzymes (6). So increasing its expression in hepatocytes leads to increased activity and expression of PEPCK and G6pase as gluconeogenesis enzymes that regulate the rate of gluconeogenesis, which results in increased hepatic glucose release due to gluconeogenesis (6).

However, the results of a study conducted by Sanchez et al. (2015) has revealed that a single session of endurance exercise leads to a significant increase in FOXO1 protein and expression in the skeletal muscle of mice, while long-term endurance trainings will lead to a significant reduction in FOXO1 expression (7). In Azad et al.'s (2016) study, the FOXO1 expression in the Vastus lateralis muscle of laboratory rats increased by 3.62 following an acute exercise, but its expression decreased by 0.56 following the 9 week training (8). In a study by Karimi et al (2019), HIIT increased FOXO expression in pancreatic of T2D rats (9). However, the effect of exercise training, especially resistance training, on its expression in subcutaneous adipose tissue is less pronounced. Therefore, the present study not only compared *FOXO1* expression in SA tissue between diabetic and non-diabetic obese rats but also examined the effect of resistance training on its expression in SA tissue as well as the blood glucose levels and insulin resistance in rats with T2D.

Materials and Methods

Experimental animals

The statistical population of the present study consists of all Wistar male rats of the animal house of Baqiyatallah University of Medical Sciences, Tehran, Iran, among which 21 ten-week-old rats with a weight of (220 ± 30 g) were purchased. Then, the studied rats were divided into 3 groups (resistance diabetic group, diabetic control group, healthy group). Diabetic rats were provided with high fat diet and they were maintained under standardized conditions (12-h light/dark cycle, 25 ± 2 °C & humidity 45-60 %).

Induction of obesity and T2D

All rats became obese by a 6-week high-fat diet (HDF). To prepare high-fat food, 1% cholesterol powder and 100% pure corn oil were added to the standard diet of field rats purchased from Parsdam Food Company (10).

After inducing obesity, 7 rats were selected as non-diabetes obese group (healthy group, n= 7), and T2D was induced by streptozotocin (STZ) intraperitoneal injection (25 ml/lg) in other rats (13). Diabetic rats were divided into control (n= 7) or exercise (resistance training, n= 7) groups. Fasting blood glucose was measured one week after diabetes induction, and blood glucose between 150-400 mg/dL was considered as a criterion for T2D (11).

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Resistance training protocol

After ensuring diabetes induction, the exercise group was climbed on a stepladder a 1 meter vertical ladder (26-step) with a gradient of 80% without resistance for 6 repetitions in 3 exercise 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 down the rats, they were climbed and descended the ladder 2 times without any resistance before and end of each exercise session. Each exercise session was performed in the form of 5 courses with 4 repetitions on each course, and the resistance was increased by attaching a weight to rats' tails. Breaks between courses were 3 min and 45 sec between repetitions. The resistance was increased gradually during training intervention (Table 1) (10). Finally, all rats were sacrificed after 10-12 hours of overnight fasting, 48 hours after the last training session. The non-diabetes and diabetic control rats were not included in the training.

Sample Collection and Biochemistry

48 hours after lasting exercise session, the fasted rats of 3 groups were anesthetized through intraperitoneal injection of 10% ketamine (50 mg/kg) along with 2% xylosine (10 mg/kg) (12). After anesthesia, Blood samples were taken directly from the heart. Then, SA tissue was extracted and immersed in RNA later to determine *FOXO1* expression. Blood samples were centrifuged for 10 minutes by 3000 rpm for serum separation to analyze insulin. Glucose was measured enzymatically (Pars Azmoon kit-Tehran). Insulin was determined by ELISA (Demeditec, Germany). Intra- assay and inter-assay coefficient of variation were 2.6% and 2.88 respectively. Fasting glucose and serum insulin were used to assess insulin resistance (HOMA-IR) (13).

RNA extraction / Real time – PCR

20 milligrams of SA tissue were ground using a mortar and pestle, and extraction was performed employing the RNeasy Protect Mini Kit (Qiagen, Germany) according to the manufacturer's protocol to purify RNA (14). The One Step SYBR Prime Script RT-PCR Kit (Takara Bio Inc, Japan) was employed according to its manufacturer to prepare the reaction product. The thermal cycle program used for the Rotor-Gene Q instrument was as follows: 42˚C for 20 minutes, 95 ˚C for two minutes, and 40 cycles with 94˚C for 10 seconds and 60˚C for 40 seconds. Temperatures from 50 to 99˚C used for the melting curve after the PCR to study the characteristics of the primers.

Statistical analysis

Data are expressed as mean and standard deviation. Data were analyzed using the Statistical Package for Social Sciences for Windows, version 22. Normality of distribution was assessed by the Shapiro-Wilk test. One-way ANOVA was used to compare body weight between the studied groups in the pre-and-post interventions. The paired T-test was also used to determine intra-group changes in body weight. One-way ANOVA was used to compare variables (insulin, glucose, *FOXO1* expression, and insulin resistance) and LSD post hoc test was used to

Table 1. Resistance training protocol based on percentage of body weight

Time	First week	Second week	Third week	Forth week	Five and six week
Resistance (Body weight %)	30	50	70	90	100

Table 2. Primer sequence

Genes	Primer sequence	Product size	T m	Gene Bank
FOXO1	**For**: CACCCTCTGCTGCCAAGATG **Rev**: GGCGAGGACTGGGTTGAC	159 bp	60	NM_001191846.3
RNA PolymraseII	**For**: ACTTTGATGACGTGGAGGAGGAC **Rev**: GTTGGCCTGCGGTCGTTC	164 bp	60	NM_031335.3

compare each variable between groups. Differences were considered to be statistically significant when $P< 0.05$.

Ethical considerations

This study was approved by Committee of Ethics in Research of Azad University of Safadasht Branch, Tehran, Iran with number of IR.SSU.REC.1396.413 and carried out in accordance with CPCSEA guidelines.

Results

Based on data by one-way ANOVA, no significant difference in body weight levels were observed between groups at baseline (*P*: 0.541). Despite a significant increase in body weight in all 3 groups, but no significant difference was observed between groups at the end of training (*P*: 0.355).

Based on data by one-way ANOVA, significant differences were observed between groups for all variables (insulin, glucose, *FOXO1* expression and insulin resistance) ($P< 0.05$).

Based on data, *FOXO1* expression in SA tissue of the diabetic control group was significantly higher than the healthy group (*P*: 0.041, effect). In other words, the induction of type 2 diabetes by a high-fat + STZ diet resulted in significant increase in *FOXO1* expression in SA tissue compared to the healthy group. On the other hand, training program led to a significant reduction in *FOXO1* expression compared to control diabetic group ($P< 0.0001$) (Figure 1).

Induction of diabetes also led to a significant increase in fasting insulin resistance and glucose as well decrease in serum insulin in diabetic rats compared to the healthy group ($P< 0.05$). In addition, based on results of Post hoc LSD, training program resulted in significant decrease in glucose ($P< 0.0001$) and insulin resistance ($P< 0.0001$) and significant increase in serum insulin (*P:* 0.002) compared to control diabetes group (Table 3).

Discussion

The findings revealed that induction of T2D led to an increase in *FOXO1* expression in subcutaneous fatty tissue, and this increase

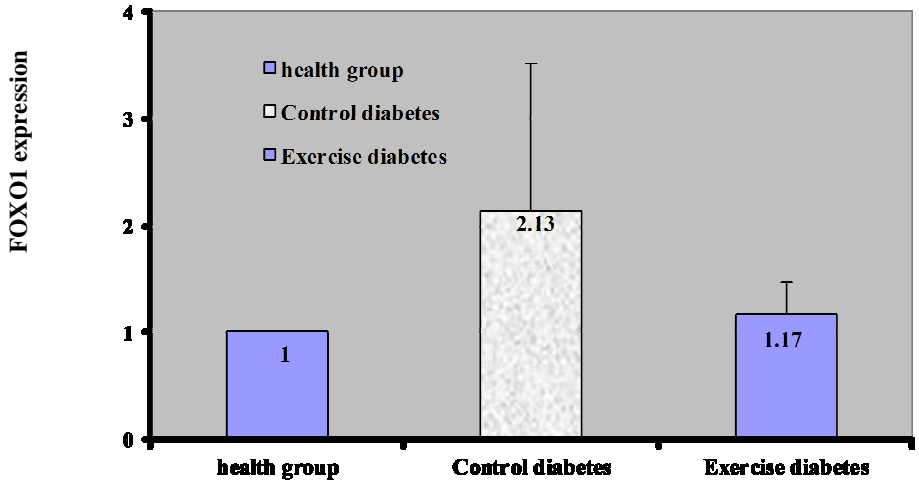

Figure 1. The change pattern of FOXO1 expression between healthy and diabetes groups

Table 3. Mean and SD of diabetes determinants of healthy and diabetes groups

Variable	Healthy group	Control diabetes	Exercise diabetes	*P*
Glucose (mg/dL)	121 (± 4)	298 (± 18)	194 (± 33)	0.021
Insulin (µIU/ml)	9 (± 0.78)	5.97 (± 0.28)	6.51 (± 0.23)	0.019
Insulin resistance (HOMA-IR)	2.69 (± 0.25)	4.39 (± 0.39)	3.13 (± 0.59)*	0.011

* data were compared by one-way ANOVA
* Values representing the levels of variables after sacrificing mice

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was associated with increased insulin resistance and fasting glucose level. These findings, in turn, support previous findings that indicated the mutual effect of genetic components on insulin function at target tissue levels. Previous molecular cell studies have repeatedly pointed to the influential role of disruption in the expression of certain genetic components in increasing insulin resistance in obese or diabetic individuals (18). Thus, their impaired expression or polymorphisms can lead to an increase in the incidence rate or an increase in the severity of diabetes due to damages to insulin secretion and the mechanisms responsible for insulin function (15).

Among them, not only *FOXO1* is potentially important in insulin secretion from β-cells but also its effects on insulin signaling pathways in target tissues strongly affect insulin resistance and glycemic profile (16). In this regard, genetic studies have pointed to the potential role of *FOXO1* in pathogenesis of T2D by claiming that it affects glycemic control and fat metabolism. However, the potential evidence that directly reports this association is limited, and this claim mostly relies on findings from in vitro studies (17). Scientific sources have suggested that FOXO1 in insulin-sensitive tissues such as liver, skeletal muscles, and adipose tissues is inversely regulated by insulin and affects the processes leading to diabetes (18). These sources have supported an increase in FOXO1 expression in diabetic patients' adipose tissue and its direct association with insulin resistance and inflammatory markers such as FOXO1 (19). Other studies have noted an increase in its expression in subcutaneous and visceral adipose tissues (20) and its importance as a vital transcription factor in insulin and glucose hemostasis (20).

In the present study, a reduction in *FOXO1* expression in the subcutaneous adipose tissue in the present study resulted in a decrease in insulin resistance and fasting glucose levels in rats receiving the intervention as compared to those not participating in the resistance training. Reduced FOXO1 expression is mentioned in the present study, while some studies have reported no change or an increase in this regard. In Slopack study (2014), the FOXO1 expression in adipose tissue of 9-week-old rats was significantly increased immediately and after two hours of recovery following a single session of endurance exercise. However, the findings in the mentioned study indicated a decrease in FOXO1 protein levels in adipose tissue from the 10^{th} session onwards. The researchers have attributed the mentioned reduction to a decline in FOXO1 nuclear reserves and an increase in cytosolic levels, or in other words, the exit of FOXO1 from the cell nucleus to the cytosol (21).

Some studies have also suggested a change in *FOXO1* expression in response to exercise trainings in other tissues of the body such as pancreas and liver. In a study by Karimi et al (2019), 12 weeks of HIIT led to a decrease in *FOXO1* expression along with an increase in serum insulin in T2D rats (9). Yarmohammadi et al (2019) also reported a decrease in *FOXO1* expression in liver hepatocytes in response to resistance training in diabetic rats (22). Furthermore, Almasi et al (2018) have noted a decrease in *FOXO1* expression in subcutaneous adipose tissue of diabetic rats in response to HIIT (23). It should be mentioned that changes in *FOXO1* expression in each of the mentioned studies have been associated with decreased fasting glucose levels.

Genetic studies have supported the potential role of *FOXO1* transcription factor in regulating insulin sensitivity in adipose tissues (16). Another study indicated that *FOXO1* expression in placental adipose tissue in women with gestational diabetes had a significant positive association with TNF-α and insulin resistance (19).

Based on the above-mentioned evidence and the findings of the present study, it seems that decreased glucose in the present study is rooted in a decrease in insulin resistance or an increase in insulin function due to a decrease in *FOXO1* in response to resistance trainings

as these findings suggest a direct relationship between changes in *FOXO1* expression with glucose and insulin resistance in response to resistance trainings. However, although measuring *FOXO1* expression as an effective factor in insulin signaling pathways is one of the strengths of the present study, but changes in *FOXO1* alone are not responsible for these changes, and understanding the mechanisms responsible for reducing insulin resistance in response to exercise requires measuring other hormonal or genetic components that affect insulin signaling pathways. Therefore, lack of measurement of other genetic components that somehow affect the function of insulin in the target tissue in response to exercise is a limitation of the present study.

Conclusions

The *FOXO1* gene expression in the subcutaneous adipose tissue of T2D male Wistar rats is higher than that of non-diabetic obese rats. Based on the findings of the present study, increased insulin resistance is somehow rooted in an increase in *FOXO1* expression in target tissues such as adipose tissues. In addition, long-term resistance trainings lead to a decrease in its expression and a decrease in blood glucose levels along with insulin resistance. Based on the available evidence, improving insulin resistance in response to resistance training in studied diabetic rats may be rooted in decreased insulin expression following these exercises.

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Conflict of Interest

The authors report no conflicts of interest.

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