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ORIGINAL ARTICLE

Blood Glucose Response to Resistance Training with Emphasis on the Hepatic TCF7L2 Gene Expression in Obese Diabetic Rats

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KEYWORDS

TCF7L2 gene expression;
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ABSTRACT: Enhanced glucose production by the liver contributes to hyperglycemia in individuals with diabetes. The purpose of this research was to explore how resistance training influences serum insulin levels, fasting glucose, insulin sensitivity, and TCF7L2 gene expression in the liver cells of rats with type 2 diabetes (T2D). To achieve this aim, T2D was induced in 14 male Wistar rats, aged 10 weeks, through an 8-week high-fat diet combined with streptozotocin (STZ) administration. Subsequently, these rats were randomly divided into two groups: an exercise group that underwent resistance training for 8 weeks, five times per week (n = 7), and a control group that did not receive any training (n = 7). 48 hours following the final training session, all rats underwent dissection. Measurements were taken for TCF7L2 gene expression, glucose, insulin, and insulin resistance, and these metrics were statistically analyzed using an independent t-test between two groups. The data indicated that, relative to the control group, resistance training significantly reduced glucose levels (P = 0.001) and insulin resistance (P = 0.009), while it increased serum insulin levels (P = 0.035) and TCF7L2 gene expression in hepatocytes (P = 0.011). Based on these finding, improved glucose and insulin resistance following resistance training in T2D can be attributed to enhance TCF7L2 gene expression in hepatocytes by training.

INTRODUCTION

Diabetes is a chronic metabolic disease that occurs when the pancreas cells are unable to produce enough insulin or when the sensitivity of glucose receptor cells to insulin decreases [1]. In diabetics, insulin resistance is associated with increased hepatic glucose production, which plays an important role in fasting hyperglycemia and severe postprandial hyperglycemia. liver tissue is the key organs for maintaining and balancing systemic glucose homeostasis in mammals. This entity has the ability to generate glucose through various mechanisms, including the degradation of glycogen (glycogenolysis) and the creation of glucose from non-carbohydrate sources like pyruvate, glycerol, lactate, and alanine (gluconeogenesis)[2]. But in diabetics, this process is accelerated to the extent that a large part of the blood glucose is dedicated to the liberation of glucose from the liver. Undoubtedly, the hormonal and enzymatic components effective in accelerating gluconeogenesis in diabetic people are different from healthy people, and the improvement of these disorders is associated with the reduction of hepatic glucose release. Increasing the activity of phosphatases plays an important role in the disruption of the gluconeogenesis process [3]. In the meantime, the role of enzymes involved in gluconeogenesis is highly effective and the activity of these enzymes is subject to genetic changes in protein levels or expression genes affecting them.

Among them, TCF7L2 is one of the transcription factors

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that strongly affects the enzymes involved in the gluconeogenesis cycle. Laboratory science researchers have pointed out that the reduction of TCF7L2 expression in the pancreas leads not only to the reduction of circulating glucose levels but also to the improvement of glucose tolerance [4]. In the pancreas, it affects the survival of beta cells, the secretion of insulin and the expression of incretin receptors. In small intestine, it affects the secretion of GLP-1 and GIP, and in the liver, it affects the release of glucose from gluconeogenesis. While the impact of alterations in TCF7L2 expression on hepatic gluconeogenesis remains incompletely understood, studies indicate that the observed decrease in blood glucose levels in experimental subjects can be attributed in part to diminished hepatic glucose production following the deletion of TCF7L2 in mice [5]. As in the study of Norton et al. (2011), in the rats in which the TCF7L2 gene was silenced in the liver hepatocytes, a 3- to 5-fold increase in hepatic glucose production was observed compared to control group, and this was primarily due to the increased expression of some genes. It is involved in the process of gluconeogenesis [5].

Insulin resistance is associated with increased hepatic glucose production, which plays an important role in fasting hyperglycemia and severe postprandial hyperglycemia, and due to the increased activity of some phosphatases in the process of gluconeogenesis, such as fructose 1 and 6 diphosphatase (Fbp1) as one of the key enzymes, phosphoenol pyruvate carboxykinase (PEPCK) and Glucose 6 phosphatase (G6Pase) are also key to gluconeogenesis [6, 7]. On the other hand, Oh et al. (2012) have pointed out that the expression of TCF7L2 in the liver hepatocytes of insulin-resistant or genetically defective mice, as well as mice with a high-fat diet, is significantly reduced [8]. It has been found that the silencing of TCF7L2 by increasing the expression key enzymes of gluconeogenesis Fbp1, G6Pase and PEPCK leads to the acceleration of this process and increase of hepatic glucose production. Meanwhile, TCF7L2 silencing seems to directly affect PEPCK expression [5]. Given the significant influence of TCF7L2 on the pathways of gluconeogenesis in the liver, it is hypothesized that increasing its expression in liver cells by inhibiting the enzyme involved in accelerating this process results in a decrease in the rate of gluconeogenesis and a decrease in the release of hepatic glucose into the bloodstream. In this area, some studies have reported its expression in the pancreas of diabetic rats to different exercise methods. Thus, in a study, 3 months interval training was associated with a decrease TCF7L2 expression in diabetic rats [9]. On the other hand, in a relatively recent study, no change in TCF7L2 expression in pancreatic was reported in response to aerobic exercise [10]. However, in the study of Karimi et al (2024), an interval training regimen spanning 8 weeks resulted in elevated TCF7L2 expression within liver hepatocytes, accompanied by reductions in glucose levels and insulin resistance in obese rats [11]. However, the effect of resistance training on insulin expression in hepatocytes has not been reported in diabetic patients.

Based on the inconsistency in the response of TCF7L2 to different training methods, as well as the lack of studies on its response to resistance training in liver tissue, the current research is conducted to assess the impact of resistance training on TCF7L2 expression in liver cells, alongside evaluating its effects on blood glucose concentrations and insulin resistance in type 2 diabetic rats.

MATERIALS AND METHODS

In this practical research study, forty male Wistar rats, each 10 weeks old and weighing approximately 220 ± 10 grams, were obtained from the institutional facility for animal housing. Type 2 diabetes was induced in all animals through a high-fat diet and intraperitoneal injections of streptozotocin (STZ). Subsequently, the subjects were randomly allocated into two groups: an exercise group (undergoing resistance training for 8 weeks, 5 days per week) and a control group (no training). The animals were kept under uniform conditions featuring a 12-hour light/dark cycle, temperatures of 25 ± 2°C, and humidity levels maintained between 45-55%. A one-week acclimatization period was allowed before the experiment began. The research protocol received approval from the Department of Exercise Physiology at the Islamic Azad University, Shahr-e-Qods Branch, Iran, and The study was carried out in strict compliance with the regulations established by the Committee for the

Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Induction of type 2 diabetes

Type 2 diabetes (T2D) was induced through an 8-week regimen of a high-fat diet (HFD) [12], followed by a single intraperitoneal injection of streptozotocin at a dose of 25 mg per kg, which was dissolved in citrate buffer with a pH of 4.5. It is important to highlight that the HFD was maintained until the conclusion of the study for two groups. Hyperglycemia was verified by increased blood glucose levels observed on the seventh day post-diabetes induction. Only those animals exhibiting fasting blood glucose levels ranging from 150 to 400 mg dL^{-1} were selected and categorized as T2D rats for inclusion in the research [12].

Resistance training protocol

Participants in the exercise group ascended a stepladder comprising 26 steps, each 1 meter in height with an incline of 80%, unencumbered by resistance, for six repetitions across three instructional sessions to familiarize them with the exercise protocol. Subsequently, they engaged in an eight-week resistance training, spanning five days per week [12]. In order to warm up and cool down the rats before and after the workout, they were climbed and descended the ladder 2 times without any resistance.

Each training session consisted of five sets, each comprising four repetitions, with resistance progressively augmented by attaching weights to the rats' tails. Intervals of three minutes between sets and 45 seconds between repetitions were maintained. The resistance was incrementally raised throughout the course of the training program (Table 1). Subsequent to the final exercise session, all rodents underwent dissection 48 hours later, after a fasting period of 10 to 12 hours overnight. It is important to mention that the diabetic control group of rats did not participate in the exercise regimen within this timeframe.

Table 1. Patterns of intensity distribution in resistance training cohorts as a function of body weight percentage [12].

Time (weeks)	Resistance (body weight %)
1	30
2	40
3	50
4	60
5	70
6	80
7	90
8	100

Sample collection and biochemical assays

48 hours subsequent to their final exercise session, following an over neigh fast of 10 to 12 hours, each rat was administered an intraperitoneal injection to initiate anesthesia. This injection comprised 10% ketamine at a concentration of 50 mg per kg and 2% xylosine at a concentration of 10 mg per kg. Once under anesthesia, cardiac puncture was performed to obtain blood specimens. Concurrently, hepatic tissue was excised and preserved in RNA later solution in preparation for subsequent gene expression analysis. The acquired blood samples were then processed to measure glucose levels and serum insulin concentrations. This involved centrifuging the serum for 5 minutes at 3,000 revolutions per minute, after which glucose measurements were conducted utilizing a Cobas 6000 Analyzer (Roche, Germany) [13]. Glucose levels were measured using an enzymatic colorimetric method that employs glucose oxidase technology (Pars Azmoon, Tehran). The intra-assay and inter-assay variability for this glucose measurement technique were 1.74% and 1.19%, respectively. Insulin concentrations were measured employing the ELISA method (provided by Demeditec, Germany), which exhibited an intra-assay variability of 2.6% and an inter-assay variability of 2.88%. The

Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was determined through the equation: HOMA-IR = [fasting blood glucose (mg dL^{-1}) x insulin (μIU mL^{-1})] / 22.5 [12].

Real time – PCR

RNA was isolated using the RNeasy Protect Mini Kit (QIAGEN) from liver tissue following the guidelines provided by the manufacturer [14]. Quantitative RT-PCR analysis of TCF mRNA levels was conducted on a Rotor-Gene 6000 system, employing the One Step SYBR PrimeScript RT-PCR Kit (Takara Co.) Following the guidelines provided by the manufacturer. RNA Polymerase II was utilized as the reference gene for normalization (Table 2).

Table 2. The primer sequence pattern of the studied gene

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

Statistical analysis

Statistical analyses were performed using a statistical software package (SPSS, Version 15.0, SPSS Inc., IL, USA). The distribution of data normality was evaluated using the Kolmogorov-Smirnov test. An independent t-test was employed to assess differences between groups. A p-value of ≤ 0.05 was deemed to indicate significant differences between the groups.

RESULTS

Variations in body weight across different groups prior to and following resistance training are detailed in Tables 1 through 4. The outcomes of the independent t-test indicated that the initial weights of the two groups did not differ significantly at the start of the investigation (P = 0.773). Analysis of intra-group variations in body weight using the paired t-test indicated significant increases in both groups. Conversely, the results from the independent t-test demonstrated a notable difference in body weight between the two groups at the conclusion of the study. Specifically, the final body weight in the resistance group was significantly greater than that in the control group (P = 0.004).

Table 3. Mean ± Standard Deviation of body weight before and after exercise training of 2 groups

Group	Pre-training	Post-training	Sig. (Paired t test)
Control	333 ± 12	378 ± 14	0.001
Exercise	331 ± 12	401 ± 9	0.001
Sig (independent t test)	0.773	0.004	-----

The primary objective of this study was to investigate the impact of resistance training on the expression of the TCF7L2 gene in liver tissue. The data analysis revealed that the expression levels of the TCF7L2 gene were substantially elevated in the group subjected to exercise compared to the control group. Specifically, eight weeks of resistance training led to a notable enhancement in TCF7L2 gene expression in comparison to the control rats (Table 4, Figure 1).

Notable disparities were observed between the two groups concerning fasting glucose, serum glucose, and insulin resistance. Conversely, resistance training led to a notable reduction in fasting glucose (Table 4, Figure 2) and insulin resistance (Table 4, Figure 3), as well as a significant rise in serum insulin when compared to the control group (Table 4, Figure 3).

Table 4. TCF7L2 gene expression and clinical markers after resistance training of exercise and control groups (Mean ± SD).

Variable	Control group	Exercise group	Sig.
Fasting glucose (mg dl^{-1})	291 ± 14	225 ± 6	0.001
Insulin resistance (HOMA-IR)	4.19 ± 0.32	3.63 ± 0.35	0.009
Serum insulin(µIU ml^{-1})	5.84 ± 48	6.51 ± 0.58	0.035
TCF7L2 gene expression	1	1.30 ± 0.27	0.011

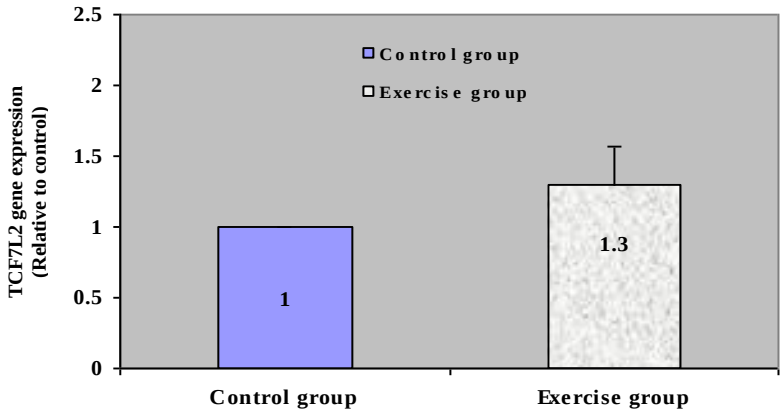

Figure 1. TCF7L2 gene expression in liver tissue in exercise rats compare to control group. Resistance training led to a decrease in hepatocytes TCF7L2 gene expressions in diabetes rats.

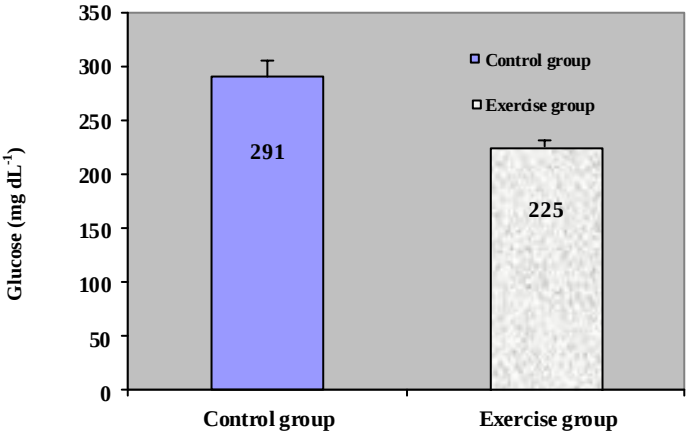

Figure 2. Blood fasting glucose after resistance training in exercise compared to control groups. Resistance training resulted in significant decrease in fasting glucose in diabetes rats.

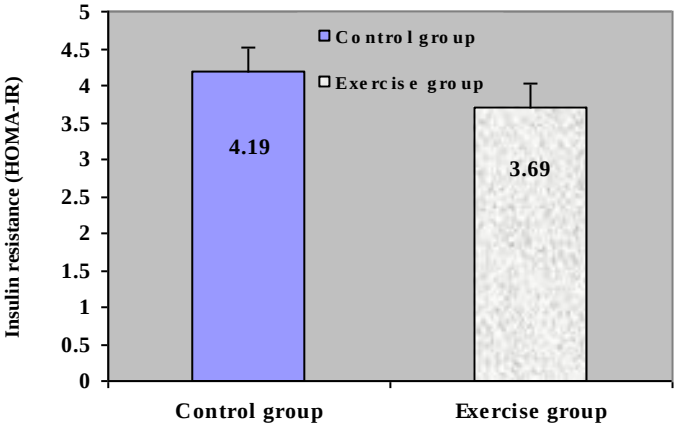

Figure 3. Insulin resistance after resistance training in exercise compared to control groups. Resistance training resulted in significant decrease in insulin resistance in diabetes rats.

DISCUSSION

Increased hepatocyte TCF7L2 expression of diabetic rats in response to resistance training is the main result of the present study. In other words, 8 weeks of resistance exercise in the form of 5 sessions per week led to an increase in TCF7L2 expression in the liver cells of T2D obese rats compared to control group. These changes were also associated with improvements in fasting glucose. Increased insulin and reducing insulin resistance are other findings of the study. Regarding the effect of resistance training on hepatocyte TCF7L2 expression, although there is no study available, there are many studies on the response or adaptation of glucose, insulin and insulin resistance to different training methods. As Eizadi et al. (2016) observed that resistance training led

to reduced fasting glucose levels and elevated serum insulin in T2D rats [15]. In the study of Karimi et al (2019), they additionally observed elevated levels of serum insulin and noted enhancements in blood glucose regulation following interval training in rats with diabete [16]. Additional research has also indicated a notable reduction in blood glucose levels and improved insulin sensitivity in diabetic rats after engaging in aerobic exercise [17].

The improvement of blood glucose along with the reduction of insulin resistance following various exercise methods has also been reported in healthy and diseased human populations. In Sheu et al.'s study (2004), 12 weeks of aerobic exercise in combination with diet led to a significant decrease in glucose with an increase in adiponectin in non-diabetic obese women [18]. In another study, 12 weeks of exercise training with three sessions of 60 minutes per week in the form of walking led to a significant decrease in fasting glucose [19]. Nevertheless, some human and animal studies have pointed out that blood glucose does not change in response to different training methods. For example, contrary to our findings, in the study of Maltais et al. (2016), 4 months of resistance training resulted in no significant alterations in glucose and insulin levels among 26 overweight senior males, despite a notable reduction in body fat mass [20].

Although several factors are effective in the response of glucose to exercise, it seems that the change in insulin function following exercise has a special place. It should be noted that in this study, apart from the decrease in fasting glucose, 8 weeks of resistance training also led to a decrease in insulin resistance. Therefore, based on scientific evidence, the improvement in glucose may be attributed to the reduction of insulin resistance. In this context, Abd El-Kader (2013) has attributed the improvement of fasting glucose and glycosylated hemoglobin following 12 weeks of moderate intensity aerobic exercise to the reduction of insulin resistance in type 2 diabetic patients [21]. In Lopes study (2016), 12 weeks of combined training (resistance + aerobic) led to a significant decrease in insulin resistance and glucose in overweight girls [22].

Apart from the fact that T2D occurs in response to a decrease in insulin secretion from the beta cells of the pancreas or dysfunction of insulin in the target tissue such as muscle tissue or fat tissue or a simultaneous defect of both, other effective factors such as increased hepatic glucose release, hyperglycemia or increased blood sugar in these patients. The elevation of glucose synthesis from non-carbohydrate sources during hepatic gluconeogenesis, coupled with the hastened glycolysis process, results in heightened glucose release from the liver, particularly in individuals with diabetes. Recently, researches have been conducted with the aim of inhibiting the liver processes that lead to the release of hepatic glucose. Meanwhile, the role of sports training is always discussed. In this context, while research into the mechanisms governing hepatic glucose release is relatively sparse, our findings indicate that TCF7L2 expression in liver tissue responds to resistance training. The scarcity of research documenting the impact of exercise training on TCF7L2 gene expression in the liver cells of either healthy or diseased rats represents a limitation of this study.

If we want to mention the responsiveness of TCF7L2 to exercise, in a relatively recent study on diabetic rats, researchers have pointed out the decrease in TCF7L2 expression in response to intermittent exercise [10]. On the other hand, in another study, the lack of effect of aerobic exercises on TCF7L2 expression in the pancreas of diabetic rats has been reported [20]. These findings highlight a gap in research concerning liver cells, while also illustrating the inconsistent responses of TCF7L2 to various exercise regimes in the pancreas. Given this context, it is challenging to make definitive statements about how TCF7L2 reacts to exercise training in liver cells. Apart from the effect of exercise training on TCF7L2 expression in liver cells, the findings of the present study indicate an increase in serum insulin levels as well as a decrease in fasting glucose levels in response to resistance training compared to the control group in the present study. Based on these results as well as other genetic studies on the effective role of TCF7L2 on the liver gluconeogenesis process, the reduction of glucose in rats of the resistance group may be attributed to the increased TCF7L2 expression in liver cells. In other words, increasing TCF7L2 expression in response to resistance training due to the decrease in the activity or TCF7L2 expression has led to a decrease in the synthesis

of glucose from non-sugar substances in the liver, which results in less release of hepatic glucose into the systemic circulation, especially in diabetics. Although one of the key strengths of this study is the assessment of insulin expression in hepatocytes following resistance training, it is not possible to reach a general conclusion about the response of the gluconeogenesis process to exercise based only on this measurement. Hence, the evaluation of other hormonal and genetic changes effective in this process is necessary for general conclusions.

CONCLUSIONS

Resistance training is associated to enhancements in fasting glucose levels in obese rats afflicted with type 2 diabetes. Based on the effective role of TCF7L2 in hepatic gluconeogenesis and the increase in its expression in hepatocytes following resistance training, the improvement of systemic glucose is probably rooted in the reduction of hepatic glucose release dependent on the inhibition of the gluconeogenesis process in response to the decrease in TCF7L2 expression.

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ETHICAL CONSIDERATION

The research protocol received approval from the Department of Exercise Physiology at the Islamic Azad University, Shahr-e-Qods Branch, Tehran, Iran. (Code: IR.IAU.QODS.R.1402.08.09)

Conflict of interest

The authors have disclosed no conflicts of interest.

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