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Research Article

Relationship between erythropoietin and fasting glucose glucose after a resistance training program in male Wistar rats with type 2

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Abstract

Background: Erythropoietin is known as a strong stimulant in the activation of satellite cells and increasing the regeneration function of muscle tissue. The purpose of this study was to investigate the relationship between erythropoietin concentration and fasting blood glucose after a resistance training program in male Wistar rats with type 2 diabetes.

Materials and Methods: Twenty-four male rats aged 6 weeks were divided into 3 groups: healthy control (n=8), diabetic control (n=8) and resistance training (n=8). Resistance exercises were performed for 8 weeks, 5 sessions per week, with an intensity of 100-30% of the weight of the rats in the resistance training group. In the last week of the training program, the maximum oxygen consumption of the rats was taken using the executive protocol on the rat treadmill. 48 hours after finishing the training program, blood samples were taken from the right ventricle of heart of the rats and erythropoietin and fasting blood glucose were evaluated. The data was statistically analyzed using Pearson's correlation and one-way analysis of variance at the alpha level of less than 0.05.

Results: The results showed that there is no significant relationship between erythropoietin and fasting blood glucose among any of the groups. Also, performing 8 weeks of resistance training in diabetic rats led to an increase in erythropoietin concentration ($P \leq 0.0001$) and a decrease in blood glucose ($P \leq 0.0001$).

Conclusion: It seems that more stimulation of EPO and regeneration of muscle tissue as well as increased energy consumption in muscle tissue is one of the possible mechanisms of blood glucose reduction caused by 8 weeks of resistance training in diabetic rats.

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1. Introduction

Erythropoietin (EPO) is a cytokine hormone that primarily activates the proliferation and growth of erythroid cells and is active in several types of non-hematopoietic cells through interaction with the EPO receptor (EPO-R). The erythropoietin receptor (EPO-R) is expressed in skeletal muscle cells, and EPO may promote myoblast differentiation and survival through activation of similar signaling cascades in hematopoietic cells (1). Expression of a specific erythropoietin receptor (EPO-R) that extends beyond hematopoietic cells defines pleiotropic functions for EPO. These functions are especially involved in the protection against oxidative stress in nerve cells, in the neovascularization and angiogenesis of the uterus, and in the maintenance and repair of the myocardium (2). Skeletal muscle exhibits a remarkable ability to regenerate, a process that has been shown to be dependent on satellite cells. Skeletal muscles at rest have quiescent satellite cells, whereas in response to growth or injury, satellite cells are activated, enter the cell cycle, and induce the proliferation of myogenic progenitor cells that either fuse with existing myofibers or form new myofibers. They are distinguished. This process is tightly regulated by the expression of key transcriptional regulators such as paired box transcription factor (Pax7) protein and myogenic regulatory factors (3). Studies have shown that EPO plays an essential role in the activation of satellite cell proliferation stimuli. Hence, EPO seems to help glucose metabolism in diabetics by repairing muscle tissue. Previous studies have implicated erythropoietin (EPO) signaling in the regulation of glucose metabolism. Whether EPO can be used to treat diabetes and its underlying mechanism are still not well understood (4). Clinical research in the field of diabetes has used erythropoietin injection as a fasting glucose reducer in diabetic samples.

But the increase in blood pressure and the risks of excessive angiogenesis caused by EPO injection limit its use as a drug, and it is recommended to use natural methods such as physical activity to increase EPO secretion (5). Exercise is mentioned as a factor to control diabetes and it has been one of the ways to control and prevent diabetes for many years (6). The effect of physical activity on the reduction of insulin resistance and the activation of some signaling pathways such as phosphoinositide 3-kinase (PI3K), AKt, AMP-activated protein kinas (AMPK) and Calcium/Calmodulin Stimulated Protein Kinase (CaMK) have been mentioned as pathways involved in the metabolism of glucose (7). Most of the studies conducted regarding the relationship between exercise and diabetes have tended to aerobic exercise as a type of exercises that creates the most metabolic adaptations in muscles. However, it has been shown that resistance training can also be effective as a non-pharmacological agent in controlling type 2 diabetes (8). In addition to exposure to altitude, exercise is one of the most important erythropoietin stimulants. Therefore, the increase of erythropoietin due to exercise can be considered as one of the possible mechanisms of the effectiveness of exercise for diabetic patients. However, what is the relationship between EPO changes and fasting blood glucose concentration in diabetic patients and to what extent is this relationship affected by resistance training, it is still not clear. This relationship can be changed through the effect of EPO on skeletal muscle function, facilitating the entry of glucose into skeletal muscles and the resulting molecular-cellular adaptations. Considering the role of resistance training on EPO changes, the present study seeks to answer the question of what is the relationship between these two variables and how does this relationship change with resistance training?

2. Materials and Methods

Subjects

The sample of the present study consisted of 24 male Wistar rats weighing 230 grams and 6 weeks old, which were transferred to the laboratory after being prepared from the Pasteur Institute of Iran, and after two weeks of familiarization and adaptation to the environment, they were divided into 3 groups. Each group consisted of 8 rats: healthy control (HC), diabetic control (DC) and resistance training (RT) and then type 2 diabetes was induced into two groups of rats. The control group did not receive any intervention. The RT and the DC groups became diabetic through a high-fat diet and STZ injection. The RT group performed resistance training according to the designed protocol. During the whole period of the research, the rats were kept and controlled at a temperature of 22±2°C, humidity 45-55% and sleep-wake cycle 12:12, with the availability of food and water.

Induction of type 2 diabetes: in rats Induction of diabetes was done in 2 groups, DC and RT, using a combination method of high-fat diet and STZ injection (HFD-STZ). For this purpose, all rats were fed a diet with 59% fat, 14% protein and 27% carbohydrates for 3 weeks (9). Normal rat food contains 570 grams of carbohydrates, 20 grams of fat, and 175 grams of protein, to which 0 grams of carbohydrates, 531 grams of fat, and 125 grams of protein were added in order to reach the percentages mentioned for inducing diabetes (9). Then, at the end, 35 mg of STZ per kilogram of body weight in a citrate buffer of 0.1 mmol/L with an acidity of 4.5, intraperitoneally after 12 hours of overnight starvation at around 9 am. was injected. Seven days after the injection of STZ, a blood sample was taken from the animal's tail to measure blood glucose by a glucometer, and the samples with blood sugar more than 300 mg/dL were determined, and it was confirmed that they were diabetic.

Resistance training protocol

In the present study, resistance training was performed using a one-meter ladder along with hanging weights from the rats' tails for 5 sessions per week. Rats climbed the ladder for three repetitions without weights and without resting between repetitions to warm up. The main part of the resistance training program consisted of 3 sets with four repetitions in each set, with 30 seconds between each repetition and 3 minutes of rest between each set. Applying resistance in the form of tying weights to the rats' tails, equivalent to 30 to 100% of the rats' body weight, was performed during the eight weeks of the training period. Also, the training intensity in the training groups increased gradually every week by increasing the amount of weight in such a way that in the first week 30%, the second week 45%, the third week 60%, the fourth week 45%, the fifth week 60%, the sixth week 75%, the seventh week 90% and the eighth week 100%, Rat body weight was applied. The angle of the ladder in these exercises was 85 degrees. After the end of the training protocol, in order to evaluate the maximum oxygen consumption, samples from a rat treadmill and a 10-step test according to the evaluation method of Leandro et al. (10) were evaluated and recorded in different groups. 48 hours after the last training session, the samples were anesthetized by a combination of ketamine (30-50 mg/kg) and xylazine (5-3 mg/kg) injections in fasting state, and the soleus muscle tissue was taken for evaluation of the expression of some genes was removed. The blood sample was also taken from the right ventricle of the heart and used to evaluate the desired variables.

Statistical Methods

In the statistical analysis section, Pearson's correlation coefficient was used to determine the relationship between variables. Also, one-way analysis of variance was used to measure the difference between groups. If there was a difference between the groups, Tuky post hoc test was used. A significance level of 5% and SPSS version 21 software was used for data analysis.

3. Results

The measured variables of the current study, including the weight of the samples, aerobic capacity, plasma concentration of EPO and FBS are listed in Table 1.

Table 1: Average and standard deviation of weight, aerobic capacity, erythropoietin concentration and fasting glucose in different research groups (M±SD).

Groups / Variables	Control	Diabetic control	Resistance training
weight (g)	312.6250±2.26385	317.5000±5.92814	310.3750±3.92565
Maximum oxygen consumption (ml/kg/min)	77.8750±2.03101	55.0000±1.30931	60.2500±2.37547
Erythropoietin concentration (ng/ml)	1.0871±.10920	.5216±.06572	1.1213±.13789
Fasting blood glucose concentration (mmol/l)	4.6597±.13092	16.5347±.29538	9.7639±.27176

Determining the relationship between EPO and FBS concentration in different research groups is shown in Table 2. The results show that there is no significant relationship between these variables in any of the groups.

Table 2: The results of Pearson's correlation test in determining the relationship between EPO and FBS concentration in different research groups

Groups	Pearson's correlation coefficient	significance
Control	-.263	.530
Diabetic control	.034	.937
Resistance training	.045	.915

Analysis of research data using one-way analysis of variance (Table 3) showed that the concentration of erythropoietin and fasting glucose in different groups have significant differences. The results of Tukey's post hoc test also showed that the concentration of erythropoietin in the diabetic group was lower than the other two groups (Table 4).

Table 3: The results of the one-way analysis of variance test regarding the concentration of erythropoietin and fasting glucose in the research groups.

	sum of squares	df	mean square	F	p
Erythropoietin (EPO)	567.766	2	283.883	4778.084	.000
Fasting blood glucose concentration (FBS)	1.815	2	.907	77.210	.000

Table 4: Tukey's post hoc test results comparing EPO and FBS concentrations in different research groups (The difference of the averages and the level of significance are specified in each case)

Variables	Groups	Diabetic control	Resistance exercise
Erythropoietin (EPO)	Control	.56550* p=.000	-.03412 p=.806
	Diabetic control	---	-.59962* p=.000
Fasting blood glucose concentration (FBS)	Control	-11.87500* p=.000	-5.10417* p=.000
	Diabetic control	---	6.77083* p=.000

4. Discussion

The first finding of the present study was that there was no significant relationship between the levels of erythropoietin and blood glucose in fasting conditions of healthy and diabetic rats. Also, resistance training could not change the amount of this relationship. Since the induction of diabetes increased the fasting glucose concentration in rats up to 3 times the normal condition, this indicates that blood glucose concentration alone cannot be a stimulus for EPO secretion. It is likely that other changes are caused by the induction of type 2 diabetes, which alters the EPO response to diabetes. Previous reports have documented the effect of EPO on lowering blood glucose levels (11,12,13). Most of these studies have been conducted on patients with diabetes or insulin resistance. In the study conducted by Katz et al. (2010), injection of large amounts of EPO led to a decrease in blood glucose in dialysis rats (5). Previous studies have reported an association between EPO levels and hypoglycemia, which indicates a potential protective effect of EPO in the treatment of diabetes (14).

It was effective (4). The mentioned study showed that in terms of ultrastructure, EPO prevents the dysfunction of pancreatic β cells, improves fragmentation of mitochondria, and increases the number of secretory granules. Administration of EPO increased the activity of antioxidant enzymes such as SOD and GSH-PX and decreased the level of MDA. Furthermore, EPO increased blood selenium in diabetic rats and produced a hematopoietic effect. The decrease in blood glucose associated with exposure to high levels of EPO may be due to an increase in the number of erythrocytes and thus their glucose uptake (15). At least two adaptive mechanisms allow mice injected with large amounts of EPO to cope with this excessive erythrocytosis. The first mechanism is the very high expression of nitric oxide (NO) and the second is the reduction of the life span of red blood cells. The second mechanism keeps red blood cells young and flexible and thus prevents excessive blood viscosity (16). Therefore, it seems that the relationship between EPO concentration and blood glucose is a cause and effect relationship during which EPO leads to the reduction of blood glucose with the mentioned mechanisms.

Another finding of the research showed that type 2 diabetes led to a significant decrease in the concentration of erythropoietin in the samples of the diabetic group. The exact mechanism of erythropoietin suppression caused by the development of type 2 diabetes is not yet known, but it seems that the increase in insulin concentration due to the development of insulin resistance in samples of the diabetic group is the most important reason for this issue (Mayes, 2015). On the other hand, resistance training was able to restore the concentration of erythropoietin to the initial levels.

The short-term and long-term effects of resistance exercise on increasing basal EPO levels have been well demonstrated (17). It has also been shown that a session of resistance activity can acutely increase many angiogenic factors such as Endothelial progenitor cells (EPCs), vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1-alpha (HIF-1α) and this response was dependent on the intensity of resistance activity (17). Therefore, it seems that resistance training, like endurance and aerobic exercises, can be effective on indicators related to hematopoiesis and angiogenesis, which was also confirmed in the present study.

Another result of the present study was changes in fasting blood glucose concentration after a resistance training program, which shows a significant difference with the control group. The results showed that while the induction of type 2 diabetes increased the FBS index up to 16 mmol/L, but the 8-week resistance training program caused a significant decrease in it and its value decreased to 11 mmol/L. The effects of endurance training and high intensity interval training on glucose metabolism in diabetic patients have been well documented (18,19,20), however, these effects have been less investigated in the case of resistance training. In a comparison conducted by Strasser et al. (2013), resistance training can improve glycemic control and insulin sensitivity possibly even more than aerobic endurance training (21). It is possible that increased lean body mass after RT is an important mediator of improved glycemic control. The increase in the number of GLUT4 transporters caused by resistance training has also been specifically discussed in various studies, because GLUT4 transporter protein expression in the plasma membrane is related to fiber volume in human skeletal muscle fibers (22).

Improvement in blood sugar control depends not only on the change in muscle mass, but also on the consequences of internal changes in the muscle. Holten et al reported improved insulin action with increased protein content of GLUT4, insulin receptor, protein kinase B-α/β, and glycogen synthase after six weeks of single-leg RT, while the untrained leg remained unchanged (23). Therefore, improving blood sugar control reduces the amount of insulin needed to clear a given amount of glucose. Resistance training can improve glucose transport in normal and insulin-resistant skeletal muscle by increasing the activation of the insulin signaling cascade (21). These exercise-induced changes improve the metabolic profile of skeletal muscle and can occur independently of significant increases in skeletal muscle mass (24).

Conclusion

In Conclusion, the results of the present study showed that resistance training helps to reduce FBS in diabetic rats with HFD-STZ method. It seems that the stimulation of EPO and the renewal of muscle tissue, as well as the increase of energy consumption in muscle tissue, is one of the possible mechanisms of this issue. The general conclusion about the effect of the mentioned variables on muscle tissue in humans and the generalization of the results of this research to humans should be done with caution, but what is certain is that resistance training can be suggested as a solution to improve disease-related indicators. Type 2 diabetes in humans noted. Future studies will clarify more facts in this regard.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: F.S., Y.K., A.B., S.A.; Methodology: F.S., Y.K., A.B., S.A.; Software: Y.K., A.B., S.A.; Validation: Y.K., A.B., S.A.; Formal analysis: F.S., Y.K., S.A.; Investigation: F.S., Y.K., S.A.; Resources: F.S., Y.K., A.B., S.A.; Data curation: F.S., Y.K., A.B., S.A.; Writing - original draft: F.S., A.B., S.A.; Writing - review & editing: F.S., Y.K., A.B., S.A.; Visualization: F.S., Y.K., A.B., S.A.; Supervision: F.S., Y.K., A.B., S.A.; Project administration: F.S., Y.K.; Funding acquisition: F.S., Y.K., A.B.

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