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Reviewing and comparing the impact of aerobic exercise (3 and 5 times per week) on insulin receptors, glucose transporter protein (GLUT4), and skeletal muscle insulin sensitivity in diabetic rats

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ABSTRACT

Objectives: The diabetes is a metabolic disease which is caused by chronic disease of blood glucose due to low insulin secretion, insulin resistance, or both of them. The sports activities have a major impact on prevention, treatment, and reduction of diabetes effects. Therefore, this study aimed to review and compare the impact of aerobic exercise (3 and 5 times per week) on some factors which are involved in glucose transition to skeletal muscles of diabetic rats.
Materials & Methods: A total of 30 Wistar rats were divided into three groups: diabetic, control (n=8); exercise group, 3 times a week (n=11); and exercise group, 5 times a week (n=11). The exercise groups ran on treadmill for 6 weeks with progressive intensity and duration. The variance analysis test was used to detect the significance of difference between groups. Also, the paired t-test was used to evaluate changes of groups in pre-test and post-test.
Results: The findings showed that the insulin sensitivity and insulin receptor levels increased significantly in aerobic exercise groups (3 and 5 times a week) compared to control group ($p<0.05$). The aerobic exercise (three times a week) did not significantly increase the levels of glucose transporter protein (GLUT4) in exercise group compared to control group. However, aerobic exercise (five times a week) increased significantly the levels of glucose transporter protein (GLUT4) in exercise group compared to control group ($p<0.05$).
Conclusions: The findings showed that aerobic exercise increased insulin receptors levels, glucose transporter proteins (GLUT4), and insulin sensitivity level. Also, the findings showed that the number of exercise session per week may be effective in management of diabetes and reduction of its destructive effects.

INTRODUCTION

The insulin resistance and impaired secretion are two major problems associated with diabetes. The insulin resistance is decreased tissue sensitivity to insulin. Normally, insulin binds to specific receptors in the cell and starts glucose metabolism-related reactions (Colberg *et al.*, 2010). Hyperglycemia may perturbcellular antioxidant defense systems and damage cells. Free radicals are formed disproportionately in diabetes by glucose oxidation, noneEnzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins (Arshadi *et al.*, 2015). In type 2 diabetes, the rate of intracellular reactions decreases; thus, the effect of insulin on tissue cells in glucose uptake and on liver cells in store glucose decreases. More insulin must be secreted to overcome insulin resistance and prevent increased levels of glucose in blood; therefore, the blood glucose level will be normal. However, if beta cells fail to supply increased insulin demand, the blood glucose level will increase and patient will suffer from type 2 diabetes (Colberg *et al.*, 2010). Physical activity increases glucose transporter protein (GLUT4) level; thus, the entry of glucose into muscle cells and its consumption will be easy.

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The studies have shown that glucose transporter protein level is higher in young athletes than on-athlete people (Bird and Hawley, 2012). Many studies suggest that long-term, regular exercise of type 2 diabetes patients leads to increased insulin sensitivity and metabolism control. Since the type II diabetes is not hereditary, a sedentary lifestyle and poor diet may cause it, and physical activity is one of the main determinants of insulin volume in body and insulin sensitivity of skeletal muscles, it can be claimed that there is a significant relationship between physical activity and insulin sensitivity. Several factors may explain this relationship. The sports activities change body composition and lead to a decrease in fat and increase in muscle size; so, it is possible that physical activity will increase insulin sensitivity and glucose consumption of muscle cells. According to above, the exercise and physical activity increases the amount of insulin in blood, the glucose transporter protein (GLUT4), and glycogen synthase enzyme activity. Therefore, it can be concluded that physical activity increases insulin sensitivity and the athletes and the people who do sports activities are less disposed to type 2 diabetes (Bird and Hawley, 2012).

Physical activity increases the number of glucose transporter protein (GLUT4), as a factor in preventing the development of insulin resistance. This leads to increased glucose transporters activity and increased insulin sensitivity (Dray *et al*., 2008). Hence, regular physical activity may increase glucose consumption in cells, especially muscle cells and overcome insulin receptor disorders (Holten *et al*., 2004; Dray *et al*., 2008).

MATERIALS AND METHODS

A total of 30 Albino-Wistar rats were divided into 6 groups, each 5 rats. They were kept in cages with 12-hour light-dark cycle 25± 2 ° C. Enough food and water was provided to them. After a week of acclimation to laboratory, they get diabetics using streptozotocin with a dose of 50 mg per kg of body weight, dissolved in 0.1 M citrate buffer, pH 4.5. After revealing the symptoms of diabetes (glucose level more than 300 mg per deciliter), they were divided into one control group and two experimental groups- 3 and 5 exercise times per week. The exercise times were performed with progressive overload, intensity, and duration. In summary, both groups started the exercise in first week from 12 meters per minute and in second week, the exercise increased 1 meter per minute. The exercise duration in 5 times per week group increased regularly 1 minute and 25 seconds per session from second week to sixth week. The exercise duration was increased from 15 minutes per week in first day of first week to 52 minutes in sixth week. The exercise duration in 3 times per week group also increased regularly 2 minute and 20 seconds per session. Adaptation period was one week, every day for 5 minutes at a speed of 5-7 meters per minute. The warmup and cool-down was also performed at the beginning and end of each session for 3-5 minutes at a speed of 5-7 meters per minute. The rats were anesthetized after 48 hours from end of activity. Then, the desired tissues (skeletal muscle and blood) were taken from body. They were immediately frozen in liquid nitrogen and transferred to a freezer -80 ° C. After completion of exercise protocol, the commercial kit made in CusabioCo. China-America with a sensitivity0.04 ng ml. was used to measure insulin receptor and GLUT4. After measuring insulin concentration using the commercial kit of MercodiaCo. in Sweden with sensitivity 0.015 micrograms per liter and measuring glucose using glucometer, the method of calculating insulin resistance using HOMA-IR formula was used to measure insulin sensitivity (Chen-Chung *et al*., 2006). The formula is as follows:

HOMA IR = serum insulin (mmol/L)*(blood glucose (mmol/L)/22.5

The insulin resistance is the opposite of insulin sensitivity. Since the number of insulin receptors on cell surface should be calculated and this is not possible in practice, researchers use HOMA-IR formula to calculate changes in insulin resistance; in this way, they realize the changes in insulin sensitivity.

The one-way analysis of variance (ANOVA) was used the groups alignment in terms of measured variables. Also, the multivariate analysis of variance (ANOVA) was used to determine the significance of relationship among measured indicators; Tokey test was used to determine the significance of difference among groups. The paired t-test was used to evaluate changes of groups in pre-test and post-test. The significance level was considered to be 0.05. The analysis of data was performed using SPSS version 16. The Excel program and descriptive statistics were used to draw charts and tables.

RESULTS

Given the significance level in paired t-test provided in Table 1, it can be seen that there is difference between post-test of insulin receptor in skeletal muscles of 3 times per week exercise group and control group (α= 0.000). This means that three times a week aerobic exercise had a significant impact on number of insulin receptors in skeletal muscles.

Given the significance level in paired t-test provided in Table 1, it can be seen that there is no difference between post-test of glucose transporter protein (GLUT4) number in skeletal muscles of 3 times per week exercise group and control group (α= 0.093). This means that three times a week aerobic exercise had no significant impact on glucose transporter protein (GLUT4) number in skeletal muscles.

Given the significance level in paired t-test provided in Table 1, it can be seen that there is difference between post-test of insulin resistance of skeletal muscles of 3 times per week exercise group and control group (α= 0.026). This means that three times a week aerobic exercise impacted on insulin resistance of skeletal muscles. Since the insulin resistance is opposite of insulin sensitivity, it can be concluded that three times a week aerobic exercise significantly impacted on insulin resistance of skeletal muscles.

Table 1: Paired t-test for insulin receptors, glucose transporter proteins (GLUT4), and insulin resistance in skeletal muscles (control group and 3 times a week exercise group) and (control group and 5 times a week exercise group).

Variables	Groups	No.	Mean	Mean difference	Degree of freedom	t-value	Sig. level
Insulin receptors levels	Diabetic control group	6	270.50	112.50	11	5.947	0.000*
	3 times a week exercise group	7	383				
	Diabetic control group	6	270.50	170.643	11	7.399	0.000*
	5 times a week exercise group	7	441.14				
Glucose transporter protein levels (GLUT4)	Diabetic control group	6	8.461	3.175	11	1.838	0.093
	3 times a week exercise group	7	11.637				
	Diabetic control group	6	8.46	5.016	11	3.58	0.004*
	5 times a week exercise group	7	13.48				
Insulin resistance	Diabetic control group	6	0.245	0.050	11	2.562	0.026*
	3 times a week exercise group	7	0.195				
	Diabetic control group	6	0.245	0.064	11	2.763	0.018*
	5 times a week exercise group	7	0.181				

* Indicates significant difference ($p< 0.05$) compared to diabetic control group.

Table 2: Multivariate analysis of variance to determine significant difference in levels of insulin receptors, glucose transporter proteins (GLUT4), and skeletal muscle's insulin resistance in two exercise groups.

Variable	Change source	Square of deviation	Degree of freedom	Mean square of deviation	F-value	Sig. level
Insulin receptors levels	Between groups	443.96010	2	221.48005	541.34	000 *.0
	Inter-groups	357.23626	17	786.1389		
Glucose transporter proteins levels) GLUT4	Between groups	339.82	2	169.41	699.5	013 *.0
	Inter-groups	800.122	17	224.7		
Insulin resistance	Between groups	014.0	2	007.0	590.5	014 *.0
	Inter-groups	022.0	17	001.0		

* Indicates significant difference ($p< 0.05$).

Given the significance level in paired t-test provided in Table 1, it can be seen that there is difference between post-test of insulin receptors of skeletal muscles of 5 times per week exercise group and control group ($\alpha= 0.000$). This means that 5 times a week aerobic exercise significantly impacted on insulin receptors of skeletal muscles.

Given the significance level in paired t-test provided in Table 1, it can be seen that there is difference between post-test of glucose transporter protein (GLUT4) number in skeletal muscles of 5 times per week exercise group and control group ($\alpha= 0.004$). This means that 5 times a week aerobic exercise had significant impact on glucose transporter protein (GLUT4) number in skeletal muscles.

Given the significance level in paired t-test provided in Table 1, it can be seen that there is difference between post-test of insulin resistance of skeletal muscles of 5 times per week exercise group and control group ($\alpha= 0.018$). This means that 5 times a week aerobic exercise impacted on insulin resistance of skeletal muscles. Since the insulin resistance is opposite of insulin sensitivity, it can be concluded that 5 times a week aerobic exercise significantly impacted on insulin resistance of skeletal muscles.

The multivariate analysis of variance was used to compare the data of groups. Given the F-value and significance level provided in Table 2 in alpha level 0.05, the impact of exercise on insulin receptors level is significant ($P= 0.000$). In other words, there is a significant difference between effect of three times a week aerobic exercise and 5 times a week aerobic exercise on skeletal muscles' insulin receptors level. Given the F-value and significance level provided in Table 2 in alpha level 0.05, the impact of exercise on glucose transporter protein (GLUT4) level is significant ($P= 0.013$). In other words, there is a significant difference between effect of three times a week aerobic exercise and 5 times a week aerobic exercise on skeletal muscles' glucose transporter protein (GLUT4) level. Given the F-value and significance level provided in Table 2 in alpha level 0.05, the impact of exercise on insulin resistance is significant ($P= 0.014$). In other words, there is a significant difference between effect of three times a week aerobic exercise and 5 times a week aerobic exercise on skeletal muscles' insulin resistance.

DISCUSSION

The reduction of glucose in blood is the main function of insulin. In the metabolism of protein and fat, it also increases cellular uptake of amino acids and increases protein and fat production. The studies show that exercise increases the number of insulin receptors and their availability; and thereby it increases the body's sensitivity to insulin. This situation decreases the need to maintain high levels of plasma insulin to move glucose into muscle cells and weakens the hormonal response in exercised individuals (Wilmor *et al*., 2008).

It is generally accepted that the improvements in insulin sensitivity associated with exercise training are related to changes in genes expression of skeletal muscles and/or activity of proteins involved in insulin signal transduction such as the AMP-activated

kinase, protein kinase B (Akt), glucose transporter (GLUT4), insulin receptor, and insulin receptor substrate 1.2 (Thorell *et al*., 1999; Lemieux *et al*., 2000).

This finding (Effect of three times a week aerobic exercise and 5 times a week aerobic exercise on insulin receptors level) is consistent with findings of Forsig *et al.* (2007) and Holten *et al*. (2004). It is also inconsistent with findings of Christian *et al*. (2002). This inconsistency may be due to difference in selected sample; in this study, obese rats were used and we used the diabetic rats.

This finding (effect of three times a week aerobic exercise on glucose transporter protein (GLUT4) level) is inconsistent with findings of Zarekar and colleagues (Zarekar *et al*., 2014), Park *et al*. (2011), Ross *et al*. (2009), Holten *et al*. (2004), Forsig *et al.* (2007), and Christine *et al* (2002). This insignificance does not mean that the 3 times a week aerobic exercise have no effect; it indicates that the effect was low. This finding (effect of 5 times a week aerobic exercise on glucose transporter protein (GLUT4) level) is consistent with findings of Zarekar and colleagues (Zarekar *et al*., 2014), Park *et al*. (2011), Rose *et al*. (2009), Holten *et al*. (2004), Forsig *et al*. (2007), and Christine *et al.* (2002). The probable reason of this difference in consistency between exercise groups is difference in number of exercise sessions and increased exercise intensity.

This finding (effect of 3 and 5 times a week aerobic exercise on insulin resistance) is consistent with findings of Christine *et al*. (2002), Colberg (2007), Asgari *et al.* (2011)), Mohebi *et al*. (2013) and Parsian *et al*. (2013). All have stated that exercise reduces insulin resistance and thus improves insulin sensitivity.

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(Colberg *et al*., 2010).It is generally accepted that applies facilitating by increasing gene levels in skeleton muscles or through increasing activity of key proteins involved in insulin signaling such as glucose 4 transmitter (GLUT4), insulin receptors and insulin receptor substrate 1/2, AMP kinase- activated, protein kinase B/ Akt which increases aerobic capacity and fat oxidation (Parimai and Ranjan, 2007). The GLUT4 protein is a major mediator for glucose uptake from blood circulation which is expressed in skeletal muscle and adipose tissue (Parimai and Ranjan, 2007). 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 (Hou *et al*., 2003). 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 (Kiraly *et al*., 2007). The increased insulin sensitivity after exercise occurs simultaneously with the accumulation of muscle glycogen stores (Hawley and Lessard, 2008). It is believed that increased muscle glycogen stores after exercise is due to increased GLUT4 (Chou *et al*., 2005; Tsai *et al*., 2006) and increased GLUT4 protein displacement from inside the cell to plasma surface (Tsai *et al*., 2006).

It should be noted that exercise training reduces plasma insulin levels, increases levels of insulin receptors on cell surface, increases glucose transporter proteins (GLUT4), increases transportation of glucose into cells, and decreases plasma glucose levels. This function is interpreted as increasing insulin sensitivity. However, this study also showed that increasing the number of exercise sessions per week may bring many other benefits.

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