Effect of an Aerobic Exercise Course on PI3K and AKT1 Expression and Neural Muscle Insulin Resistance in Diabetic Rats

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Abstract

Objective: The purpose of the present study was to investigate eight weeks of aerobic workout on PI3K and AKT expression as well as insulin resistance (IR) of muscle in diabetic rats by nicotinamide - streptozotocin.

Materials and Methods: This laboratory study was conducted on 14 male Wistar rats (8 to 10 weeks) with a weight range of 201 to 250 g and induction of type 2 diabetes (one week)**.** These mice were classified into 2 groups: aerobic training and control group. No exercise was given to the control group during the study, while the aerobic exercise program was run for 5 weeks a week with a gradual increase of speed (10 to 25 m / min) and time (15 to 40 minutes) in running treadmill was performed for the aerobic training group. Assay of gene expression in both study groups was by rt-PCR. For statistical analysis, the SPSS 19 software was used. The variables were compared between the two groups using one-way ANOVA.

Results: The findings showed that there was no significant difference between the two groups in terms of IR in diabetic rats (3.85 (± 0.39) vs. 5.26 (± 0.55); *P*-value= 0.345). The expression of AKT (2.37 (± 2.33) vs. 1.000 (± 0.001); *P*-value= 0.042) and PI3K (2.87 (± 2.54) vs. 1.000 (± 0.001); *P*-value= 0.028) in the neural muscle of the training group compared with control group had a significant increase.

Conclusion: It seems that performing eight weeks of aerobic exercise could be a strong stimulus for PI3K and AKT gene expression.

Keywords: Exercise, AKT, PIK3R3, Insulin resistance, Diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is a complicated metabolic disorder that influences various organs and is characterized by hyperglycemia (1). Nowadays, there is no persistent and effective treatment for this disease; therefore, the mechanisms that may display a role in the progression of diabetes and executable ways for treating this disease should be identified (2).

Protein kinase B (AKT) is a group of serine or threonine kinases that play a vital role in

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signaling pathway of phosphoinositide 3-kinase (PI3K) (3). According to studies, balance in the PI3K/ AKT signaling pathway is essential for normal metabolism, and damage to this pathway leads to increased obesity, IR, and T2DM. The IR, in turn, exacerbates damage in the PI3K/ AKT pathway and makes a destructive cycle (4).

Aerobic exercise is the best practice model for interacting with diabetics who can improve many of the side effects associated with the disease (5). Evidence suggests that the mechanism of action of aerobic exercise on glucose homeostasis and insulin function mainly depends on muscle function (6). Muscle makes up about half of your body weight and the main place of consumption is glucose (7). Muscle contraction that occurs as a result of aerobic exercise has an insulin-like role, it causes a lot of glucose to enter the cell and produce energy (7).

Given that the highest amount of glucose in the bloodstream is removed by skeletal muscle (7), and since the effects of diabetes and exercise, especially aerobic workout, on the activation of the PI3K/ AKT pathway in the muscles of diabetics have not yet been determined. Hence, this study discusses the role of PI3K/ AKT signaling in skeletal muscle, adipose tissue, liver, brain, and pancreas. It also provides evidence of factors affecting the PI3K/ AKT pathway that may be effective in treating obesity and T2DM.

Materials and Methods

This laboratory study was performed on 30 male Wistar rats (age: 10 weeks / mean weight 230 ±10 g) who were randomly divided into two groups (control, aerobic). In order to adapt the animal to the laboratory environment, rats were initially maintained in the laboratory for 2 weeks without intervention. During the first sessions and exercises in the experimental groups, we encountered attrition; Eight mice in each group died during the study. Therefore, the final sample size of 7 male rats in each group was determined. After separating the rats into diabetic and healthy groups, T2DM was induced in the intra-peritoneal injection of nicotinamide and Streptozotocin (STZ) in diabetic groups.

The group included 7 male 10-week old male Wistar rats who, after familiarizing themselves with the lab environment and inducing T2DM, participated in an aerobic training course from the 12th week. The exercise program was conducted for 8 weeks in 5 sessions per week with a gradual increase in speed (10 to 25 m / min) and time (15 to 40 minutes) in the form of running on the treadmill. All mice were described 48 hours after the last workout. The diabetic control group consists of 7 male 10-week old male Wistar rats with T2DM.

There was a reverse primer in the kit. But the Forward primer is designed with iodine. In fact, the forward primer is the same as the adult microRNA sequence, but it should be checked for the melting temperature (Tm), so that if its melting temperature is not matched to the reverse primer, changes to its structure are given. After designing the primer by a geneticist using Primer 3 software, the order was made to make the company a pioneer and was prepared after a week. In addition, the RNA-polymerz2 gene was used as control gene. Table 2 shows the pattern of primers.

RNA extraction

The RNA was extracted from the neural tissue of Rneasy protect mini kit (QIAGEN) according to the company's instructions. So, we scraped 20 milligrams of tissue from scalpel into microchips, and then the RNA was extracted using the RNeasy Protect kit in accordance with the instructions of the German manufacturer.

To extract RNA, 20 mg of crushed tissue was inserted into the microtype and The RNA was

Table 1. Aerobic exercise protocol

Week	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
Speed (m/min)	10	10	15	15	20	20	25	25
Duration (min)	15	15	20	20	20	20	40	40

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then extracted using the RNeasy Protect mini kit (QIAGEN) according to the manufacturer's instructions. To determine IGF1 mRNA, AKT1 mRNA and PI3K mRNA by RT-Real time PCR, Rotorgen 6000 system was used using the One Step SYBR TAKARA single-step kit according to the manufacturer's instructions. Thermal cycle protocol used by Rotogen device in Real time-PCR includes: 42° for 20 minutes, 95° for 2 minutes, 40 cycles at 94° for 10 seconds and was 60° for 40 seconds. RNA Polymrase II was used as a control gene to determine the expression of the studied genes. CT of the reactions was extracted and recorded by Real time-PCR software. A comparative ΔΔCT method was used to quantify the expression of mRNA.

Statistical analysis

The Kolmogorov-Smirnov test was used to ensure the normal distribution of data. Descriptive statistics were used to describe the data and draw charts. One-way ANOVA was used to evaluate the variables between diabetic groups (control, aerobic), and LSD test was used to perform complementary tests if needed. The significance level was considered as< 0.05. Statistical analyzes were performed using SPSS/ Win software version 19.

Ethical considerations

The protocol of this study was approved by the Ethics Committee of the Institute of Physical Education and Sports Sciences (IR.SSRC.REC.1398.047).

Results

Information on body weight, IR, AKT, and PI3K was reported in table 3. Comparison of body weight values in the duodenal axis of diabetic rats demonstrated a considerable increase in the endurance workout group before and after the intervention (*P*-value= 0.006). Also, at the end of the study, body weight showed a remarkable difference between the two groups (*P*-value= 0.001). There was no statistically significant difference between the IR (Fasting Insulin> 60 pmol/l) in the intervention and control groups (*P*-value= 0.345). In another word, endurance training could not significantly reduce IR in diabetic rats. This trial demonstrated that compared to the control group, AKT expression (*P*-value= 0.042) and PI3K expression (*P*-value= 0.028) were significantly increased in the duodenal muscle of diabetic rats in the intervention group. The changes of these variables were doubled in the endurance-training group (Table 3).

Discussion

Findings from this study indicated that there is

Table 2. Primer pattern used in research.

Genes	Primer sequence	Product size	T m	Gene Bank
AKT1	**For**: AGGAGGTCATCGTTGCCAAG **Rev**: GCTCACGAGACAGGTGGAAG	159 bp	60	NM_001191052.1
PI3K	**For**: ACTGAGATGGAGACACGGAAC **Rev**: GCATCCAAGGGTCCAGTTAGTG	159 bp	60	NM_001191052.1
RNA polymraseII	**For**: ACTTTGATGACGTGGAGGAGGAC **Rev**: GTTGGCCTGCGGTCGTTC	164 bp	60	NM_008759265.1

Table 3. The comparison of mean (± standard deviations) of variables

Variables	Group	Baseline	After 6 weeks	*P*-value*	*P*-value**
Weight	Endurance training	230.85 (± 2.47)	284.85 (± 10.77)	0.006	0.001
	control	230.42 (± 2.82)	292.25 (± 8.05)	0.104	
IR	workout	-	3.85 (± 0.39)	-	0.345
	control	-	5.26 (± 0.55)	-	
AKT	workout	-	2.37 (± 2.33)	-	0.042
	control	-	1.000 (± 0.000)	-	
PI3K	workout	-	2.87 (± 2.54)	-	0.028
	control	-	1.000 (± 0.001)	-	

**P*-value: within groups. ** *P*- value: between groups. *P*-value was obtained from Independent samples T-test (2-tailed).
Abbreviations: IR: Insulin Resistance; AKT: Calorie restriction; PI3K: Body Mass Index.

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a significant difference after 8 weeks of aerobic exercise activity between the control group and aerobic training in body weight changes and expression of AKT1 and PI3K1. IR due to T2DM leads to impaired function of beta cells (8). The results of many studies show that high-intensity exercise is a very important factor in improving insulin levels (9); therefore, endurance exercise can improve muscle performance, workout capacity, and ultimately prevent disease and early mortality (10).

Results of our study is in line with the results of some studies (11). Kazior et al. expressed that seven weeks of endurance-resistance training significantly increased oxygen uptake and levels of AKT (12). Chavanelle et al. demonstrated a considerable increase in the expression of AKT protein in the bipartite and fetal muscle by continuous high-intensity exercise. Another study reported that exercise activates AKT and reduces oxidative stress and thus reduces apoptosis in neurons (13). Chih-Hsueh et al. displayed that physical activity and resveratrol supplementation lead to increased activity of the PI3K / AKT signaling pathway (14).

While the results obtained from some other studies are inconsistent with the findings of our research. Sherafati Moghadam et al. in a study showed that four weeks of HIIT in neural muscle of T2DM rats did not cause a significant difference in AKT1 protein levels (15). Mascher et al. conducted a study to assess the rate of protein synthesis deficit in the early recovery period after a severe aerobic workout in the lake of dietary supplements. The findings of this study showed that stimulation of the mTOR signaling pathway had no significant effect on AKT phosphorylation (16). Shadmehri et al. stated that there was no significant difference in the 8-week endurance training in AKT1 protein content (17).

Clinical studies have shown that PI3K pathway activity is reduced in the skeletal muscle of T2DM patients (18). Exercise activates AMPK, increases insulin receptors substrate 1 and 2 (IRS1 and IRS2) and PI3K/ AKT in skeletal muscle (19); Thus, exercise increases the expression of the GLUT4 gene by activating the AMPK pathway and improves glucose uptake into muscle cells (20, 21). Reinforcement of the PI3K/ AKT pathway and BCL-2 family also prevents diabetes-induced apoptosis (22). On the other hand, due to the effect of T2DM on protein homeostasis and impaired protein synthesis, physical activity leads to improved IR, T2DM, and ultimately improved protein synthesis (23).

This study has some limitations. Small sample size, short median time, and non-consideration of calorie intake by rats are among the limitations of the present study, which is suggested to be examined in future research. In addition, the use of alternative pathways to regulate glucose metabolism can determine the intracellular defect in the insulin signal cascade and thus help in the treatment of obesity and T2DM.

Conclusions

Co-activity is a strong stimulus that can make changes in signal transduction and cellular metabolism, which varies with intensity, type, and duration of exercise. Therefore, the selection of sports activities with different conditions (severity, type, and duration) is important for biochemical and morphological adaptation in skeletal muscle.

Endurance and aerobic exercises, by making their biochemical changes in the muscles, increasing capillary density, and increasing oxidative enzymes, can improve the transport and metabolism of glucose and increase the ability of insulin binding to muscle cell receptors, and in the result is a reduction in the need for insulin. Therefore, it seems that the implementation of eight weeks of aerobic exercise can be a strong stimulant for the expression of PI3K and AKT gene in neural muscle in T2DM rats.

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Conflict of Interest
None.

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