doi:10.22100/ijhs.v10i2.1111
Original Article

IJHS 2024;10(2):8-13
ijhs.shmu.ac.ir

IJHS
International Journal of Health Studies

The Effect of Resistance Training on Some Genes Related to Insulin Secretion in the Pancreas of Type 2 Diabetic Rats

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Received: 5 May 2024
Accepted: 20 May 2024

Abstract

Background: *Recently, the role of genetic components in the synthesis and secretion of insulin from the pancreatic islet cell has been raised many times. This study aimed to determine the effect of resistance training on serum insulin, emphasizing changes in TCF7L2 and GLUT2 expression in pancreas tissue in type 2 diabetic (T2D) rats.*

Methods: *In this study, T2D was induced by intraperitoneal injection of nicotinamide and streptozotocin to 14 male Wistar rats and divided into exercise and control groups. Resistance training lasted 10 weeks (5 times/weekly) for the exercise groups. After resistance intervention TCF7L2 and GLUT2 genes expression in pancreatic tissue, serum insulin, beta cell function, and fasting were compared between groups by independent t-test.*

Results: *Compared to the control group, resistance training resulted in a significant decrease in TCF7L2 expression (P-value=0.006) and an increase in GLUT2 expression (P-value=0.016). In addition, a significant decrease in glucose (P-value=0.001) and an increase in serum insulin (P-value=0.002) as well as beta cell function (P-value=0.001) in the exercise group was observed in the control group.*

Conclusions: *Based on these findings, glucose and serum insulin improvement may be attributed to the increase and decrease of GLUT2 and TCF7L2 expression in pancreatic tissue in response to resistance training.*

Keywords: Resistance exercise, Gene expression, Type 2 diabetes, Insulin.
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Please cite this paper as: Eizadi M, Ghofrani MH, Farazandeh M. The Effect of Resistance Training on Some Genes Related to Insulin Secretion in the Pancreas of Type 2 Diabetic Rats. Int J Health Stud 2024;10(2):8-13.

Introduction

T2D is a multi-caused disease, one of its prominent risk factors is the prevalence of obesity[2]. There is also the question of why not all obese people become diabetic (T2D) or why some T2D patients have normal weight. In this context, some studies have revealed that some genetic components pave the way for the occurrence of T2D even in the absence of obesity[3]. So that they do not affect the body weight, but they severely change the function of beta cells and insulin secretion[4].

However, until now, the main mechanisms responsible for the defect or dysfunction of beta cells that lead to the reduction of insulin synthesis and secretion are not well understood. On the other hand, it is known that this abnormality is in response to the decrease in the mass or number of these cells or signaling pathways effective in insulin transcription in the pancreas[5]. Among transcription factors, TCF7L2 gene polymorphisms strongly affect insulin synthesis in pancreatic beta cells[6]. So increasing its expression increases the risk of T2D by 1.46 fold[7]. Although no significant difference in the expression of TCF7L2 gene in visceral fat tissue and subcutaneous fat has been reported between diabetics and non-diabetics, as well as between obese and non-obese people[8], some studies show a 5-fold increase in the expression of this gene in pancreatic cells of patients. They have reported that T2D is associated with a decrease in insulin secretion compared to healthy people[9]. Also, a strong correlation has been reported between the reduction of insulin secretion and the function of beta cells with the reduction of GLUT2 expression in the beta cells of some animal species with T2D[10]. On the other hand, some sources have pointed to a kind of inverse relationship between the protein levels or TCF7L2 expression and GLUT2[11]. Thus, increasing TCF7L2 expression in pancreatic beta cells leads to a decrease in GLUT2[11]. Meanwhile, some researchers have reported the response of some genetic or hormonal factors effective in insulin secretion to other external stimuli such as exercising with different methods[12,13], although the findings are more or less contradictory. For example, in the study of Lee et al (2015), 12 weeks of low-intensity aerobic training led to a decrease in glucose and serum leptin, as well as a significant increase in GLP-1 in adolescent boys with T2D[14]. On the other hand, in the study of Eizadi et al (2017), a decrease in TCF7L2 expression in pancreatic tissue has been reported in response to long-term interval exercise in T2D rats[15], which is one of the beneficial effects of exercise on genetic factors effective in insulin secretion.

Despite the existing research findings, there is no study on the simultaneous effect of exercise training on the expression of TCF7L2 and GLUT2 in the pancreatic tissue of T2D rats. Therefore, considering the potential role of TCF7L2 and GLUT2 expression in the synthesis and secretion of insulin from pancreatic cells, as well as the lack of a study with exercise intervention in this field, this study aimed to determine the effect of 10 weeks resistance training on their expression in pancreatic tissue as well as fasting glucose and serum insulin were measured in T2D rats.

Materials and Methods

Experimental animals: The statistical population of this experimental study consisted of male Wistar rats from the Pasteur Institute, Tehran. The research sample consisted of 16 rats with 10 weeks old (220±10 grams) which were randomly selected. The studied rats were kept in a room with dimensions of 5x10 meters (12 hours of light and 12 hours of darkness, light starts at 6 in the evening and turns off at 6 in the morning) with a temperature of (22±3 centigrade) and humidity in the range of 30 to 50 and had free access to standard food and water.

Induction of type 2 diabetes: After 2 weeks of familiarization with the laboratory environment, T2D induced by intraperitoneal injection of nicotinamide (110 mg/kg body weight) and STZ in citrate buffer with pH=4.5 (60 mg/kg body weight after an overnight fast (16). T2D was confirmed by the detection of elevated blood glucose levels on day 7 post-injection, and only animals with fasting blood glucose levels between 150-400 mg/dl were identified as diabetic[17]. Diabetic rats were randomly divided into control and resistance training groups.

Resistance training protocol: Resistance exercises according to Table 2 were performed for 10 weeks with 5 sessions per week in the form of climbing a step ladder in the form of 3 sets with 6 repetitions in each period (Table 1). The resistance was applied by attaching a weight as a percentage of the body weight to the rat's tail. The rest time between sets is 3 minutes and the rest time between repetitions in each period is 45 seconds[18]. 48 hours after the last training session, all rats were dissected.

Table 1. Resistance exercise program based on body weight percentage

Exercise session (Week)	1-2	3-4	5-6	7-8	9-10
Resistance (body weight %)	20	40	60	80	100

Tissue and blood sampling: All animals were anesthetized and dissected 48 h after the last training session (overnight fast) intraperitoneal ketamine injection (10%) along with xylosine (2%) (50 mg/kg and 10 mg/kg respectively), then they were dissected. Blood samples were derived through cardiac puncture. Then, pancreas tissue was removed and immersed in RNA later to determine GLUT2 and TCF7L2 expression. Insulin and glucose were assessed by ELISA (Demeditec, Germany) and glucose oxidase method (Pars Azmoonf kit, Tehran).

RNA extraction /Real time–PCR: For purify RNA, 20 milligrams of pancreas tissue were ground using a mortar and pestle. Then extraction was performed employing the RNeasy Protect Mini Kit (Qiagen, Germany) according to protocol (Table 2). RNA Polymerase II was used as a control gene.

Table 2. Primer sequence

Genes	Primer sequence	Product size	T m	Gene Bank
GLUT2	For: GCATGTCTGTTACCCCAGGATAG Rev: AGAGGAGTAACAAGCTCAAGGTG	159 bp	60	NM_001191052.1
TCF7L2	For: CGTCCATGGTCCCTTCCTC Rev: ACTTCAATCAAGCAGGGGCAC	164 bp	60	XM_008759265.1
RNA PolymraseII	For: ACTTTGATGACGTGGAGGAGGAC Rev: GTTGGCCTGCGGTCGTTC	164 bp	60	XM_008759265.1

Statistical analysis: Data comparison was done using an independent t-test in the SPSS/Win version 22 software environment. The Kolmogorov-Smirnov test was used to ensure the normal distribution of the data. The significant level was set at 0.05.

Results

Pre and post-training of body weight for 2 groups are shown in Table 3. Independent t-test revealed no statistically significant difference in body weight between the groups at pre-training. Conversely, after the training period, a statistically significant difference in body weight was observed between the two groups. So, the body weight in the exercise group is significantly higher than the control group.

Table 3. Pre and post-training of body weight of 2 groups (Mean±SD)

Group	Pre-training	Post-training	P-value
Control	221±4	256±7	0.205
Resistance	223±2	276±4	0.001
P-value	0.954	0.001	-----

The effect of resistance training on GLUT2 and TCF7L2 gene expression in pancreas tissue was the main aim of the study. The statistical comparison revealed that GLUT2 and TCF7L2 expression were significantly higher and lower in exercise than control group respectively. In other words, 10 weeks of resistance training resulted in a significant increase in GLUT2 expression (Fig 1) and a decrease in TCF7L2 expression (Fig 2) compared with the control group (Table 4).

Significant differences were also observed between the 2 groups concerning fasting glucose and serum insulin. On the other hand, resistance intervention resulted in a significant decrease in fasting glucose (Fig 3) and serum insulin (Fig 4) compared with control group (Table 4).

Table 4. Fasting glucose and insulin resistance after training intervention of exercise and control groups (Mean±SD)

Variable	Control group	Exercise group	P-value
Fasting glucose (mg/dl)	295±11	209±11	0.001
Serum Insulin (µIU/ml)	4.63±0.65	6.31±0.88	0.002
Beta cell function (HOMA-BF)	7.20±0.99	15.69±2.42	0.001
GLUT2 expression	1	1.39±0.36	0.016
TCF7L2 expression	1	0.74±0.21	0.006

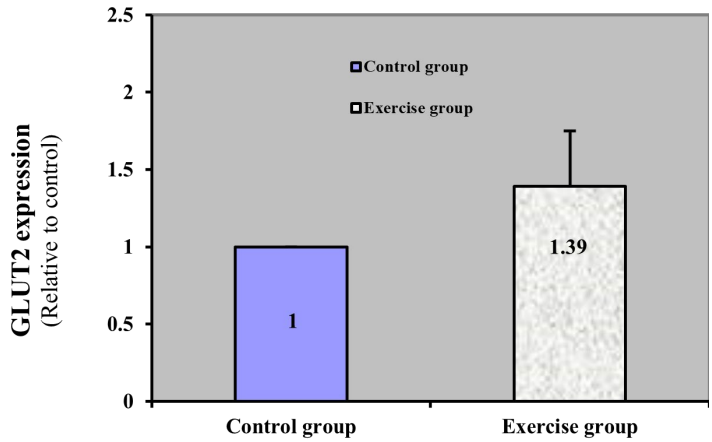

Figure 1. GLUT2 gene expression in pancreas tissue in exercise rats compare to control group

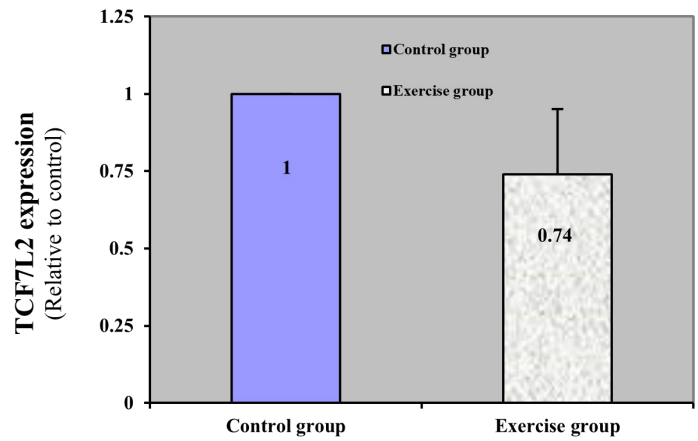

Figure 2. TCF7L2 gene expression in pancreas tissue in exercise rats compare to control group

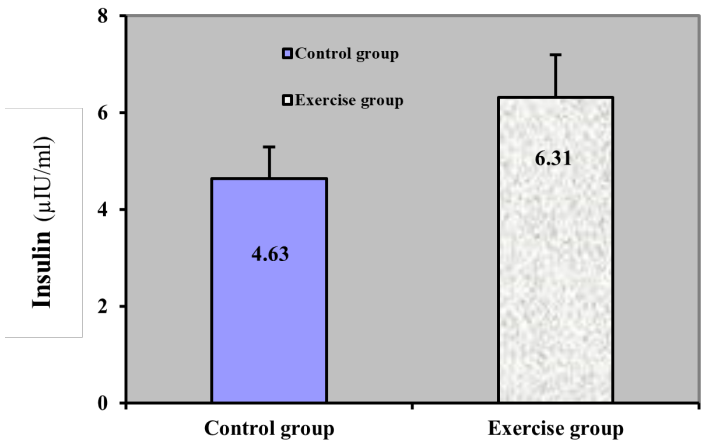

Figure 3. Fasting glucose after resistance training in studied groups

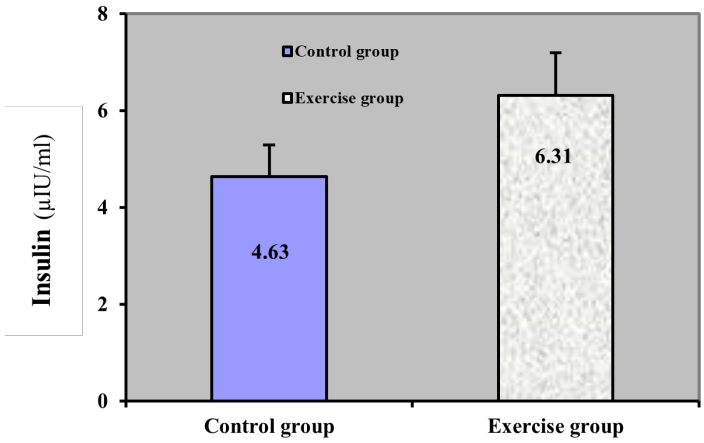

Figure 4. Serum insulin after resistance training in studied groups

Discussion

The increase in GLUT2 expression along with the decrease in TCF7L2 expression in response to resistance training are the main findings of the study. These findings, along with the increase in serum insulin and the significant decrease in fasting glucose, indicated the effectiveness of resistance training in T2D rats. The increase in serum insulin may be attributed to improved beta cell function in response to the exercise intervention. Because exercise increases the mass of beta cells through the process of hyperplasia and reduces cell death[19]. In this context, Eizadi et al (2017) have attributed the increase in serum insulin in response to exercise in T2D patients to the improvement of beta cell function[15]. On the other hand, gene linkage studies have attributed the dependence of beta cell function and insulin synthesis to effective transcription factors in insulin signaling pathways in beta cells. In other words, although the function of beta cells is affected by some reversible factors, there is enough evidence that the disorder and inability of beta cells to secrete enough insulin have a genetic basis and promote the onset of diabetes[20].

Based on this evidence, the increase in beta cell function in the present study may be attributed to the increase in GLUT2 expression and decrease in TCF7L2 expression in the pancreas following exercise intervention. In this context, Rashet et al (2021) have reported an increase in GLUT2 expression in the pancreas of diabetic rats in response to aerobic exercise[21]. Sokhanvar et al (2020) have pointed out the increase in GLUT2 expression in the pancreatic tissue of obese diabetic rats in response to aerobic exercise[22]. Simoes et al (2020) have also reported the improvement of insulin function and insulin receptors as well as the increase in GLUT2 expression in response to aerobic exercise[23]. However, no study has reported the effect of resistance training on GLUT2 expression in pancreatic tissue of T2D rats. On the other hand, although Eizadi et al, (2017) reported a decrease in TCF7L2 expression following interval exercise in T2D rats[15], these researchers

observed no change in its expression following aerobic exercise[24].

Despite these evidences, the role of GLUT2 in pancreatic cells in insulin secretion is prominent. Insulin is primarily synthesized and secreted in response to glucose, or in other words, increased glucose entry into beta cells facilitated by GLUT2[25]. This is despite the fact that other factors such as free fatty acids and amino acids lead to the acceleration of glucose-dependent insulin secretion[26]. It has been found that in the adult pancreas, PDX1 inactivates the insulin gene and other genes sensitive to glucose and is effective in glucose metabolism, such as GLUT2 and glucokinase[27]. GLUT2 is expressed as the main glucose transporter in beta cells and enables the two-way flow of glucose and other food sugars such as fructose and galactose due to its high absorption capacity. Glucose transport is the first step in glucose-dependent insulin secretion. Decreased expression of GLUT2 in human pancreatic beta cells is associated with hyperglycemia and damage to glucose-dependent insulin secretion[10]. A direct correlation between decreased glucose-dependent insulin secretion and decreased expression of GLUT2 in beta cells has been observed in some animal species with T2D[10].

Clinical studies have indicated that GLUT2 is necessary for the glucose-dependent insulin secretion process and its absence is associated with hyperglycemia. Based on the aforementioned evidence and based on the findings of the present study, the increase in serum insulin in response to resistance training can be attributed to the increase in insulin synthesis in the pancreas in response to the increase in GLUT2 expression related to resistance training. The increase in serum insulin in diabetic rats in the present study may be attributed to the decrease in TCF7L2 expression in response to resistance training. In this context, Eizadi et al (2017) have attributed the increase in serum insulin and the improvement of glucose in trained diabetic rats to the decrease in TCF7L2 expression following interval training[15]. This theory is strengthened by the fact that genetic studies have reported TCF7L2 as the strongest risk factor in T2D[6,28]. It has been mentioned that single nucleotide polymorphisms in the TCF7L2 gene have a strong relationship with the risk of T2D[6]. Thus, TCF7L2 variants are associated with damage to beta cells and the process of insulin production and secretion, damage to the conversion of proinsulin to insulin, increased insulin resistance, and hyperglycemia[29].

On the other hand, genetic studies have introduced GLUT2 as one of the target genes of TCF7L2[11]. Based on this evidence, the decrease in TCF7L2 expression following resistance training may be associated with the effect on protein levels or GLUT2 expression in beta cells. Ho et al., have reported that high concentrations of extracellular glucose increase GLUT2 endocytosis, which leads to insulin secretion in parallel with the increase in GLUT2 expression[30]. So the initial phase of glucose-dependent insulin secretion in mice lacking GLUT2 is not visible in beta cells[31]. Despite the existence of many studies, the exact mechanism that explains the effect of different training methods on insulin secretion and beta cell function is still not visible. Undoubtedly, this response is not unrelated to mechanisms responsible for cellular-molecular or genetic adaptations.

Resistance exercises are associated with the improvement of fasting glucose in T2D rats. This improvement may be attributed to the increase in serum insulin in response to this training method. On the other hand, resistance training leads to a decrease in TCF7L2 expression along with an increase in GLUT2 expression in the pancreas of diabetic rats. Based on the effective role of these transcription factors and based on the findings of the study, the improvement of serum insulin may be attributed to these genetic changes in the pancreas of diabetic rats following resistance training. Despite the mentioned evidence, understanding and knowledge of the main mechanisms responsible for changes in insulin synthesis in response to exercise training require more studies in this field.

Ethical Considerations

The study was approved by Ethics Committee of Islamic Azad University, (Ethic Code: IR.IAU.K.REC.1401.111).

Acknowledgment

The authors thank the Pasteur Institute for their cooperation in measuring gene expression and biochemical variables.

Conflict of Interest

The authors declared no conflict of interest.

Funding

This research was funded by Islamic Azad University.

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