Research in Molecular Medicine (RMM)
Volume 6, Issue no. 2, DOI 10.18502/rmm.v6i2.4539
Production and Hosting by Knowledge E

Research Article

Effects of Exercises on *IL-6* and *GLUT4* Expression in Diabetic Rats

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Abstract

Background: Diabetes mellitus is one of the most common metabolic disorders worldwide. Empirical evidence has shown exercise to be of value in ameliorating symptoms of diabetes, but the underlying molecular mechanisms involved have not been well-studied.

To evaluate the effects of continuous and interval exercises on blood glucose and insulin levels and on *IL-6* and *GLUT4* expression in diabetic rats.

Materials and Methods: Old Wistar rats with streptozotocin-induced diabetes were randomly divided into three groups; namely, the patient (non-exercise control) group, the interval exercise group, and the continuous exercise group. The blood insulin level was measured using a specific ELISA kit, whereas the blood glucose level was determined with an AutoAnalyzer apparatus. Expression of the *IL-6* and *GLUT4* genes in skeletal muscle was determined using real-time PCR. All data were analyzed using SPSS software.

Results: The expression of *IL-6* and *GLUT4* was significantly decreased in skeletal muscle of the diabetic rats ($p < 0.001$). Continuous (9.97-fold; $p = 0.000$) and interval (7.11-fold; $p = 0.000$) exercises significantly increased the expression of *IL-6* relative to that in the animals of the patient group. Furthermore, continuous (9.36-fold; $p = 0.000$) and interval (7.65-fold; $p = 0.001$) exercises also significantly increased the expression of *GLUT4* relative to that of the patient group. Both types of exercise training were associated with a significant decrease in the blood glucose and insulin levels compared with the levels in the patient group ($p < 0.0001$).

Conclusions: Diabetes is significantly associated with IL-6 and GLUT4 downregulation, but this can be reversed through continuous and interval exercises, which also help to lower the blood glucose and insulin levels significantly.

Keywords: Continuous Exercise, Diabetes, *GLUT4*, *IL-6*, Interval Exercise

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Editor-in-Chief:
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1. Introduction

Diabetes mellitus (DM), a common type of metabolic disorder, is characterized by high blood glucose levels and insulin resistance. The incidence of DM is increasing, where it has been estimated that this disease will affect more than 300 million people by 2030 [1, 2]. Type II diabetes mellitus (T2DM) is caused by a defect in pancreatic

beta-cells that leads to their failure to compensate for blood insulin resistance. Aside from chronic hyperglycemia, T2DM can be associated with several conditions, such as hypertension, inflammation, hyperlipidemia, ketoacidosis, atherosclerosis, nonalcoholic fatty liver disease, coronary artery disease, stroke, and cardiovascular disease [3, 4]. Recent investigations have indicated that multiple risk factors, such as obesity, high body mass index, blood glucose tolerance, insulin resistance, aging, genetics, nutritional habits, and a sedentary lifestyle, are involved in the pathogenesis and development of DM [5, 6]. Nevertheless, the exact cellular and molecular mechanisms of T2DM are unclear.

Glucose transporter type 4 (GLUT4) is a transmembrane protein that plays a pivotal role in glucose removal from the blood circulation and therefore serves as a key regulator of glucose homeostasis [7, 8]. It is preferentially expressed in skeletal muscle and adipose tissue [9]. Many studies have shown that the reduced expression of GLUT4 is associated with blood glucose accumulation, insulin resistance, and diabetes development [10, 11]. Several studies have indicated that in addition to GLUT4, interleukin-6 (IL-6) has a critical role in DM as well. IL-6 is a proinflammatory cytokine that is involved in various pathological conditions [12]. There are conflicting results regarding the expression of IL-6 in skeletal muscle of patients with diabetes. Previously, it was thought that increased IL-6 expression was associated with inflammation, glucose intolerance, insulin resistance, and diabetes development. IL-6 was considered as a risk factor for the development of insulin resistance and T2DM [6, 13]. Recent evidence has indicated that IL-6 is a key regulator that affects glucose homeostasis and metabolism through its action in skeletal muscle.

Given the regulatory functions of IL-6 and GLUT4 in glucose homeostasis and DM, they can be considered as therapeutic targets in treatment strategies for T2DM. Exercise training is one of the first clinical approaches for the prevention and treatment of metabolic disorders such as T2DM [14]. However, the exact cellular and molecular effects of exercises on T2DM are not clear. Given the critical roles of IL-6 and GLUT4 in glucose homeostasis and diabetes, we assumed that exercise training might exert effects through the improvement of *IL-6* and *GLUT4* gene expression. Thus, we designed this study to compare the effects of continuous and interval exercises on blood glucose and insulin levels, as well as on *IL-6* and *GLUT4* gene expression in skeletal muscle of rats with T2DM.

2. Materials and Methods

2.1. Animals and induction of diabetes

Twenty-one male Wistar rats (age: 40–45 weeks; body weight: 250–300 g) were selected from the laboratory animal research center at Islamic Azad University of Sari (Ethical approval number: NO.19.33.2018). This study was approved by the animal care and use committee of Islamic Azad University, Sari Branch. The rats were housed 3 per cage (30 × 15 × 15 cm) in a climate controlled room (ambient temperature of 22 ± 2°C, humidity of 50 ± 5, and a 12:12 light:dark cycle). To generate the animal model of

diabetes, the rats were injected intravenously with 50 mg/kg of streptozotocin (1000 mg, ZellBio Company, Ulm, Germany) [15]. Diabetes was induced in these animals within 48 h after the injection (blood glucose > 250 mg/dl). The diabetic rats were then randomly divided into 3 groups; namely, a diabetic control group (hereinafter patient group), a diabetic group subjected to continuous exercise (MIT group), and a diabetic group subjected to interval exercise (HIT group). Rats in the patient group were not subjected to exercises.

2.2. Exercise training

To diminish stress in the animals, the rats in the exercise groups were adapted to a rodent treadmill for 5 days (at a speed of 10 m/min at 0% inclination for 5 min/day) [16, 17]. The process of exercise training is described in our previous study [18]. Rats in the HIT group were trained for 3 days/week for 8 weeks. The speed and time of exercises were gradually increased by 2 m/min and 2 min, respectively, per week. The training speed eventually reached 28 m/min in the last week. A rest time of 2 min was provided between the exercise intervals. Rats in the MIT group were trained for 5 days/week for 8 weeks. The exercise was initiated for 5 min at a velocity of 15 m/min. The speed and time of training were gradually increased by 1–2 m/min and 1–2 min, respectively, per week. The exercise training speed eventually reached 28 m/min in the last week. A warm-up and a cool-down period were provided at 5 m/min during the beginning and end of the interval and continuous training exercises.

2.3. Sample collection and measurements

At 48 h after the end of exercise training, the rats were anesthetized with ketamine (30–50 mg/kg) and xylazine (3–5 mg/kg). From each rat, a skeletal muscle sample (100–150 g) was obtained and homogenized in phosphate buffer (pH 7.0) at 4°C with a homogenizer (UP100H, Hielscher Ultrasonics, Berlin, Germany). The homogenized tissue was centrifuged at 12000 rpm at 4°C for 15 min [19], and the supernatant was collected for gene expression analysis. Additionally, a blood sample was collected from the abdominal aorta for measurement of the blood insulin and glucose levels. The blood insulin level was measured using the Rat Insulin ELISA Kit (ZellBio Company), whereas the blood glucose level was determined with an AutoAnalyzer apparatus.

2.4. RNA isolation, cDNA synthesis, and real-time PCR

The RNX-Plus Kit (RN7713C, SinaClon BioScience Co., Karaj, Iran) was used to extract total RNA from the skeletal muscle samples of all rats. The NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Newington, NH, USA) was applied to estimate the quantity and quality of extracted RNA. cDNA was then synthesized from the extracted RNA using random hexamer primers (Thermo Fisher Scientific, Dreieich, Germany) and Revert Aid Reverse Transcriptase (Thermo Fisher Scientific, Germany) at 42°C for 1 h. The amplification process was performed using a Rotor Gene 6000

thermocycler (Corbett Research, Mortlake, NSW, Australia) and Real Q-PCR 29 Master Mix Kit (Ampliqon, Odense, Denmark) in 40 cycles. Each reaction contained 5 µl of master mix and 100 nM of primers. The primer sequences used are summarized in Table 1. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was applied as a reference gene, where its mRNA level was used to normalize the mRNA levels of all samples. The $2^{-\Delta Ct}$ method was applied to calculate the relative expression of the studied genes [20] according to the following formula: ΔCt = mean Ct (studied gene) - mean Ct (reference gene), where the $2^{-\Delta Ct}$ value was considered as the relative expression of the studied genes.

Table 1: Primer sequences for the studied genes.

Gene name		Sequences
IL-6	Forward	5′-ACTTTTAGGCGTGGCTGATG-3′
	Reverse	5′- TTTTGCTGCTCACTGTATTTTATTTT-3′
GLUT4	Forward	5′-TGTATTCTTACTCTACCGCAC-3′
	Reverse	5′-GCACAAAGTGACTGGATGAAC-3′
GAPDH	Forward	5′-AAGTTCAACGGCACAGTCAAGG-3′
	Reverse	5′-CATACTCAGCACCAGCATCACC-3′

2.5. Statistical analysis

The blood glucose and insulin levels are presented as the means ± standard deviations. Comparison of the means of all data (blood glucose and insulin levels, and gene expression patterns) between groups was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Data were analyzed using SPSS software (version 19, IBM, Armonk, NY, USA). A p-value of <0.05 was considered as being statistically significant.

3. Results

The mean level of blood glucose in each experimental group is shown in Fig. 1. A significant difference in the mean levels was observed between all groups ($p < 0.0001$). The continuous and interval training exercises significantly decreased the blood glucose levels (222.66 ± 12.39 and 214.17 ± 9.98 mg/dl, respectively) relative to the levels in the patient group (362.02 ± 21.99 mg/dl; $p < 0.0001$). However, there was no significant difference in the levels between the HIT and MIT groups ($p = 0.7$; Fig. 1).

There was a significant difference in the mean levels of blood insulin between the groups ($p = 0.003$; Fig. 2), being significant decreased in the MIT and HIT groups (12.39 ± 0.95 and 9.98 ± 0.71 mU/L, respectively) compared with that in the patient group (21.99 ± 1.26 mU/L; $p < 0.01$). However, there was no significant difference in the blood insulin levels between the HIT and MIT groups ($p = 0.47$; Fig. 2).

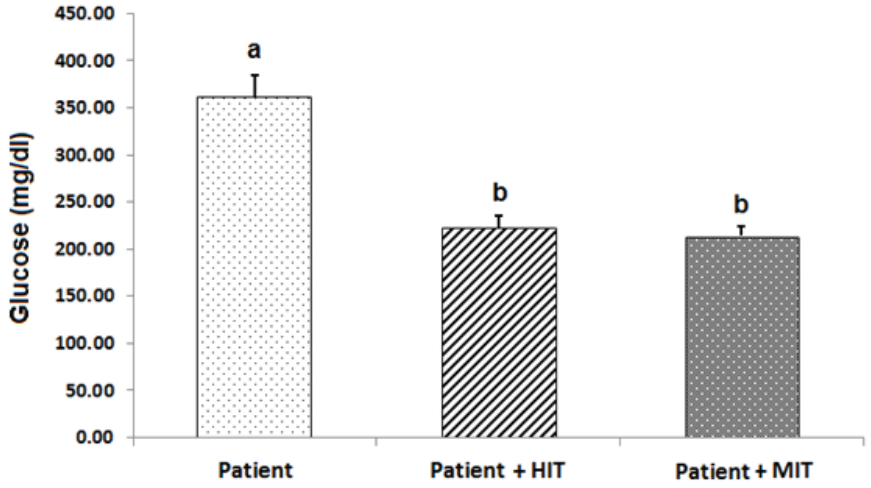

Figure 1: Comparison of the blood glucose levels between the groups. The mean blood glucose level was in the order a > b.

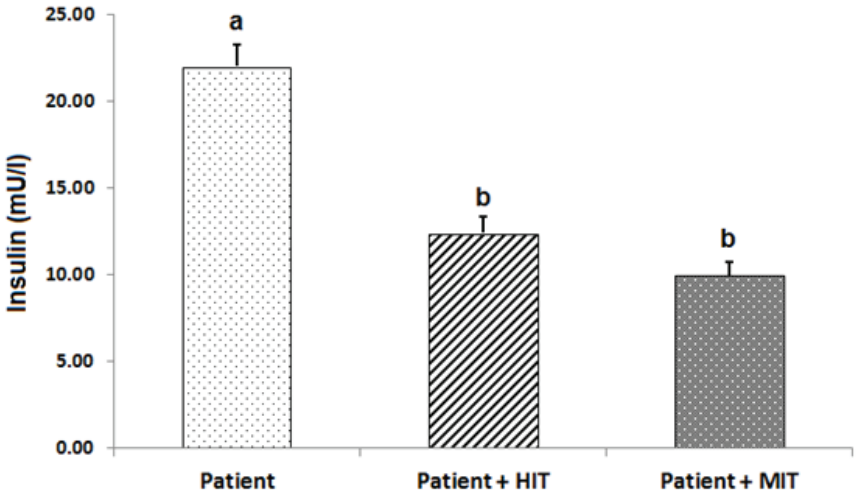

Figure 2: Comparison of the blood insulin levels between the groups. The mean blood insulin level was in the order a > b.

The comparison of the *IL-6* expression levels in all three groups is shown in Fig. 3. A significant difference was found in the expression of *IL-6* between the groups ($p < 0.001$). The fold-change ratio of *IL-6* expression in each group is summarized in Table 2, where it can be seen that the continuous and interval exercises had significantly increased the expression of this gene compared with that in the patient group (Table 2). There was no significant difference in the expression of *IL-6* between the HIT and MIT groups (Fig. 3).

The comparison of the *GLUT4* expression levels is shown in Fig. 4. The patient group had a significantly lower mean level of *GLUT4* expression than the groups subjected to aerobic exercise, as was also evident from its lower fold-change ratio (Table 3). There was no significant difference in the expression of *GLUT4* between the HIT and MIT groups (Fig. 4).

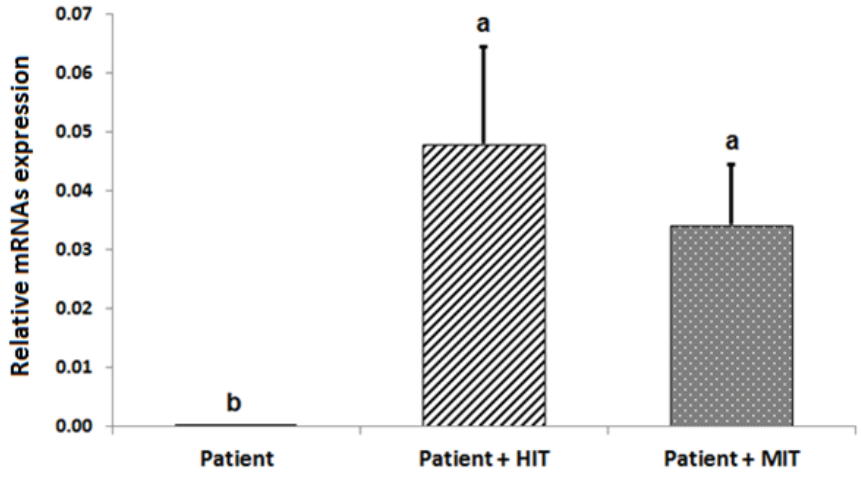

Figure 3: Comparison of the *IL-6* expression levels between the groups. Gene expression was detected by RT-PCR. There was no significant difference in the *IL-6* mRNA levels between groups with similar symbols (a-b). The mean *IL-6* mRNA level was in the order a > b.

Table 2: Fold-change ratio of *IL-6* expression between the groups.

	Fold-change ratio	Up-/downregulation	p-value
HIT vs. Patient	9.97	Upregulation	0.000
MIT vs. Patient	7.11	Upregulation	0.000
HIT vs. MIT	1.4	Upregulation	0.07

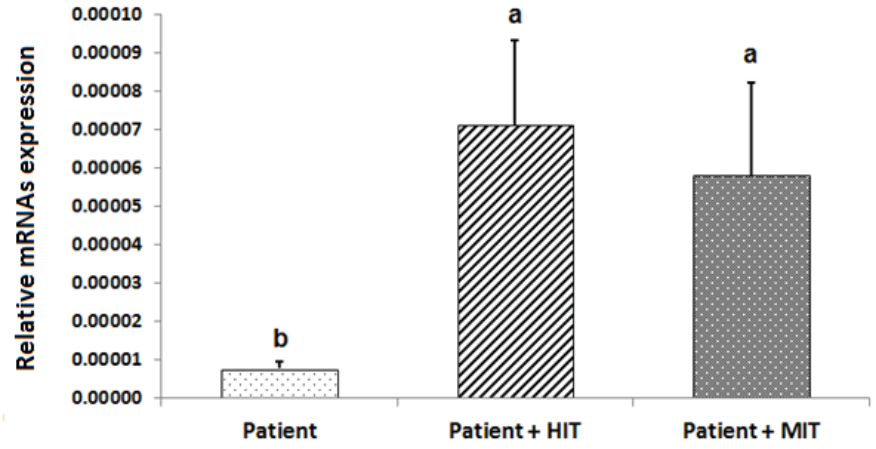

Figure 4: Comparison of the *GLUT4* expression levels between the groups. Gene expression was detected by RT-PCR. There was no significant difference in the *GLUT4* mRNA levels between groups with similar symbols (a-b). The mean *GLUT4* mRNA level was in the order a > b.

Table 3: Fold-change ratio of *GLUT4* expression between the groups.

	Fold-change ratio	Up-/downregulation	p-value
HIT vs. Patient	9.36	Upregulation	0.000
MIT vs. Patient	7.65	Upregulation	0.001
HIT vs. MIT	1.22	Upregulation	0.29

4. Discussion

In this research, we evaluated the effects of continuous and interval exercises on blood glucose and insulin levels, as well as on *IL-6* and *GLUT4* gene expression in skeletal muscle of diabetic rats. Our data showed that diabetes was significantly associated with the reduced expression of *IL-6* and *GLUT4* mRNAs. We also found that aerobic exercise significantly improved the expression of *IL-6* and *GLUT4* genes, while at the same time significantly decreasing the blood glucose and insulin levels. These data suggest the importance of aerobic exercise in the regulation of blood glucose and insulin levels as well as *IL-6* and *GLUT4* expression in skeletal muscle tissue.

IL-6 is an essential cytokine that regulates glucose homeostasis. Therefore, its reduced expression in skeletal muscle tissue seems to be a main reason for insulin resistance and diabetes development. Initially, it was thought that IL-6 had negative effects on glucose homeostasis and subsequently caused insulin resistance and diabetes. Later, it was found that IL-6 downregulation was associated with glucose intolerance, leading to the development of diabetes and obesity [21]. Some studies have demonstrated that IL-6 increases insulin secretion, possibly by increasing the activity of the phospholipase C–inositol triphosphate-dependent pathway and also by increasing glucagon-like peptide 1 secretion from L cells and pancreatic alpha cells [22, 23]. More recently, Kurauti *et al*. [24] reported that IL-6 knockout mice displayed lower insulin clearance in the liver and skeletal muscle owing to the decreased expression and activity of insulin-degrading enzyme. These findings indicate that IL-6 is a critical factor for the maintenance of normal carbohydrate and lipid metabolism and prevention of diabetes development.

We also found *GLUT4* downregulation in the skeletal muscle of diabetic rats. Many studies have demonstrated the lower expression of GLUT4 in T2DM [25, 26]. As mentioned above, GLUT4 plays a critical role in glucose homeostasis. Atkinson *et al*. [27] showed that GLUT4 overexpression improved insulin sensitivity and fasting triglyceridemia levels in rats. Therefore, these studies indicate that the decreased expression of GLUT4 is correlated with insulin resistance and diabetes.

Given the critical roles of IL-6 and GLUT4 in insulin resistance and glucose homeostasis, they can be considered as good therapeutic targets for treatment strategies against diabetes. The exercise-mediated increase of *IL-6* and *GLUT4* expression, along with the associated decrease in blood glucose and insulin levels, suggests the importance of aerobic exercises in insulin clearance and glucose homeostasis. In support of this theory, Kurauti *et al*. [24] revealed that exercise training increased the expression of IL-6 and its secretion from skeletal muscle, as well as the expression and activity of insulin-degrading enzyme. Studies have also shown that exercise training and muscle contractions increase IL-6 secretion into the bloodstream and modulate glucose homeostasis [28]. In addition to IL-6, many studies have considered the effect of different types of exercises on GLUT4 expression. For example, Kraniou *et al*. [29] showed that acute exercise increased *GLUT4* mRNA and GLUT4 protein in human skeletal muscle. In another study, Ren *et al*. [30] found that swimming exercise caused a rapid increase in GLUT4 expression in rat skeletal muscle. Similarly, Hussey *et al*. [31] demonstrated that

aerobic exercise increased GLUT4 expression in adipose tissue and skeletal muscle of human patients with T2DM.

According to previous studies and our findings, aerobic exercises, whether by continuous or interval training, can ameliorate insulin resistance and the pathogenesis of T2DM. However, the exact mechanism involved is not well understood. The exercise-mediated upregulation of IL-6 and GLUT4 in skeletal muscle is probably a significant mechanism by which exercise training increases insulin sensitivity and glucose clearance. Exercise training causes the overexpression of IL-6 and its secretion from skeletal muscle, which in turn stimulates GLUT4 expression, glucose clearance, and diabetes amelioration. A recent experimental study showed that IL-6 injection increased the GLUT4 expression in mouse skeletal muscle, which was associated with an enhancement of insulin sensitivity [32]. Therefore, our findings suggest that continuous and interval exercises decrease blood glucose levels and insulin resistance by improving the expression of *IL-6* and *GLUT4* in the skeletal muscle of diabetic subjects. However, further studies are needed to determine the exact mechanism of exercise action either at the gene or protein expression levels.

In conclusion, the results of the current study revealed that DM was significantly associated with decreased *IL-6* and *GLUT4* gene expression in skeletal muscle. Continuous and interval exercises significantly increased the expression of these genes in the skeletal muscle of diabetic rats, which was subsequently associated with decreased levels of blood glucose and insulin. Therefore, the pharmacological activation of *IL-6* and *GLUT4*, which has been implicated in the pathogenesis of DM, may be a potential therapeutic strategy for treating this disease.

Acknowledgment

This work was supported by a grant provided from the Department of Exercise Physiology, Islamic Azad University, Sari Branch, Sari, Iran. We would also like to thank the staff of the exercise physiology centers of Islamic Azad University, Sari, Iran.

Conflicts of Interest

The authors declare no conflicts of interest.

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