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Comparing the Effect of Continuous and Intermittent Exercise Training Regimens on soleus GLUT4, AMPK and Insulin Receptor in Streptozotocin-Induced Diabetic Rats

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Abstract

Background: The impact of continuous and intermittent training on diabetes mellitus condition and its mechanism is not well understood. The aim of the present study was to assess the changes in glucose uptake after 6 weeks of continuous and intermittent exercise training protocols in healthy and streptozotocin (STZ)-induced diabetic rats.

Method: Sixty male albino Wistar rats (13 weeks old) were randomly divided into six groups including healthy control, healthy continuous, healthy intermittent, diabetic control, diabetic continuous, and diabetic intermittent groups. Animals ran continuously and intermittently on treadmill for 6 weeks. They got diabetes using STZ (50 mg per kg of body weight).

Results: STZ increased blood glucose levels and insulin resistance in diabetic rats. In contrast, STZ reduced insulin, insulin receptor (IR), glucose transporter type 4 (GLUT4), and 5' adenosine monophosphate-activated protein kinase (AMPK) levels in diabetic rats. However, both continuous and intermittent exercise training protocols improved insulin resistance and prevented the reduction of GLUT4 and AMPK in diabetic rats. Neither of continuous and intermittent exercise trainings had any effect on insulin and IR receptor.

Conclusions: Continuous and intermittent exercise trainings comparably reduce blood glucose and subsequently improve insulin resistance by increasing GLUT4 and AMPK independent of insulin and its receptors.

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Introduction

Diabetes mellitus is a metabolic disorder that is widespread in the world and is associated with increased blood glucose, inadequate secretion, and dysfunction of insulin (1). Type 2 diabetes results from pancreatic beta cell destruction, leading to insulin deficiency. There is a direct relationship between chronic complications of diabetes and high levels of blood glucose (2). Blood sugar causes non-enzymatic binding of glucose to proteins inside and outside the cells (3,4).

The insulin transports glucose into the cells (5). Activation of phosphoinositid 3-kinase (PI3K) induced by binding insulin to insulin receptor (IR) causes fast and intense glucose transporter type 4 (GLUT4) translocation to plasma membrane (3,4). This leads to an increase of glucose uptake in muscle and adipose tissue. In addition, there is a growing body of evidence demonstrating that dysregulation of 5′ adenosine monophosphate-activated protein kinase (AMPK), an enzyme that plays a pivotal role in cellular energy homeostasis, in relevant tissues is a key component of the development of metabolic syndrome and type 2 diabetes mellitus (6). This protein signals to stimulate glucose uptake in skeletal muscles, fatty acid oxidation in adipose (and other) tissues, and reduces hepatic glucose production. These metabolic effects induced by AMPK are associated with lowering blood glucose levels in hyperglycemic individuals (7). There is substantial evidence suggesting that AMPK is dysregulated in animals and humans with metabolic syndrome or T2D, and AMPK activation (physiological or pharmacological) can improve insulin sensitivity and metabolic health (6,7). In turn, an increase in AMPK leads to a greater translocation of GLUT4 to the cell surface membrane and more glucose uptake (8). In addition to insulin, muscle contracture or exercise is also shown to increase glucose uptake (9). In reality, reduced insulin resistance (HOMA-IR) occurs after exercise simultaneously with accumulation of muscle glycogen stores (9).

It is believed that increased muscle glycogen stores after exercise is due to an increase in expression (9,10) and translocation of GLUT4 to plasma membrane (10). In this context, the increase in GLUT4 content has been reported in skeletal muscle of healthy and diabetic rats following 6 weeks running on treadmill at moderate intensity (5). Moreover, it has been shown that resistance exercise with 70-80% of one maximum repetition leads to 40% and 21% increases in GLUT4 and IR contents in diabetic patients' muscles, respectively (11). Similarly, improved glucose uptake and reduced HOMA-IR in obese rats following long duration of running on treadmill has been reported by others (12). In addition, it has been shown that running on treadmill and swimming for 3 weeks resulted in 47% and 44% increases in GLUT4 protein concentration in rat soleus muscle, respectively (13). Besides, running on treadmill with moderate intensity for 30 min a day, 5 days a week for 6 weeks increased the expression of GLUT4 in the cell membrane and myonuclei in skeletal muscle fibers of STZ-induced diabetic rats (5). In contrast, GLUT4 reduces in the heart and adipocytes after one week of detraining, while the same effect is seen in the gastrocnemius muscle within 2 weeks of detraining (14). Finally, evidence shows that exercise reduces HOMA-IR in diabetic rats in independent ways from GLUT4 (15). However, the actual mechanisms behind this translocation after exercise training are unclear (5).

Today, there is no doubt that improved glycemic control in diabetic patients can decrease the incidence of chronic complications of diabetes. Although the effect of aerobic exercise training on glucose uptake and HOMA-IR is obvious, the effects of two types of aerobic exercise trainings, i.e., continuous and intermittent, are not clear. Endurance training has been shown to reduce insulin resistance in type 2 diabetes mellitus patients. While these results are encouraging, individuals with moderate to severe insulin resistance are often unable to sustain prolonged periods of exercise at high intensity. Consequently, intermittent exercise has been suggested as an alternative training modality that may be

better tolerated in patients (16). Intermittent exercise is characterized by repeated short bouts of exercise separated by periods of rest and may be more suited to type 2 diabetes mellitus patients whose daily activities typically require short bursts of exertion interspersed with periods of recovery (16,17). Especially, it has been revealed that intermittent exercise may have the potential to increase carbohydrate metabolism over more traditional continuous muscle contraction and also offer a potentially more palatable form of exercise (17). The importance of this issue becomes more evident given the fact that many diabetic patients do not have enough ability to perform exercise training continuously. Thus, it is necessary to examine the effects of different kinds of exercise training on health improvement of diabetic patients. Hence, this study aims to investigate the effects of continuous and intermittent exercise training on GLUT4, AMPK, IR, insulin, HOMA-IR and finally glucose uptake in soleus muscle of diabetic rats. The purpose is to answer these questions: What is the actual mechanism of improved glucose level after exercise training in diabetic rats? And what type of exercise training is more effective?

Materials and Methods

Animals

The procedures used in the present study were in accordance with the guidelines for the use of laboratory animals ("Principles of laboratory animal care", NIH publication No. 23-86; revised 1996), and the experimental protocol was approved by the ethics committee of Shahid Chamran University of Ahvaz (EE/97.24.3.17656/sc.uac.ir). Moreover, we made an effort to minimize animal suffering and used a small number of animals to fulfill the purposes of the study. Hence, sixty male albino Wistar rats (13 weeks old, 260±41 gr) were prepared from the animal center of Kermanshah University of Medical Sciences in Iran. Animals were maintained in a temperature-controlled room (21±2◦C) with an artificial 12:12-h dark-light cycle (light on between 07:00 and 19:00). To avoid stress and physiological conditions, the samples were stored for 2 weeks under the new conditions. In the second week, the animals were trained to run on the treadmill (5 days, 10 min/day at a speed of 10 m/min). After acclimatization, 30 rats randomly got diabetes using Streptozotocin (STZ; ALX-380-010-G001, Enzo Life Sciences; Farmingdale, NY, USA) (50 mg per kg of body weight solved in citrate buffer 1.0 Molar, pH 4.5). After a week, the blood samples were taken with a small wound on the tail; blood glucose levels were measured with a glucometer (Easy Glucometer, South Korea). After disclosure of diabetes (glucose greater than 300 mg per deciliter), the animals were randomly divided into six equal groups (n=10) of control (C), sedentary diabetic (SD), healthy continuous (HC), diabetic continuous (DC), healthy intermittent (HI), and diabetic intermittent (DI).

Continuous and Intermittent Exercise Training Protocol

Exercise training was performed on a 5-lane treadmill because the intensity and duration of exercise could be controlled easily. Continuous and intermittent exercise trainings were performed on the basis of overload principle for 6 weeks, 3 sessions per week (Table 1). Warm-up and cool-down were performed at 7 m/min for 3 min at the beginning and end of the exercise training. Animals were trained through mild electrical shock stimulation to avoid approaching and resting in the end section of the device.

Table 1. The process of performing training protocols

Week	Intermittent training	Continuous training
Week 1	2 intervals, 12 m/min, 7 min	14 min,12 m/min
Week 2	2 intervals, 12 m/min, 9.5 min	19 min, 12 m/min
Week 3	2 intervals, 13 m/min, 12 min	24 min, 13 m/min
Week 4	2 intervals, 14 m/min, 14.5 min	29 min, 14 m/min
Week 5	2 intervals, 15 m/min, 12 min	36 min, 15 m/min
Week 6	2 intervals, 16 m/min, 14.5 min	43.5 min,16 m/min

Sample Collection

To avoid data misinterpretation due to the remaining effects of the last exercise session, the rats were anesthetized with ether inhalation 48 hours after the last exercise session. Five ml of blood was taken directly from the heart and was immediately centrifuged (Eppendorf Centrifuge, Germany) for 10 min at 3000 × g at 2-8 ℃. The soleus muscle of each rat was removed and washed by normal saline to remove excess surface blood. It was homogenized and kept at -80 °C for further analyses.

Biochemistry Assay

We used the commercially sandwich enzyme-linked immunosorbent assay kits (Cusabio Biotech CO., LTD. Sino-American) to measure the total protein of GLUT4, IR and AMPK in the soleus muscle. Also, insulin level was determined by commercially sandwich enzyme-linked immunosorbent assay kit (Mercodia CO., LTD. Sweden). All assays were carried out according to the manufacturers' instructions. The absorbance of these dependent variables was measured by Anthos 2020 microplate reader (Biochrom CO, England). Each sample was assayed in duplicate and tissue data were expressed as picograms per milligram protein. Insulin resistance index (HOMA-IR) was calculated according to the formula: fasting insulin (micro unit/ml) × fasting glucose (mg/dl) ÷ 405 (18).

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences, version 16. First, the Shapiro-Wilk Test was used to determine the normality of data in all groups. According to the results of this test, statistical significance was determined at $P<0.05$ using one-way analysis of variance followed by Tukey post-hoc comparison to test the differences between the groups.

Results

Injection of STZ resulted in weight loss in SD rats (229 ± 21) compared to the C group (290 ± 26 gr) (P=0.014). However, there were no significant difference between body weight of HC (304±36 gr) (P=0.946), HI (283±37 gr) (P=0.998), DC (270±25 gr) (P=0.845), DI (269±40 gr) (P=0.884), and C groups (Figure 1).

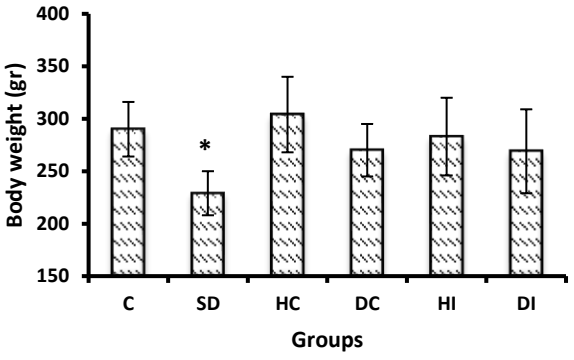

Figure 1. Body weight of healthy and STZ-induced diabetic rats after 6 weeks of continuous and intermittent exercise trainings. Abbreviations: C, Control; SD, Sedentary Diabetic; HC, Healthy Continuous, DC,

Diabetic Continuous; HI, Healthy Intermittent; DI, Diabetic Intermittent.

The asterisk (*) indicates a significant difference from C.

The results showed a significant difference in glucose levels among different groups ($F_{5,37}$=86.00, P=0.001). Glucose level in SD (327±43.12 mg/dl) was significantly higher than C group (98±15.78 mg/dl). However, there was no significant difference between glucose levels of DC (132±32.38 mg/dl) and DI (128±23.66 mg/dl) groups and HC (97±15.29 mg/dl) and HI (96±14.72 mg/dl) groups, respectively. This means that both continuous and intermittent exercise training protocols reduced glucose levels in STZ-induced diabetic rats. Also, there was no significant difference between HC an HI groups (P=0.998) (Figure 2).

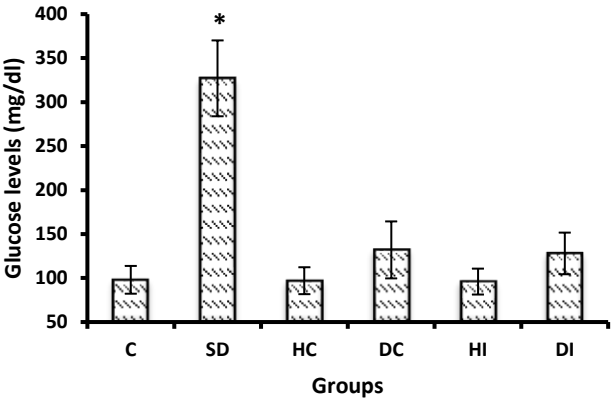

Figure 2. Comparison of glucose levels in healthy and STZ-induced diabetic rats after 6 weeks of continuous and intermittent exercise trainings. Abbreviations: C, Control; SD, Sedentary Diabetic; HC, Healthy Continuous, DC, Diabetic Continuous; HI, Healthy Intermittent; DI, Diabetic Intermittent. The asterisk (*) indicates a significant difference from C.

A significant difference was found in insulin levels in different groups ($F_{5,37}$=10.80, P=0.001). Insulin levels in SD (0.417±0.1 µIU/ml), DC (0.509±0.1 µIU/ml), and DI (0.487±0.08 µIU/ml) were significantly lower than C group (0.695±0.1 µIU/ml). Furthermore, insulin levels in DC and DI groups were significantly lower than those of HC (0.706±0.08 µIU/ml) and HI groups (0.739±0.1 µIU/ml), respectively. Also, there was no significant difference between insulin levels of HC group, HI group and C group (Figure . 3). This indicates that continuous and intermittent exercise trainings did not have any significant effect on insulin levels in STZ-induced diabetic rats.

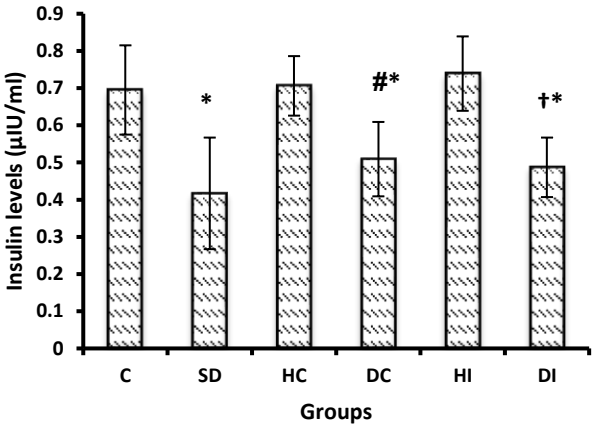

Figure 3. Comparison of serum insulin in healthy and STZ-induced diabetic rats after 6 weeks of continuous and intermittent exercise trainings. Abbreviations: C, Control; SD, Sedentary Diabetic; HC, Healthy Continuous, DC, Diabetic Continuous; HI, Healthy Intermittent; DI, Diabetic Intermittent. The asterisk (*) indicates a significant difference from C. The hash sign (#) indicates a significant difference from HC. The dagger (†) indicates a significant difference from HI.

Also, there was a significant difference in HOMA-IR levels between different groups ($F_{5,37}$=10.80, P=0.001). HOMA-IR in SD (0.334±0.12) was significantly higher than

that of C group (0.169±0.03). However, there was no significant difference in terms of HOMA-IR between DC (0.159±0.01) and HC (0.169±0.03) and between DI (0.150±0.02) and HI (0.172±0.01) groups. This means that both exercise training protocols reduced HOMA-IR level in STZ-induced diabetic rats. Finally, there was no significant difference between HC and HI groups in terms of HOMA-IR level (Figure . 4).

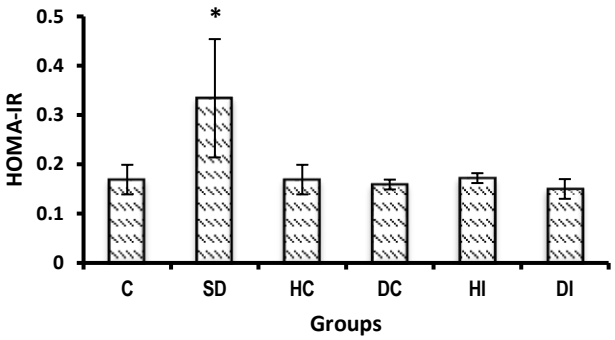

Figure 4. Comparison of HOMA-IR levels in healthy and STZ-induced diabetic rats after 6 weeks of continuous and intermittent exercise trainings. Abbreviations: C, Control; SD, Sedentary Diabetic; HC, Healthy Continuous, DC, Diabetic Continuous; HI, Healthy Intermittent; DI, Diabetic Intermittent. The asterisk (*) indicates a significant difference from C.

The results also showed a significant difference in IR levels of soleus muscle in different groups ($F_{5,37}$=9.66, P=0.001). IR levels in SD (289±54 pg/mg), DC (339±44 pg/mg), and DI (331±39 pg/mg) were significantly lower than that of the C group (443±66 pg/mg). Furthermore, IR levels in DC and DI groups were significantly lower than IR levels in HC (435±81 pg/mg) and HI groups (453±51 pg/mg), respectively. Also, there was no significant difference between HC group, HI group, and C group (Figure . 5). This means that neither the continuous nor the intermittent exercise protocols had any significant effect on IR levels in STZ-induced diabetic rats.

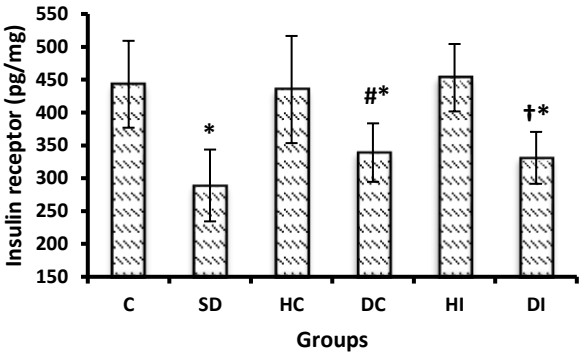

Figure 5. Comparison of IR of soleus muscle in healthy and STZ-induced diabetic rats after 6 weeks of continuous and intermittent exercise trainings. Abbreviations: C, Control; SD, Sedentary Diabetic; HC, Healthy Continuous, DC, Diabetic Continuous; HI, Healthy Intermittent; DI, Diabetic Intermittent. The asterisk (*) indicates a significant difference from C. The hash sign (#) indicates a significant difference from HC. The dagger (†) indicates a significant difference from HI.

Statistical analyses indicated a significant difference in GLUT4 levels of soleus muscle in different groups ($F_{5,37}$=20.14, P=0.001). GLUT4 levels in SD (5.82±1.74 pg/mg) were significantly lower than that of the C group (10.75±2.33 pg/mg). Although continuous (16.49±1.77 pg/mg) and intermittent (15.42±2.52 pg/mg) exercise trainings increased GLUT4 levels in healthy rats to a greater extent than in the C group, there was no significant difference between the effects of these two types of training. In addition, GLUT4 levels in HC and HI groups were significantly higher than those of DC (9.24±2.64 pg/mg) and DI groups (10.08±3 pg/mg), respectively. There was no significant difference in GLUT4 levels of DC, DI, and C groups. This means that both exercise training protocols prevent GLUT4 reduction in STZ-induced diabetic rats (Figure . 6).

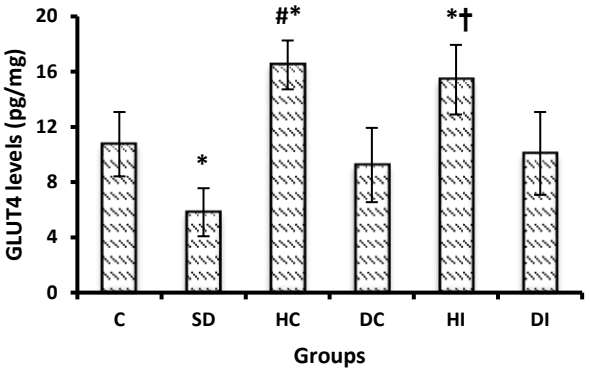

Figure 6. Comparison of GLUT4 of soleus muscle in healthy and STZ-induced diabetic rats after 6 weeks of continuous and intermittent exercise trainings. Abbreviations: C, Control; SD, Sedentary Diabetic; HC, Healthy Continuous, DC, Diabetic Continuous; HI, Healthy Intermittent; DI, Diabetic Intermittent. The asterisk (*) indicates a significant difference from C. The hash sign (#) indicates a significant difference from DC. The dagger (†) indicates a significant difference from DI.

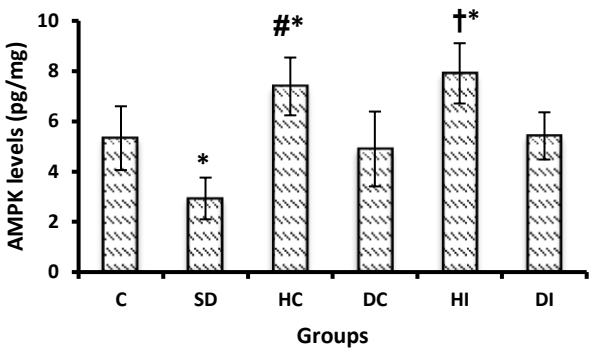

Figure 7. Comparison of AMPK levels of soleus muscle in healthy and STZ-induced diabetic rats after 6 weeks of continuous and intermittent exercise trainings. Abbreviations: C, Control; SD, Sedentary Diabetic; HC, Healthy Continuous, DC, Diabetic Continuous; HI, Healthy Intermittent; DI, Diabetic Intermittent. The asterisk (*) indicates a significant difference from C. The hash sign (#) indicates a significant difference from DC. The dagger (†) indicates a significant difference from DI.

Finally, there was a significant difference between AMPK levels of soleus muscle among different groups ($F_{5,37}$=16.2, P=0.001). It was determined that AMPK levels in SD (2.93±0.83 pg/mg) were significantly lower than that of the C group (5.33±1.27 pg/mg). Although, continuous (7.39±1.15 pg/mg) and intermittent (7.91±1.20 pg/mg) trainings increased AMPK in healthy rats compared to C group, there was no significant difference between the two types of training. In addition, AMPK levels in HC and HI groups were significantly higher than those of DC (4.90±1.49 pg/mg) and DI groups (5.42±0.94 pg/mg), respectively. There was no significant difference in AMPK levels between DC and DI groups as well as C group. This means that both exercise training protocols prevent the reduction in AMPK levels of STZ-induced diabetic rats (Figure . 7).

Discussion

In recent years, there has been growing evidence that low and high intensity aerobic (5,9, 10,19) and resistance (11,20) exercise trainings are beneficial to diabetic patients. Here, in an experimental animal model, it was revealed that both continuous and intermittent exercise trainings enhance insulin sensitivity and glucose uptake by promoting GLUT4 and AMPK independent of insulin and its receptors.

Our findings show that the body weight of rats was reduced after diabetes. However, both continuous and intermittent exercise trainings prevented it. Our results are consistent with studies that show an increase in body weight after low and high-intensity training (19), and resistance exercise training (11,20) in STZ-induced diabetic rats. Diabetes results in accelerated loss of muscle function and muscle protein synthesis, and peripheral nerve dysfunction can result in decreased muscle mass and strength (21,22). In

addition, increased inflammatory cytokines during diabetes reduces protein synthesis in muscle (19,20). Furthermore, diabetes-related disorders increase catabolic stimulus and reduce anabolic stimulus in patients (23). In contrast, exercise training or muscle contractures enhance fire and recruitment of motor unit by reducing inflammatory cytokines (19,20), and increasing anti-inflammatory cytokines (20). Moreover, it has been shown that AMPK has an important role in regulating muscle mass and regeneration (24). Therefore, part of the weight gain in trained diabetic rats may be due to an increase in AMPK. Taken together, these factors lead to increased use of muscles and enhance protein synthesis in diabetic rats (11,20).

STZ increased blood glucose levels and HOMA-IR. Conversely, it reduced insulin and IR in diabetic rats. Continuous and intermittent exercise trainings did not have any significant effect on insulin and IR in healthy and STZ-induced diabetic rats. Our finding is consistent with other studies that reported a reduction in blood glucose levels and HOMA-IR following short- (25) and long-term (26,27) endurance (25) and resistance (11) exercise trainings. Our findings are inconsistent with those of other studies that show an increase in IR in adipose tissue after high intensity interval training (24). Due to insulin and IR reductions in STZ-induced diabetic rats, increased glucose uptake in the present study was determined merely by insulin-independent glucose transport (28).

Glucose is transmitted into the cells by facilitated diffusion and GLUT4, which is distributed throughout the muscle fibers (29). In this context, we showed that both continuous and intermittent exercise training protocols increased GLTU4 in healthy and STZ-induced diabetic rats. Similarly, we observed an increase in GLUT4 after 6 weeks of resistance training (11) and running on treadmill at moderate intensity (5). In addition, it has been reported that Wistar rats submitted to swim training had improved glucose tolerance and increased total GLUT4 expression in the gastrocnemius (30) and epitrochlearis (31) muscle. These adaptations resulted in part from increased tyrosine phosphorylation of insulin receptors and its IRS1 and IRS2 substrates, as well as its association with PI3K protein (30,31). However, our findings are inconsistent with one study that showed no significant changes in muscle GLUT4 after 1 day or 1 week of treadmill exercise training in rats, suggesting that exercise load was insufficient. Nevertheless, 6 weeks of training increased GLUT4 protein content 1.4- and 1.7-fold in the soleus and red vastus lateralis in rats, respectively (32). Therefore, part of discrepancy in results may be attributed to the exercise training period. Part of increased glucose uptake and subsequently insulin sensitivity induced by exercise training may be due to increases in vesicle-associated membrane protein, which enhances translocation of GLUT4 to cell membrane (33). Although insulin can enhance GLUT4 gene expression in 3T3-F442A cells (34), insulin was reduced in the present study. Therefore, insulin does not have any effect on increased GLUT4 in the present study. In reality, it has been shown that changes in intracellular calcium and AMPK after muscle contracture increase the activation of myocyte enhancer factor 2 (MEF2), GLUT4 Enhancer Factor (GEF) (35,36) and Peroxisome proliferator-Gamma Coactivator-1α (PGC-1α) (37). In addition, an increase has been reported in PGC1 mRNA expression in the soleus of diabetic rats after chronic exercise (3). GLUT4 promoter has sets of DNA sequences that bind to MEF2 and GEF (35,36) and PGC-1α (37).

Manipulation of the AMPK pathway is an attractive approach to increase glucose uptake in muscle and subsequently improve glycemia in subjects with diabetes (38). Interestingly, we observed an increase in AMPK levels after

continuous and intermittent exercise training in healthy and STZ-induced diabetic rats and this change was associated with a clinically significant decrease in blood glucose concentrations. This finding is consistent with the report on increased phosphorylation and expression of AMPK, and decreased phosphorylation and expression of AMPK substrate, i.e., acetyl CoA carboxylase in the soleus of diabetic rats after both acute and chronic exercise training (3). In addition, trained subjects have a higher expression of α1 AMPK than untrained individuals (39). Intense endurance training of young healthy males for 3 weeks results in increases in α1 and α2 AMPK protein expression and Acetyl-CoA carboxylase β phosphorylation. The latter strongly suggests that the basal activity of AMPK was increased (40). Furthermore, cycle ergometer at 72% of maximum workload increases AMPK activity in vastus lateralis of diabetic patients (38) as well as the nuclear AMPK content (4). This suggests nuclear translocation of AMPK mediate gene and protein expression of GLUT4 after exercise training (4). In contrast, our findings are inconsistent with one study that showed short-term exercise training at moderate intensity reduces AMPK signaling during prolonged exercise independent of muscle glycogen (41). Moreover, 3 weeks of intensified exercise training did not alter basal AMPK protein expression and activity in skeletal muscle 24 h after the last bout of exercise compared with pre-training values in middle-aged and well-trained athletes (42). No significant changes were observed following 12-wk training program in human subjects on insulin sensitivity and protein content or phosphorylation of the AMPK (43). AMPK acts as a sensor and a regulator of intracellular energy metabolism. Muscle contracture induced by exercise training increases ATP consumption (38). Subsequently, ATP/AMP ratio decreases and AMPK activity increases. Later, AMPK helps to improve glucose uptake in muscles by increasing translocation and gene expression of GLUT4 (3,4). In addition, AMPK could control elevated blood glucose level in the body of diabetic patients by inhibiting hepatic glucose output and increasing muscle glucose uptake (44). These results suggest that both continuous and intermittent exercise training protocols improve glucose uptake and insulin sensitivity by increasing GLUT4 and AMPK independent of insulin and its receptors.

Conclusion

Continuous and intermittent exercise trainings comparably reduce blood glucose and subsequently improve insulin resistance by increasing GLUT4 and AMPK independent of insulin and its receptors. Although evidence is not available in support of this notion, it appears that there is no significant difference between intermittent and continuous exercise trainings in terms of glucose uptake and insulin sensitivity in diabetic patients. In fact, many diabetic patients are not able to perform exercise training continuously. These results suggest that diabetic patients can perform intermittent, rather than continuous, exercise to achieve similarly positive results.

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