The Effect of Resistance Training on G6Pase Gene Expression in Liver Hepatocytes, Glucose and Insulin Resistance Levels in Type 2 Diabetic Rats

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Abstract

Objective: The aim of this study was to determine the effect of 12 weeks resistance training on G6Pase expression in liver cells, as well as glucose and insulin levels in type 2 diabetic rats.

Materials and Methods: In this experimental study, 16 wistar rats were selected as the research sample. After injection of nicotinamide and streptozocin to induce diabetes, the rats were randomly divided into two groups of resistance training and control. The resistance group participated in a course of resistance training for up to 12 week in five sessions per week, with intensity of 75% and a time of 30 to 45 minutes. Finally, 48 hours after the last exercise session, G6Pase expression in liver cells, as well as glucose and insulin levels were measured in both groups.

Results: Comparison of resistance and control training groups showed a decrease in glucose levels (*P*-value= 0.001) and increased insulin levels (*P*-value= 0.001). Exercise also reduced the expression of G6Pase in liver cells in the resistance training group (*P-value=* 0.001).

Conclusion: Based on the results of the study, it is recommended that diabetics use resistance training under the supervision of a specialist to reduce the negative effects of diabetes.

Keywords: Resistance training, Type 2 diabetes, Gluconeogenesis, G6Pase gene expression

Introduction

Type 2 diabetes is the most common endemic disease due to non-glucose intolerance, which affects the balance between reserves and insulin requirements. Several factors play a role in the development of this disease.Obesity increases the risk of developing the disease by increasing insulin resistance and increasing blood glucose levels. Also, other factors such as hormonal, genetic, metabolic and enzymatic disorders can also be effective in the development of type 2 diabetes (1). In this regard, most studies have sought to understand how hormonal or metabolic factors affect insulin function and synthesize or release it from beta cells. But less attention has been paid to glucose production processes by some body tissues (such as liver) that especially in diabetic patients, lead to

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hyperglycemia .In fact, an increase in glucose, which is mainly due to increased glucose release, is a major feature of type 2 diabetes (3,5).

The liver is one of the key mechanisms for maintaining and stabilizing the systemic glucose hemostasis in the body that is able to produce glucose by some pathways such as breaking glycogen (glycogenolysis) and the synthesis of glucose from non-carbohydrate precursors such as pyruvate, glycerol, lactate and alanine (gluconeogenesis) (4). The rate of gluconeogenesis is controlled and regulated by the activity of some enzymes such as phosphoanol pyruvate carboxy kinase (PEPCK), fructose 1 and 6 diphosphatase, and glucose 6-phosphatase (G6Pase). This indicates the key role of these enzymes in the regulation of glucose hemostasis and thus diabetes (8,5) Also, the genetic coding of these proteins is strongly controlled by the transcription of certain key hormones, particularly insulin, glucagon, adrenaline (epinephrine), and glucocorticoids. (5,10).

Given the negative impact of diabetes on individual and social life, researchers are always looking for ways to minimize, prevent and treat diabetes. In this regard, various methods such as medication and various sports exercises have been used and contradictory conclusions have been obtained.

So far, several studies have been conducted to prevent, improve or reduce the severity of diabetes in people with this disease. In the meantime, most metabolic or hormonal components have been studied to maintain equilibrium at systemic levels and as a consequence of the effect on insulin function, depending on the type of population, and have yielded different results. The findings of most studies in this area, which have been conducted with sports interventions, are two-way and heterogeneous, and there is still no general consensus on this issue (8,9). Some findings have reported the role of regular exercise in increasing adiponectin levels (10), and decreasing fasting glucose, HbA1c (11 & 12) and CRP (13) in type 2 diabetic patients. Some others have mentioned the lack of effect of sports activities on these components (14-16).

So far, there have been few studies on the expression of effective enzymes in gluconeogenesis or glycolysis processes in liver hepatocytes in type 2 diabetic patients. But the focus was more on sports intervention studies on other tissues in the body and relation to the effect of other sports exercises such as aerobics and HIIT. (17).

Given the above and the negative role of diabetes in society on the one hand, the importance of liver and G6Pase enzyme in glucose metabolism on the other hand, and the lack of research on the impact of resistance training on research variables, the researcher seeks to answer the question of whether 12 weeks Does endurance training affect the expression of the G6Pase gene in liver hepatocytes, glucose levels, and beta cell function in type 2 diabetic rats?

It is hoped that by conducting such research, a clear view of the impact of resistance training on research variables will be available to researchers, physicians, and diabetics so that they can use it to properly reduce the negative effects of diabetes.

Materials and Methods

The present study was experimental. For do this research 16 male wistar rats (weighing 220 ± 220 gr and 10 weeks old) obtained from Laboratory Animal Breeding and Duplication Center of Tehran Pasteur Institute. The protocol implemented in this study was approved by the Ethics Committee of Physical Education Research Institute From the Institute of Physical Education in 2018. (Trace Code: 69863). All rats were put up in triple cages made of Plexiglas with a lid (Dimensions 25 * 27 * 43 cm) for 2 weeks at the Animal Lab and under controlled light conditions (12 hours of light and 12 hours of darkness, Start lighting at 18 o'clock until 6 Am).The temperature was maintained at 22 ± 3 ° C and the moisture at a range of 30 to 60 ° C. While all rats freely had access to standard

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food and water, the Helsinki Statement on Animal Laboratory was respected. Then type 2 diabetes was induced by intra-peritoneal injection using nicotinamide injection at a dose of 110 mg / kg body weight and streptozotocin at a dose of 60 mg / kg in diabetic groups. One week after diabetes induction, fasting blood glucose and glucose levels between 150 and 400 mg / dL were considered as a measure to ensure that mice were diagnosed with type 2 diabetes (18). Before applying the training program to homogenize the two training and the control groups, measurement was done using a weighing scale for Japanese animals (accurately 0.01 g). The training program used in this study included resistance training. In the resistance training group, 8 male Wistar 10-week-old diabetic rats participated in the training sessions for 12 weeks in 5 sessions per week in 3 courses with 6 repetitions per period. The Rest intervals between the courses was 3 minutes and the Rest intervals between repetitions in each period was 45 seconds.

The training program was as follows:

- In the first week, repetitions were performed with 10% of body weight.
- In the second and third weeks, repetitions were performed with 20% of body weight.
- In the fourth and fifth weeks, repetitions were performed with 40% of body weight.
- In the sixth and seventh weeks, repetitions were performed with 60% of body weight.
-In the eighth and ninth weeks, repetitions were performed with 80% of body weight.
- From the tenth to the twelfth week, repetitions were performed with 100% body weight.

The control group also consisted of 8 male 10 week old male Wistar rats who were diabetic intraperitoneally injected and were not involved in any training program and were simultaneously described as resistance group. The control group also consisted of 8 male 10 week old male Wistar rats who were diabetic intraperitoneally injected and were not involved in any training program and Necropsy was done simultaneously with resistance group. 48 hours after the last training session (10-12 hours fasting), the rats were anesthetized by intraperitoneal injection of ketamine 10% and xylose 2% in each group. Then the chest of the animal was split and to ensure the least amount of hurt, the blood sample was taken directly from the animal's heart. The rat liver tissue was also sampled and washed in a physiological serum in Micro tubes (1.8ml) containing RNAlater^{TM} (RNA Stabilization reagent 50 mL) liquid with a 20% ratio and transferred to the laboratory for genetic tests. Blood samples were centrifuged with 1000 × g for 20 minutes for Serum separation and serum glucose was kept at a temperature of 80 °C. The concentration of glucose was measured by glucose oxidase enzyme colorimetric method using glucose kit of Pars Tehran Company. Internal and external test Coefficient of glucose variation were 1.74 and 1.19% respectively and the sensitivity was 5 mg/dL. The serum insulin was measured accordance with the demeditec diagnostic Insulin ELIZA standards developed by Germany. The coefficient of variation of the internal and the external insulin test were 2.6 and 2.88, respectively, and the sensitivity was 1.76. Also, in relation to the expression of the G6Pase gene, at first the Forward Primer, the same microRNA mature sequence, was originally designed for the Real Time PCR process by a geneticist, and then the order was made by the company, and was prepared after a week. In addition, the RNA-polymerz2 gene was used as control gene. To analyze the results, independent T-test was used to determine the intergroup variations. All statistical analyzes are performed using SPSS / Win version 22.

Ethical considerations

This study was approved by the ethics committee of Islamic Azad University of Tehran and registered in the Iranian Registry of Clinical Trial with registration number of IR.SSRI.REC.1398.646.

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Results

Findings in relation to gene expression showed that resistance training resulted in a significant reduction of expression of the G6Pase enzyme in the liver cells of the resistance group compared to the control group.

Resistance group and control group had a significant difference in fasting glucose levels. Resistance exercise led to a significant decrease in fasting glucose in the training group.

The findings indicated that resistance training led to a significant increase in the performance of insulin levels in the resistance group compared to the control group. These results are presented in Table 1.

Discussion

Since increased glucose production from non-carbohydrate pathways in the liver gluconeogenesis process as well as accelerated glycolysis, ultimately leads to increased glucose elimination in the liver, especially in diabetic patients (2), studies have recently been conducted to control the liver processes that release glucose in the diet. In addition, the role of sports has always been the subject. Although studies in this area have been conducted on pathways involved in the release of glucose in the liver. In addition, the role of sports has always been the subject. But the findings of the study revealed that the expression of genes involved in the liver gluconeogenesis process affects if exercise training. As a result, 12 weeks resistance training in 5 sessions per week increasing G6Pase expression in liver cells in type 2 diabetic rats than in the control group that did not participate in the training program. So far, very limited studies have been conducted in this regard. In a study Chang et al (2006) show that regular endurance exercises lead to increased insulin sensitivity in insulin resistant rats, compared to obese groups that did not participate in the exercise program. Also, expression of gene and PEPCK protein in lean rats that participated in regular endurance exercises decreased significantly compared to the lean control group (19). Marinho et al (2012) also cited their findings that long-term endurance sports exercises, independent of weight loss, improve insulin signaling pathways in the liver tissue. Also, the beneficial effects of exercise on insulin function along

with reduced gluconeogeneic expression of genes were identified. So that Long-term endurance exercises resulted in decreased expression of G6Pase (20). In addition, the balance and improvement in protein levels and the expression of genes involved in the liver gluconeogenesis severely affects blood glucose levels, apart from resistance or insulin sensitivity in target tissues such as skeletal muscle and adipose tissue. As a result, the reduction of blood glucose levels following exercise may reduce the expression of genes involved in the liver gluconeogenesis pathway. In a study by De Moura et al (2013), the effect of a long-term exercise session on protein levels and between some of the genes that are effective in the release of glucose in the elderly was studied. These findings, consistent with the results of this study, showed a significant decrease in G6Pase protein levels after exercise tests in the liver tissue (21). The contribution of the liver to blood glucose levels is so high that clinical studies have shown liver gluconeogenesis to be the most important process in balancing blood glucose levels, and has shown pathological changes in liver glucose production as the main characteristics of type 2 diabetes. As far as the pharmacological interventions of the

Table 1. Comperasion of variables in resistance and control groups

Variables	Control Group (n=8) Mean ± SE	Resistance Group (n=8) Mean ± SE	*P*-value
G6Pase expression	1	0.78± (0.19)	0.01*
glucose (mg/dl)	294± (11)	213± (18)	< 0.0001*
insulin (µIU/ml)	6.06± (0.80)	4.06± (0.21)	< 0.0001*
Insulin Resistance	3.19± (0.53)	2.95± (0.22)	> 0.0001*

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expression of key gluconeogenesis enzymes, such as PEPCK and G6Pase, are described as an effective strategy for the treatment of metabolic abnormalities associated with this disease. However, such interventions require more understanding of the molecular mechanisms involved in regulating this process. Some researchers have pointed to metabolic and hormonal pathways that are effective in liver gluconeogenesis. Glucagon and corticotroids are considered to be responsible for increasing the rate of liver gluconeogenesis due to increased expression of PEPCK and G6Pase. The PGC-1 activating protein acts as an important mediator in regulating this process. In return, Insulin reduce the expression of PEPCK and G6Pase genes by activates PI 3-kinase. Nevertheless, pathways independent of PI 3-kinase also inhibit the expression of gluconeogeneic enzymes (22). Thus, the reduction of the expression of clinical genes in response to exercise is likely to be rooted in changes in other transcriptional components. Since FOXO1 and HNF4α play an important role in stimulating the expression of G6Pase and PEPCK, sport seems to play an important role in reducing the expression of G6P and PCK by accelerating FOXO1 phosphorylation and decreasing FOXO1 level by reducing gluconeogenesis in the liver (23). Therefore, insulin signaling pathways play an important role in controlling the expression of gluconeogenic genes such as G6Pase and PEPCK that regulate the rate of liver gluconeogenesis (6). Generally, the coding or genetic coding of G6Pase and PEPCK is strongly controlled by the transcription of some key hormones such as insulin, glucagon, adrenaline (epinephrine), and glucocorticoids. To the extent that in postnutrition, insulin is the most important regulatory factor for inhibiting gluconeogenesis and producing liver glucose (5,7,24). In other words, although free fatty acids, glucocorticoids and glucagon lead to increased expression of G6Pase and PEPCK, insulin strongly inhibits the expression of these genes (25,26). Although the recognition of the exact effect of the sport protocols on these variables in animal samples seems to be difficult to measure. In one study, improvement in insulin secretion capacity from isolated islet pancreas was reported following eight weeks of swimming training (27), which was associated with a decrease in blood glucose levels in type 1 diabetic rats (28). It has also been shown that eight weeks of running on treadmill lead to increased activity of growth pathways, cellular survival in the pancreatic islets (29) and increased anaplerotic enzyme activity (30). Based on this evidence, insulin-dependent glycemic control is thought to be more relevant to type 2 diabetics that have impaired beta cell function or have insufficient levels of synthesis and secretion of insulin. Of course, these reasons are different depending on the type of exercise, the duration of exercise and the intensity of that. King et al also argued that regular exercises at the 65-85% VO2max intensity range led to an adaptation in beta cells to increase insulin secretion in response to glucose (31). It seems that insulin responses to stimuli such as glucose or exercise training in different populations, such as healthy or patient, athlete or non-athlete are different. On the other hand, glucose or insulin's response to exercise training is different. Almeida (2012) reported that multiple sessions of resistance training lead to increased insulin secretion from the pancreatic islets (32). In this regard, Eizadi et al (2017) reported an increase in serum insulin as well as a reduction in blood glucose in response to long-term HIIT exercises in type 2 diabetic rats (17). Also, Rashidi et al (2016) reported an effect of 12 weeks of aerobic exercise on increased serum insulin levels and decreased blood glucose in type 2 diabetic rats (33).

In general, The mechanism of action of different types of exercise on glucose homeostasis is similar. Resistance training increases muscle mass and strength, thereby improving insulin sensitivity and glycemic control. Also, resistance training increases glucose uptake by active muscles and

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stimulates GLUT-4 and its transfer to the cell membrane, and rapid glucose uptake increases active skeletal muscle by protein carriers (34).
In the present study, resistance training seems to stimulate glucose metabolism and thus lead to changes in blood glucose levels. Because blood sugar is affected by hepatic glycogenolysis (due to the presence of the enzyme glucose phosphatase), it can be said that the intensity and duration of the resistance training program in the present study may have caused changes in the glycogenolysis process. However, the changes do not appear to be significant enough to lead to a significant change in insulin resistance, perhaps the duration of training should be changed to see a significant change in insulin resistance. Of course, these are speculations that need further research.

Conclusions

In conclusion, according to the results of the study, it can be concluded that prolonged resistance training is effective in improving glucose and insulin levels due to the effect of genetic factors effective on glucose elimination of glucose in type 2 diabetic patients. However, further studies are needed in this area, and it is suggested that future studies compare the different training programs (interval, aerobic, and combination exercises) with the use of effective nutritional supplements at the levels Protein, and expression of transcription factors affecting glycemic profiles.

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Conflict of Interest

There are no conflicts of interest.

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