

## The comparison of continuous and intermittent training impact on glucose-4 transporter protein level and insulin sensitivity in diabetic rats

Mohammad Esmail Afzalpour<sup>1\*</sup>, Mohammad Reza Yousefi<sup>2</sup>, Hossein Abtahi Eivari<sup>3</sup>, Saeed Ilbeigi<sup>1</sup>

1. Department of Physical Education and Sport Sciences, University of Birjand, Birjand, Iran
2. Department of Exercise Physiology, University of Birjand, Birjand, Iran
3. Department of Clinical Biochemistry, Gonabad University of Medical Sciences, Gonabad, Iran

\*Corresponding author: Tel: +98 56324062517 Fax: +98 5632202032

Address: Department of Physical Education and Sport Sciences, Faculty of Physical Education and Sport Sciences, University of Birjand, POB 615-97175, Birjand, Iran

E-mail: mafzalpour@birjand.ac.ir

Received: 2016/01/22 revised: 2016/02/24 accepted: 2016/05/9

### Abstract

**Introduction:** Diabetes mellitus (DM) is now seen as a worldwide epidemic disease with high prevalence. Exercise training (ET) is known to promote beneficial changes in diabetic patients. The aim of this study was to compare the impact of continuous and intermittent aerobic training on glucose-4 transporter protein (GLUT-4) levels and insulin sensitivity in diabetic rats.

**Materials and methods:** This research was an experimental and interventional study. A number of 60 male rats weighing 180 to 310 grams and 13 weeks old were divided into six groups: healthy control, healthy continuous, healthy intermittent, diabetic control, diabetic continuous, and diabetic intermittent groups. The exercise protocol in both continuous and intermittent groups was aerobic training for six weeks which was conducted considering overload principle. The data were analyzed using one-way analysis of variance test in  $P < 0.05$  level.

**Results:** The results showed that despite the significant difference between insulin resistance and GLUT4 level among diabetic rats in different groups compared to control group, there was no significant difference between the impact of continuous and intermittent training on these indices ( $P=1.00$ ).

**Conclusion:** Through increasing the GLUT4 protein content, the continuous and intermittent training improved insulin resistance.

**Keywords:** GLUT4, Insulin sensitivity, Continuous training, Intermittent training

### Introduction

The mellitus diabetes is a metabolic disorder that is widespread in the world and is associated with increase in blood glucose, inadequate secretion, and dysfunction of insulin (1). The type 2 diabetes is a result of pancreatic beta cells destruction resulting in insulin deficiency. The type 2 diabetes is diagnosed considering insulin resistance and relative reduction of insulin level. The chronic high blood sugar has many complications which lead to damages in various organs

and disorder in their function including 10 years less lifetime (2, 3), 10 times more common amputation (4), 9% death (5), and visual impairment and blindness (6). There is a direct relationship between chronic complications of diabetes and high level of blood glucose (7). The high blood sugar causes non-enzymatic binding of glucose to proteins inside and outside the cells (8). Today, there is no doubt that improved glycemic control in diabetic patient results

in decreasing incidence of chronic complications of this disease (9).

The transportation of glucose into the muscle fiber is done by glucose transporter proteins (GLUTs); the GLUT4 is the most important glucose transporter isoform in skeletal muscles. The insulin and exercise stimulate fast and intense GLUT4 translocation to plasma membrane and cause glucose uptake in muscle and adipose tissue (10). The increased insulin sensitivity occurs after exercise simultaneously with accumulation of muscle glycogen stores (11). It is believed that the increased muscle glycogen stores after the exercise is due to increased GLUT4 (12, 13) and increased GLUT4 protein translocation from inside the cell to plasma surface [13]. In this context, Park et al (2011) reported the increased GLUT4 in skeletal muscle of healthy and diabetic mice after 6 weeks running on a treadmill at a speed of 15 meters per minute (14). Moreover, Holten et al (2004) showed that resistance exercise with 70% to 80% of one maximum repetition led to 40% and 21% increase of GLUT4 and insulin receptors content in diabetic patients' muscles (15). Also, Christine (2002) showed that 7 week running on a treadmill at a speed of 22 meters per minute increases the GLUT4 level and improves insulin resistance in obese mice (16). In contrast, Rudnick (1990) showed that there was no change in GLUT4 content of soleus muscle in three weeks of running on treadmills (17). Moreover, Vannucci et al (1998) reported that the GLUT4 level is decreased due to exercise-induced insulin reduction (18). Finally, Ivy (2004) showed that exercise improves insulin sensitivity in independent ways from GLUT4 in diabetic rats (19).

According to studies conducted in this field and different results in previous studies, this study aims to investigate the impact of two aerobic (continuous and intermittent) exercises on insulin sensitivity and GLUT4 indices in soleus muscle of diabetic rats and answer this

question: which aerobic exercise (continuous and intermittent) protocol is more useful for diabetic rats?

### Materials and methods

A total of 60 male rats weighting between 180 to 310 grams and 13-week old were bought from Laboratory Animals Breeding Centre. These animals were kept in a place with 12-hour light-dark cycle,  $21 \pm 2$  ° C temperature, and a relative humidity of 45 to 55 percent (20). During the study, the animals were given enough food and water. To avoid stress and physiological conditions, the sample was stored for 2 weeks under the new conditions. The subjects were kept for 2 weeks in new conditions to avoid stress and change in physiological conditions. In the second week, the animals were trained to work on the treadmill. The training program was conducted for 5 days (at a speed of 10 meters per minute, once a day for 10 minutes) (21). After a period of adaptation to laboratory, 30 rats (except healthy control, healthy intermittent, and healthy continuous groups) got diabetic using Streptozotocin (STZ) drug with a dose of 50 mg per kg of body weight, solved in citrate buffer 1.0 Molar and acidity 4.5. After a week, the blood samples were taken with a small wound on the tail; the blood glucose levels were measured with a glucometer. After disclosure of diabetes (glucose more than 300 mg per deciliter) in animals (22). The rats were randomly divided into six healthy control, diabetic control, healthy intermittent, diabetic intermittent, healthy continuous, diabetic continuous (each group of 10 rats) groups based on the type of training protocol. The exercise protocol of both continuous and intermittent groups was six weeks of aerobic exercise which was performed with progressive intensity and duration and considering gradual overload principle (Table 1). Throughout the training period, the slope of treadmill was considered at zero degree. The interval of rest between training sessions in intermittent group was

considered to be one to one-quarter. The exercise groups ran at the beginning of each training session for 3 minutes at a speed of 7 meters per minute for warming up. The treadmill speed was increased 2 meters per minute to achieve the desirable speed. The speed of treadmill was reduced

reversely to initial speed at the end of each exercise session for cooling down. To avoid possible effects of electric shock (1 to 3 volts) on study results, the animals were trained through audio stimulation to avoid approaching and resting at the end section of device (23).

**Table 1.** The process of performing training protocols (24).

Intermittent training	Continuous training	week
2 repeats for 7 minutes and 12 meters per minute	14 minutes at a speed of 12 meters per minute	1
2 repeats for 9.5 minutes and 12 meters per minute	19 minutes at a speed of 12 meters per minute	2
2 repeats for 12 minutes and 13 meters per minute	24 minutes at a speed of 13 meters per minute	3
2 repeats for 14.5 minutes and 14 meters per minute	29 minutes at a speed of 14 meters per minute	4
3 repeats for 12 minutes and 15 meters per minute	36 minutes at a speed of 15 meters per minute	5
3 repeats for 14.5 minutes and 16 meters per minute	43.5 minutes at a speed of 16 meters per minute	6

**Table 2.** The mean and standard deviation of dependent variables in separate rat groups.

Group	Healthy control	Diabetic control	Continuous training	Diabetic continuous training	Intermittent training	Diabetic intermittent training
Weight (g)	269±40	283±37	270±25	304±36	229 ±21	290±26
Insulin resistance	0.150±0.02	0.172±0.01	0.159±0.01	0.169±0.03	0.334±0.12	0.169±0.03
GLUT4 (Pg / mg)	10.08±3	15.42±2.52	9.24±2.64	16.49±1.77	5.82±1.74	10.75±2.33

However, 48 hours after last exercise session followed by 12 hours fasting, the rats were anesthetized with inhaling ether solution in inside of glass chamber. Cutting the skin of abdomen and chest area and opening abdominal cavity, about 10 ml of blood was taken directly from the heart and was transferred to test tube. The collected samples were immediately centrifuged (at 3000 rpm for 10 min) and were used to assess the insulin level. After dissection, their soleus muscle was also taken by surgery knife. It was washed, homogenized, and frozen in liquid nitrogen and was kept at -80 ° C for subsequent analyses (25). In the present study, the commercial kit of Sweden Mercodia Company with sensitivity of 0.015 micrograms per liter and commercial kit of China-American Cosabio Company with sensitivity of 0.04 ng per ml were used to measure insulin level and a GLUT4, respectively. The

samples were analyzed using Sandwich ELISA method. The insulin resistance is opposite to insulin sensitivity. It is not possible to calculate insulin sensitivity by calculating the number of insulin receptors on the cell surface. Therefore, the HOMA-IR formula (26) was used to calculate insulin resistance changes and insulin sensitivity changes.

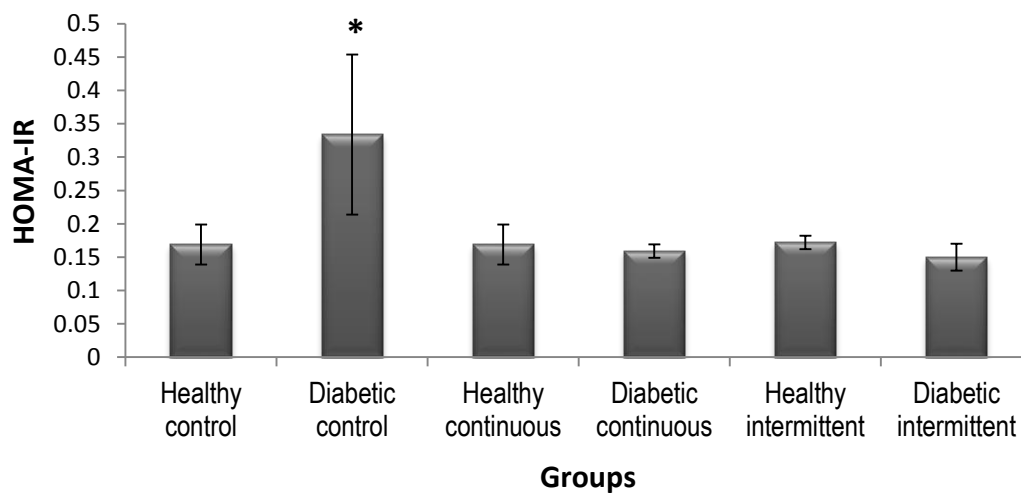
$$\text{HOMA-IR} = \frac{\text{fasting insulin (Micro unit/ml)} \times \text{fasting glucose (mg / dl)}}{405}$$

The data were analyzed using Statistical Package for Social Sciences (SPSS), version 16. First, the Shapiro-Wilk Test was used to determine the distribution of data in all groups. According to results of this test (Table 2), the one-way analysis of variance (ANOVA) was used to determine between-groups changes of dependent variables at P<0.05 level. Also, the Tukey test was used to determine the difference between pairs.

## Results

The results showed a significant difference in insulin resistance levels among different groups ( $P=0.001$ ,  $F_{5, 37}= 10.80$ ). Comparing the pairs in Tukey test showed that insulin resistance in diabetic control mice is significantly higher than that of healthy control group ( $P=0.001$ ). However, there was no significant difference between insulin resistance of diabetic continuous training group and healthy continuous exercise group ( $P=0.99$ ) and diabetic intermittent training group and

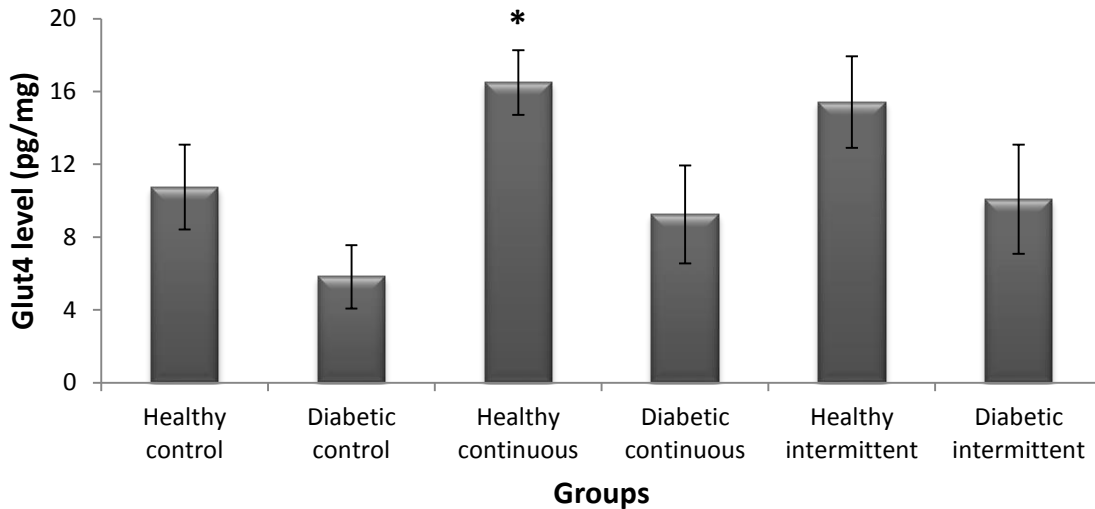
healthy intermittent training group ( $p=0.96$ ). This means that continuous and intermittent training reduce insulin resistance level in diabetic mice compared to diabetic control group (Figure 1). Also, the continuous ( $P=1.00$ ) and intermittent ( $P=0.96$ ) training had not significant effect on insulin resistance level of healthy mice compared to healthy control group; there was no significant difference between the impact of both continuous and intermittent training on insulin resistance ( $P=1.00$ ).



**Figure 1.** The comparison of blood insulin resistance level of diabetic rats after 6 weeks continuous and intermittent training. \*Significant difference with control group at  $P<0.05$ .

The results showed a significant difference in GLUT4 of soleus muscle in different research groups ( $P=0.001$ ,  $F_{5, 37}= 20.14$ ). Comparing the pairs in Tukey test showed that GLUT4 of soleus muscle in diabetic control mice is significantly lower than healthy control group ( $P=0.006$ ). Although the results showed that the continuous ( $P=0.001$ ) and intermittent ( $P=0.004$ ) training increase GLUT4 of soleus muscle in healthy mice compared to healthy control group, there was no significant difference between the effects of these two type of training ( $P=0.94$ ). In addition,

GLUT4 of soleus muscle in continuous training group was significantly higher than diabetic continuous training group ( $P=0.001$ ); also, GLUT4 of soleus muscle in intermittent training group was significantly higher than diabetic intermittent training group ( $P=0.002$ ) (Figure 2). There was no significant difference between GLUT4 of soleus muscle in diabetic continuous training group ( $P=0.81$ ), diabetic intermittent training group ( $P=0.99$ ), and healthy control group.



**Figure 2.** The comparison of GLUT4 of soleus muscle in healthy and diabetic rats after 6 weeks continuous and intermittent training. \*Significant difference with control group at  $P < 0.05$ .

## Discussion

The insulin resistance of diabetic control mice was higher than that of healthy control group. However, there was no significant difference between insulin resistance of diabetic continuous training group and that of continuous training group, and between diabetic intermittent training group and intermittent training group. In addition, the results showed that GLUT4 of soleus muscle in diabetic control group was lower than that of healthy control group. Although the continuous and intermittent training increased GLUT4 of soleus muscle in healthy mice compared to healthy control group, there was no significant difference between these two types of training. In a study conducted on rats, the increased level of GLUT4 in plantaris muscle and no change in soleus muscle was observed after three weeks of training on a treadmill (17). In this context, Vannucci et al. suggested that due to reduced level of insulin after exercise, GLUT4 was significantly reduced in rabbits (18). In addition, Ivy (2004) stated that exercise improves insulin sensitivity, but causes no change in insulin signaling and GLUT4 transmission to membrane surface in muscles of rats. It can be found that the

results of present research on GLUT4 are not consistent with above findings. This may be due to difference in training program type and studied muscle. However, the research shows that muscle fiber type is involved in type of response. For example, white cords are less affected by aerobic activity in terms of GLUT4 and insulin sensitivity. However, the results of present study are consistent with the findings of Durant (2002) increase of GLUT4 transportation to cell membrane (27), Caponi et al (2013) increase of GLUT4 (28), Park et al. (2011) increase of GLUT4 (14), Zarekar et al (2014) increase of GLUT4 and insulin sensitivity (29), and Catherine et al. (2013) increase of GLUT4 and improved insulin sensitivity (30). The GLUT protein is a major mediator for glucose uptake from blood circulation which is expressed in skeletal muscle and adipose tissue (31). The evidence has shown that the total amount of GLUT4 protein and its displacement to muscle fiber membrane determines the amount of muscle glucose uptake in response to insulin. It is now widely accepted that this protein molecule plays a key role in whole-body insulin sensitivity and glucose tolerance (32).

There are two routes for stimulating glucose uptake by muscle. During the rest, the glucose uptake by muscle depends on insulin and its main role is rebuilding muscle glycogen stores. During exercise, the muscle contraction increases blood glucose uptake to help muscle glycogenolysis. Since both routes are separate, the blood glucose uptake in active muscle is natural in patients with type 2 diabetes which their insulin-dependent absorption is impaired. The uptake of glucose into muscle is high even after exercise, because the routes which stimulate glucose uptake remain active for hours after exercise (33, 34). Due to increased GLUT4 protein, increased displacement, and increased exposure of these transport proteins at the cell surface, the muscle contraction increases membrane permeability to glucose and improves insulin action in glucose metabolism (35). The increased insulin sensitivity after exercise occurs simultaneously with the accumulation of muscle glycogen stores (11). It is believed that increased muscle glycogen stores after exercise is due to increased GLUT4 (12, 13) and increased GLUT4 protein displacement from inside the cell to plasma surface (13).

The findings of some studies show that exercise (36, 37) may impact on AMPK activity and AMPK impact on protein GLUT4 (37). However, it can be said that continuous and intermittent exercises increase AMPK activity and stimulate GLUT4 protein in these groups.

In terms of increased insulin sensitivity as one of the findings, it can be said that some mechanisms increase insulin action after aerobic (continuous and intermittent) exercise including increased insulin receptors signaling, increased protein of glucose transporter (GLUT4), increased

activity of glycogen synthase and hexokinase, decreased release and increased deletion of free fatty acids, increased release of glucose from blood into the muscle due to increased muscle capillaries, and changes in composition of muscle to increase glucose uptake (26). Given the rise in GLUT 4 protein levels in this study, the increased GLUT4 level is probably one of the reasons for improvement of insulin action.

Both the insulin dependent and independent routes in diabetic patients are impaired in glucose uptake, because diabetes reduces GLUT4 level and increases resistance to insulin. In contrast, the exercise acts as an alternative to compensate low levels of these indicators. In addition, although diabetes reduces GLUT4 displacement to muscle cell membrane, the exercise compensates this transportation and displacement through stimulating insulin independent route.

### Conclusion

Given that the present study showed that the continuous and intermittent training impact significantly on GLUT4 protein levels and insulin sensitivity, it seems that training may overcome the damage caused by diabetes on AMPK and increases GLUT4 protein. In general, considering the positive impact of training on GLUT4 protein levels in diabetic rats, it is suggested that the findings of this study be used in clinical trials; if the results will be positive and useful, they should be considered as a complementary way in the management of diabetes. However, due to the lack of significant difference between the effects of intermittent and continuous training, the diabetics may perform any of these training to achieve positive results.

### References

1. Abou-Seif MA, Youssef AA. Evaluation of some biochemical changes in diabetic patients. *Clin Chim Acta*. 2004; 346(2): 161-70.

2. Furuya M1, Hayashino Y, Tsujii S, Ishii H, Fukuhara S. omparative validity of the WHO-5 Well-Being Index and two-question instrument for screening depressive symptoms in patients with type 2 diabetes. *Acta Diabetol.* 2013;50(2):117-21.
3. Rosenbloom AL, Joe JR, Young RS, Winter WE. Emerging epidemic of type 2 diabetes in youth. *Diabetes Care.* 1999; 22(2):345-54.
4. Siitonen OI, Niskanen LK, Laakso M, Siitonen JT, Pyörälä K. Lower-Extremity Amputations in Diabetic and Nondiabetic Patients, *Diabetes Care.* 1993; 16(1):16-20.
5. Kastorini CM, Panagiotakos DB. Mediterranean diet and diabetes prevention: Myth or fact? *World J Diabetes.* 2010; 1(3): 65-7.
6. Dabelea D, Hanson RL, Bennett PH, Roumain J, Knowler WC, Pettitt DJ. Increasing prevalence of type 2 diabetes in American Indian children. *Diabetology.* 1998; 41:904-910.
7. Gomez-Perez FJ, Aguilar-Salinas CA, Almeda-Valdes P, Cuevas-Ramos D, Lerman Garber I, Rull JA. HbA1c for the diagnosis of diabetes mellitus in a developing country. *Arch Med Res.* 2010; 41(4): 302-8.
8. Chait A, Bierman E. Pathogenesis of macrovascular disease in diabetes. *Joslin's Diabetes Mellitus.* Lea and Febiger: Philadel. 1994; 648-64.
9. American Diabetes Association. Economic Consequences of Diabetes Mellitus in the U.S. *Diabetes Care.* 1998; 21:296-309.
10. Augustin R. The protein family of glucose transport facilitators: It's not only about glucose after all. *IUBMB Life.* 2010;62(5):315-33.
11. Hawley JA, Lessard SJ. Exercise training-induced improvements in insulin action. *Acta Physiol (Oxf).* 2008;192(1):127-35.
12. Chou CH, Tsai YL, Hou CW, Lee HH, Chang WH, Lin TW, et al. Glycogen overload by postexercise insulin administration abolished the exercise-induced increase in GLUT4 protein. *J Biomed Sci.* 2005;12(6):991-8.
13. Tsai YL, Hou CW, Liao YH, Chen CY, Lin FC, Lee WC, et al. Exercise training exacerbates tourniquet ischemia-induced decreases in GLUT4 expression and muscle atrophy in rats. *Life Sci.* 2006;78(25):2953-9.
14. Park ST, Kim K, Yoon JH, Lee S. Effect of exercise on GLUT4 expression of skeletal muscle in streptozotocin-induced diabetic rats. *Physiology.* 2011; 14(1): 113-22.
15. Holten MK, Zacho M, Gaster M, Juel C, Wojtaszewski JFP, Dela F. Strength training increases insulin-mediated glucose uptake, GLUT4 content, and Insulin signaling in skeletal muscle in patients with type 2 diabetes. *Diabetes.* 2004; 53(2): 294-305.
16. Christine CY, Hunt D, Hancock J, Garcia-Macedo R, Mandarino LJ, Ivy JL. Exercise training improves muscle insulin resistance but not insulin receptor signaling in obese zucker rats. *J Appl Physio.* 2002; 92(2): 736-44.
17. Rodnick KJ, Holloszy JO, Mondon CE, James DE. Glucose transporters and maximal transport are increased in endurance-trained rat soleus. *Appl Physio.* 1992; 39 (11):1425-9.
18. Vannucci SJ, Koehler-Stec EM, Li K, Reynolds TH, Clark R, Simpson IA. GLUT4 glucose transporter expression in rodent brain: effect of diabetes. *Brain Res.* 1998; 22;797 (1):1-11.
19. Ivy JL. Muscle insulin resistance amended with exercise training: role of GLUT4 expression. *Med Sci Sports Exerc.* 2004; 36(7):1207-11.
20. Kraniou G, Cameron-Smith C, Hargreaves M. Effect of short-term training on GLUT4 mRNA and protein expression in human skeletal muscle. *Exp Physiol.* 2004; 89(5): 559-63.
21. Borzykh AA, Kuzmin IV, Martianov AA, Borovik AS, Sharova AP, Tarasova OS, et al. Changes of rat

- respiratory and locomotory muscles during aerobic exercise training in continuous and interval regimens. *Biofizika*. 2012;57( 5): 684-9.
22. Srinivasan K, Visvanad B, Lydia Asrad CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacol Res*. 2005; 52(4): 313-20.
23. Siamilis S, Jakus J, Nyakas C, Costa A, Mihalik B, Falus A, et al. The effect of exercise and oxidant-antioxidant intervention on the levels of neurotrophins and free radicals in spinal cord of rats. *Spinal Cord*. 2009; 47(6): 453-7.
24. Shabkhiz F, Ravasi AA, Hassan ZM, Taghikhani M, Razav TA. The effect of aerobic continuous and interval training and detraining on some indexes of the cellular immune system in female wistar rats. *Sport Sci*. 2008; 1(1): 17-26.
25. Ogonovszky H, Berkes I, Kumagai S, Kaneko T, Tahara S, Goto S, et al. The effects of moderate-, strenuous- and over-training on oxidative stress markers, DNA repair, and memory, in rat brain. *Neurochem Int*. 2005; 46(8): 635-40.
26. Hosseini SRA, Mir E, Hejazi K, Sayeedi MM. [The Effect of eight weeks combined training on some insulin resistance markers in middle-aged men]. *Med J Mashhad Uni Med Sci*. 2015; 58 (3): 129-36.(Persian)
27. Durante PE, Mustard KJ, Park SH, Winder WW, Hardie DG. Effects of endurance training on activity and expression of AMP-activated protein kinase isoforms in rat muscles. *Am J Physiol Endocrinol Metab*. 2002; 283(1): 178-86.
28. Caponi PW, Lehnen AM, Pinto GH, Borges J, Markoski M, Machado UF, et al. Aerobic exercise training induces metabolic benefits in rats with metabolic syndrome independent of dietary changes. *Clinics*. 2013; 68(7): 1010-7.
29. Zarekar M, Saghebjo M, Foadodini M, Hedayati M. [Combined effect of aerobic training and pistacia atlantica extract on GLUT-4 protein expression and muscle glycogen in diabetic rats]. *Iran J Endocri Metab*. 2014; 16 (4): 245-53. (Persian)
30. Katharine E, Hall KE, Matthew W, McDonald MW, Kenneth N, Gris  KN, et al. The role of resistance and aerobic exercise training on insulin sensitivity measures in STZ-induced Type 1 diabetic rodents. *Metabolism*. 2013; 62(10): 1485-94.
31. Huang S, Czech MP. The GLUT4 glucose transporter. *Cell Metab*. 2007; 5(4): 237-52.
32. Hou CW, Chou SW, Ho HY, Lee WH, Lin CH, Kuo CH. Interactive effect of exercise training and growth hormone administration on glucose tolerance and muscle GLUT4 protein expression in rats. *J Biomed Sci*. 2003; 10(6): 689-96.
33. Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. *Ann Rev Med*. 1998; 49: 235-61.
34. Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, et al. Exercise and type 2 diabetes. *Diabetes Care*. 2010; 33(10): 147-67.
35. Kiraly MA, Bates HE, Yue JT, Goche-Montes D, Fediuc S, Park E, et al. Attenuation of type 2 diabetes mellitus in the male Zucker diabetic fatty rat: the effects of stress and non-volitional exercise. *Metabolism*. 2007; 56(6): 732-44.
36. Eguchi T, Kumagai C, Fujihara T, Takemasa T, Ozawa T, Numata O. Black tea high-molecular-weight polyphenol stimulates exercise training-induced improvement of endurance capacity in mouse via the link between AMPK and GLUT4. *PLoS One*. 2013; 8(7): 69480.



37. Teixeira-Lemos E, Nunes S, Teixeira F, Reis F. Regular physical exercise training assists in preventing type 2 diabetes development: focus on its antioxidant and antiinflammatory properties. *Cardiovasc Diabetol.* 2011; 10-2.
38. Richter EA, Hargreaves M. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol Rev.* 2013; 93(3): 993-1017.