Effect of 12 Weeks Aerobic Training on FOXO1 Gene Expression in Pancreatic Tissue of Type 2 Diabetes Wistar Rats

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Abstract

Objective: Exercise as a non-pharmacological treatment plays an important role in regulating and reducing the inflammatory cytokine associated with beta cell function. Genetics is one of the most important and effective factors in the incidence of diabetes, in most cases. The present study aims to explain the effect of 12 weeks aerobic training on FOXO1 expression in pancreatic tissue, insulin and blood glucose levels in male wistar rats with type 2 diabetes mellitus.

Materials and Methods: In this study, 16 male wistar rats were divided into diabetic control and diabetic training groups. The two groups were diabetic with nicotine amide and streptozotocin injections and the training group did aerobic exercises for treadmill for 12 weeks.

Results: The results of the study showed a significant increase in FOXO1 expression after 12 weeks of aerobic training (*P*-value: 0.027), which resulted in a significant decrease in blood glucose concentration (*P*-value: 0.0001).

Conclusion: The induction of type 2 diabetes leads to a reduction in the expression of FOXO1 gene in the tissues of the pancreas in experimental rats, which is associated with a decrease in serum levels of insulin and an increase in blood glucose levels. On the other hand, 12 weeks of aerobic training of 5 sessions per week leads to a significant increase in the expression of FOXO1 gene, with decreasing glucose and increased serum insulin in the pancreatic tissue of diabetic rats.

Keywords: FOXO1 gene expression, Type 2 diabetes mellitus, Aerobic exercise, Pancreatic beta cells

Introduction

In the last decade, the potential role of transcription factors in the secretion of insulin from beta cells has been raised in diabetic or pre-diabetic individuals. Meanwhile, it has been shown that FOXO1 protects pancreatic cells against hyperglycemia oxidative stress (1,2). This phenomenon is caused by the activation or enhancement of the expression of MAFA and NeuroD, which are introduced as two

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transcription factors of the insulin gene (3-5). The change in the Expression of the FOXO1 nucleus of beta cell hyperplasia was documented in insulin resistant mice (6). This information provides evidence that FOXO1 adjusts the mass of beta cells and also determines that FOXO1 levels in diabetic rats decrease (4). Hyperglycemia, Oxidative stress, and the activity of c-Jun N-terminal kinases (JNKs) lead to the introduction of FOXO1 from cytosol to the nucleus, which in turn results in the release of pancreatic and duodenal homeobox1 (PDX-1) from the nucleus of the pancreatic cells to the cytoplasm.

FOXO1, PDX-1 and MAFA are all three of the most important transcription factors affecting the synthesis and secretion of insulin from beta cells (7). This shape somehow reflects the potential molecular mechanism of inhibiting insulin biosynthesis in type 2 diabetes (T2DM). Under conditions of diabetes, chronic hyperglycemia leads to oxidative stress, which results in inhibition of biosynthesis and insulin secretion, which is associated with decreased expression of MAFA and PDX1. On the other hand, hyperglycemia and oxidative stress lead to the release of FOXO1 from cytosol to the nucleus of beta cells, which in turn leads to the withdrawal of PDX-1 from the nucleus of the pancreatic cells to the cytoplasm(8). The inhibition of FOXO1 expression along with the reduction of the expression of MAFA and PDX1, along with other genetic changes, reduce the synthesis and secretion of insulin from beta cells (7,8). Given the crucial role of FOXO1 transcription factor in protecting and functioning beta cells and secreting insulin, the question arises as to whether exercises can be effective in increasing the expression of this factor. Therefore, the present study aims to explain the effect of 12 weeks aerobic training on FOXO1 expression in pancreatic tissue, insulin and blood glucose levels in male wistar rats with T2DM.

Materials and Methods

The studied population was selected from all male rats of the animal's house of the Institute of Pasteur. Sixteen male wistar rats (at the age of ten weeks and weighting 220 ± 20 gr) were randomly selected to participate in the study. Subsequently, wistar rats, all of which have similar physical and age characteristics, were randomly assigned to two groups including the diabetic control group and the aerobic diabetic group. Rats in Animals' Laboratory of Parand Islamic Azad University in a 5-by-10-meter-wide room under controlled light conditions (12 hours of light and 12 hours of darkness, 6-evening lighting start and 6-morning darkness start) with temperature (22 ± 3°C) and moisture maintained at a range of 30 to 60. At first, the rats became acquainted with the environment for 2 weeks with the living conditions of the animal house and how to run on the treadmill. Then, after a fasting night (12 hours), nicotinamide and streptozotocin were used to induce T2DM. Initially, a solution of nicotinamide at a dose of 110 mg per kg of rat mice was injected peritoneal; after 15 minutes, the freshly prepared STZ solution in the citrate buffer with PH = 4.5 was also injected intraperitoneally. One week after diabetes induction, fasting blood glucose and glucose levels above 150 mg / dL were considered as a measure to ensure that mice were diagnosed with T2DM (1,9,10).

A training program for 12 weeks aerobic exercise of 5 sessions per week with gradual increase in speed (18-26 m / min) and time (10 to 55 minutes) in the form of running on treadmill with the aim of determining its effect on the function of beta cells and expression. The relative proportions of FOXO1 in the pancreatic tissue were compared to the control group that did not participate in the training program. About 48 hours after the last training session (10-12 hours fasting), the rats in each group were anesthetized by intraperitoneal injection of ketamine 10%, at a dose of 50 mg / kg blended with zylosin 2% at a dose of 10 mg / kg. After assuring anesthesia, the animal's chest was taken by a split surgical blade and blood samples were taken directly from the

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animal's heart. Blood samples were centrifuged at 1000 × gr for 20 minutes to isolate the serum and stored at 80°C for glucose and insulin measurement. Then the chest of the animal was split and the pancreatic tissue of the rats was sampled and after washing in a physiologic serum in a 1.8 microtiter containing RNAlater stabilization solution, immersed in a ratio of 20% and transferred to the laboratory for genetic testing. Also, beta cell function was calculated from the insertion of fasting insulin and glucose in the software (HOMA2-calculator). All statistical analyzes were performed using SPSS software version 16. The Kolmogorov Smirnov test was used to ensure the normal distribution of data. Data analysis was performed using independent T-test. *P*-value less than 0.05 were significant.

All procedures of this study were approved by the ethics committee in the research institute of physical education and sport sciences (IR.SSRC.REC.1398.001).

Results

Table 1 shows the pattern of body weight changes before and after aerobic training in the aerobic diabetic group and the diabetic control group.

• In diabetic control group, rats weight increased significantly compared to pre-test (*P*-value: 0.001).

• In the aerobic diabetic group, rats weight increased significantly compared to the pre-test (*P*-value: 0.001).

According to Table 2, aerobic exercise significantly increased the relative expression of FOXO1 in the pancreatic tissue of the aerobic diabetic group compared to the diabetic control group.

Table 2 also shows that aerobic training led to a significant decrease in fasting glucose and also a significant increase in serum insulin levels in the aerobic diabetic group compared to the diabetic control group.

Discussion

Several studies have been conducted to determine the effect of short-term and long-term training methods on blood glucose and insulin levels in T2DM patients, although conflicting findings are observed. In the present study, 12 weeks of aerobic training in 5 sessions per week resulted in a significant decrease in fasting glucose and a significant increase in serum insulin levels in T2DM rats compared to those who did not attend the training program. This is while most studies have been supporting fasting glucose decrease in response to long-term exercise training (2,11,12).

Hence, it seems that if any internal or external intervention can somehow increase the function of beta cells, it will lead to an increase in the synthesis and secretion of insulin from these cells, which, along with the reduction of insulin resistance or increased insulin sensitivity to reduced levels glucose in the blood leads to these patients (13-15). Some transcription factors such as FTO, MAFA, PPARY, GLUT4, IRS-1 and FOXO1 play a central role in signaling pathways and insulin function in target tissues. Also, we could mention to genes such as TCF7L2, MTOR-B,

Table 1. Body weight changes (g) in pre and post training conditions

Groups	Before training Mean (±SD)	After training Mean (±SD)	*P*-value
Diabetic control	220 ± 3.34	254 ±5.96	<0.001
Aerobic diabetic	225 ± 2.61	241 ±2.24	<0.001

Table 2. Relative expression of FOXO1, serum insulin levels and fasting glucose levels in aerobic and control diabetic groups

Variable	Diabetic control group Mean (±SD)	Aerobic diabetic group Mean (±SD)	*P*-value
Relative expression of FOXO1	1	2.58±1.66	0.027*
Insulin (µIU / ml)	4.06± 0.21	5.11±0.25	0.000*
Glucose (mg / dL)	294± 11	240±14	0.000*

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PDX-1, PGC-1α and GLUT-2, which synthesize and secrete insulin from beta cells (16-18). Among them, FOXO1 is one of the genes discussed in the study, which plays an important role in the processes of transcription and insulin synthesis in beta cells of the pancreas. In this regard, although studies on the effect of various training methods on the genes involved in the synthesis and secretion of insulin from the pancreatic beta cells are limited, few reports have been made on changes in the expression and protein of genetic components at the target tissue levels. so that In a study of a training session, endurance training resulted in a significant increase in protein and expression of FOXO1 in skeletal muscle of mice, while long-term endurance exercises led to a significant reduction in FOXO1 expression (19). This researchers pointed out that changes in protein levels and expression of FOXO1 were associated with changes in blood glucose levels and insulin resistance in the studied mice. This researchers also introduced FOXO1 as one of the most important FOXO isoforms in insulin-sensitive tissues such as liver, muscle, and fatty tissue, which is inversely regulated by stimulation of insulin. Insulin signal damage to FOXO1 is associated with a series of important reactions which has abnormalities associated with type 2 diabetes (20). It is hypothesized that a change in the expression of these transcription factors in response to ongoing exercise training will improve the components of diabetes determination such as glucose levels and synthesis or serum insulin levels. Some studies have reported the ability of FOXO-1 gene expression in other body tissues, such as skeletal muscle or fatty tissue with exercise training. For example, a study aimed at determining the immediate response and compatibility of FOXO1 expression in the external muscle to a period of exercise in rats. The findings revealed that both immediate and longitude extroverted exercises (9 weeks) in the form of running on treadmill with negative slope leads to a significant change in the expression of FOXO1 in the vastus lateralis of the experimental rats, with the difference that immediate training increased FOXO1 expression by 3.62 and long-term exercises reduce the expression of FOXO1 by 0.56% compared to the control group for 9 weeks (21).

Also, the effect of endurance exercise on the expression of FOXO1 gene and protein expression in the vastus lateralis of the 9-week-old mice was measured which measured the effect of immediate training on FOXO1 expression in 9-week-old mice. Each training session lasted 60 minutes at a speed of 25 meters per minute. The findings showed that one training session and 2 hours of recovery immediately increased FOXO1expression. FOXO1 protein levels increased significantly only 2 hours after the test (22).Despite the lack of direct evidence of the role of aerobic exercises on the expression of FOXO1 in the pancreatic tissue of diabetic rats, the findings of the present study support the hypothesis that this type of exercise leads to an increase in its expression in pancreatic tissue at the same time as the increase in insulin levels in the serum.

Based on these findings, it seems that increased serum insulin in response to aerobic intervention in this study also has some roots in the expression of FOXO1 expression. FOXO1 plays a central role in processes that are dependent on the synthesis and secretion of insulin from beta cells. (23).

Conclusions

The induction of T2DM leads to a reduction in the expression of FOXO1 gene in the tissues of the pancreas in experimental rats, which is associated with a decrease in serum levels of insulin and an increase in blood glucose levels. On the other hand, 12 weeks of aerobic training of 5 sessions per week leads to a significant increase in the expression of FOXO1 gene, with decreasing glucose and increased serum insulin in the pancreatic tissue of diabetic rats. Based on the available evidence, an increase in insulin levels can be

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attributed to a significant increase in the expression of FOXO1 genes in pancreatic beta cells. The increased expression of these genes is associated with increased synthesis and release of insulin from these cells.

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Conflict of Interest
There is no conflict of interest.

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