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Effect of Eight Weeks of High Intensity Interval Training on Insulin Resistance and *IRS1* Gene Expression in Gastrocnemius Muscle of Obese Wistar Rats

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ABSTRACT

Background and objectives: The role of genetic components in expression of proteins involved in signaling pathways of fat and carbohydrate metabolism has been well-demonstrated. The aim of this study was to determine effects of high intensity interval training (HIIT) on glucose, insulin, and insulin resistance levels as well as *IRS1* expression in gastrocnemius muscle of obese Wistar rats.

Methods: The study included 14 male, Wistar rats (aged 10 weeks) weighting 220 ± 20 g. Obesity was induced in all rats via exposure to a high-fat diet for six weeks. Then, the rats were randomly divided into a HIIT group (n=7) and a control group (n=7). The rats in the HIIT group performed treadmill running, five sessions a week, for eight weeks. Levels of fasting glucose, serum insulin, insulin resistance, and *IRS1* expression in the gastrocnemius muscle of the rats were measured after the last training session. Data were analyzed by the independent t-test at statistical significance of 0.05.

Results: The HIIT intervention significantly decreased fasting glucose compared with the control group (p<0.0001). It also resulted in a significant decrease in serum insulin levels and insulin resistance compared with the control group (p<0.0001). Moreover, the HIIT training significantly increased *IRS1* expression (p=0.030) in the gastrocnemius muscle of rats.

Conclusion: Based on the available evidence, the increase in insulin function and the decrease in insulin resistance can be attributed to increased *IRS1* expression in the gastrocnemius muscle following HIIT training.

Keywords: High intensity interval training, Obesity, muscles.

INTRODUCTION

Scientific studies have identified obesity as an effective factor in the development of type 2 diabetes (1).

Hyperinsulinemia due to increased insulin release from the pancreas and insulin resistance are among the most common endocrine disorders in obesity (2). Glucose transport from the bloodstream to target tissues, especially skeletal muscles, is one of the most important functions of insulin (3). Studies in recent decades have demonstrated the role of genetic factors in the incidence of obesity and obesity-related metabolic diseases. It is believed that impaired expression of some genes may affect lipolysis or insulin function, thereby altering carbohydrate and fat metabolism. For example, genetic components such as *FOXO1*, *PPARy*, and *FTO* affect energy homeostasis as well as glucose and fat metabolism in target tissues of insulin, such as skeletal muscle and adipose tissue (4, 5).

In fact, the association between PPARy and FTO levels with obesity, lipid profile, and insulin resistance has been well-demonstrated (5, 6). In the meantime, the insulin receptor substrate 1 (*IRS1*) gene is also of great importance as a cytoplasmic substrate of both insulin and insulin like growth factor 1 receptor components (7).

Insulin exerts a wide range of growth and metabolic effects by binding to its receptor and activating the tyrosine kinase property. This ultimately leads to phosphorylation of tyrosine kinase residues on the surface of anchor proteins, including IRS proteins (8). According to studies, IRS1 plays important roles in insulin signaling pathways. Some studies have revealed that altered expression of IRS1 in insulin signaling pathways, especially along the PI3K kinase pathway, may lead to insulin resistance (7, 9). It has been reported that IRS1 protein expression increases after one day of exercise activity and declines by 50% after 16 hours of chronic exercise (five days of swimming) (10). High-intensity interval training (HIIT) is a highly time-efficient model of exercise that stimulates many metabolic adaptations to endurance and regular exercises (11).

It is also effective in reducing body fat and insulin resistance (12).

The present study investigates effects of eight weeks of HIIT on insulin signaling, glucose level, and *IRS1* gene expression in obese rats.

MATERIALS AND METHODS

This experimental study was carried out on 14 10-week old male, Wistar rats (weighing 220 ± 20 g). The rats were purchased from the Baqiyatallah University of Medical Sciences, Tehran, Iran**.** Obesity was induced via exposure to a high-fat diet containing 1% cholesterol powder and 1% pure corn oil (Pars dam Food Company, Iran) for six weeks (13). Then, the rats were randomly divided into a training group (n=7) and a control group (n=7). The rats were kept in a 12:12-hour light–dark cycle, at controlled temperature (22±3 °C) and humidity (30%). The rats were handled by one person throughout the study period. All procedures were carried out according to the ethical considerations in the care of laboratory animals. The study protocol was approved by the ethics committee of the Institute of Physical Education and Sport Sciences (ethical code: IR.SSRI.REC.1399.651).

The rats became familiar with the laboratory environment for one week. They were also familiarized with running on treadmill for one week. The main training intervention started at the beginning of the 19th week. Rats in the training group were subjected to 30 minutes of treadmill running, five sessions a week, for eight weeks, with 40 seconds repetition and a 2-minute active rest between each repetition (14). The control group did not participate in any training. Both groups received the high-fat diet until the end of the study. Finally, all rats were dissected 48 hours after the last training session.

The details of the HIIT exercise program were as follows:

- In weeks 1 and 2: 8 repetitions of 40 seconds at speed of 25 m/min, with 2 minutes active rest between repetitions at 10 m/min (5% slope).
- In weeks 3 and 4: 10 repetitions of 40 seconds at speed of 28 m/min, with 2 minutes active rest between repetitions at 10 m/min (10% slope).
- In weeks 5 and 6: 10 repetitions of 40 seconds at speed of 32 m/min, with 2 minutes active rest between repetitions at 10 m/min (10% slope).
- In weeks 7 and 8: 10 repetitions of 40 seconds at speed of 36 m/min, with 2 minutes active rest between repetitions at 10 m/min (10% slope).

After overnight fasting, the rats were anesthetized by intraperitoneal injection of ketamine-xylosine, and blood samples were taken directly from the animal. The gastrocnemius muscle was then removed, washed with physiological serum, and placed in a microtube containing RNAlater^{TM} fluid with a ratio of 20%. Glucose concentration was measured by glucose oxidase method using a commercial kit (Pars Azmoun Co., Iran). Serum insulin was measured by enzyme-linked immunosorbent assay using a commercial kit (Demeditec Diagnostic, Germany). RNA extraction was performed using the RNeasy Mini Kit (Qiagen, Germancy) according to the manufacturer's instructions (15). Next, *IRS1* mRNA was quantified by RT-Real time PCR in the 6000 Rotagen System using the One Step SYBR kit (TAKARA BIO Inc. Japan) according to the company's instructions. The sequences of the primers used in the study are shown in table 1. Statistical analysis of data was performed using SPSS (version 16). The Kolmogorov-Smirnov test was used to assess normal distribution of data. The independent t-test was used to compare pretest and posttest values of body weight between the groups. The paired t-test was used to determine intra-group changes. The independent t-test was used to compare the dependent variables (glucose, serum insulin and IRS1) between the study groups. A *p*-value of less than 0.05 was considered statistically significant.

Table 1- The sequences of the primers used in the study

Genes	Primer sequence (5` to 3`)	Product size	Tm (°C)	Gene Bank
IRS1	Forward: GGCCATGAGCGATGAGTTTC Reverse: GGCGGAGGATTGTTGAGATG	159 bp	60	NM_001191052.1
RNA PolymraseII	Forward: ACTTTGATGACGTGGAGGAGGAC Reverse: GTTGGCCTGCGGTCGTTC	164 bp	60	XM_008759265.1

RESULTS

Based the results of the independent t-test, there was no significant difference in body weight between the two groups in the pretest stage (p=0.632).

The results of the paired t-test showed a significant increase in body weight at the posttest stage compared to the pretest stage. However, there was no significant difference in the body weight between the two groups at the posttest stage (p=0.126).The expression of *IRS1* in the gastrocnemius muscle differed significantly between the two groups (p=0.03). In other words, the HIIT training significantly increased *IRS1* expression in the gastrocnemius muscle of obese Wistar rats (Tables 3).

Table 2- Body weight (g) before and after the intervention in the study groups

Group	Pre-intervention	Post-intervention	*p*-value (paired t-test)
Control	275 ± 8.50	370 ± 4.93	<0.0001
HIIT	277 ± 6.6	364 ± 7.48	<0.0001
Sig (T test)	P = 0.632	P = 0.126	-

Data are presented as mean ± standard deviation.

Table 3- Relative expression of *IRS1* in the gastrocnemius muscle of rats in the HIIT and control groups

Variable	Control group	HIIT group	*p*-value
Relative expression of *IRS1*	1	1.44 ±0.47	0.03

Fasting blood glucose, insulin, and insulin resistance were significantly lower in the HIIT group than in the control group (Table 4).

Table 4- Fasting glucose levels in the HIIT and control groups

Variables	Control group	HIIT group	*p*-value
Glucose (mg/dL)	120 ±4	96 ±5	<0.0001
Insulin (µIU/ml)	9.04 ±0.72	6.37 ±1.15	<0.0001
Insulin resistance (HOMA-IR)	2.68 ±0.22	1.53 ±0.34	<0.0001

DISCUSSION

The findings of the present study indicated a decrease in fasting glucose level and insulin resistance following eight weeks of HIIT in insulin-resistant obese rats. Racil et al. (2013) reported a decrease in triglyceride, total cholesterol, low-density lipoprotein, and insulin resistance in response to four weeks of moderate to high interval exercise in obese men (16). Tan et al. (2016) also reported significant reductions in glucose, triglyceride, and body fat mass along with increased lipoprotein lipase activity after 10 weeks of aerobic exercise in overweight women (17). In another study, 12 weeks of moderate-intensity aerobic training decreased glycosylated hemoglobin, glucose, and insulin resistance in type 2 diabetic men and women (18). Contrary to these studies, Maltais et al. (2016) reported that four months of resistance training did not change glucose and insulin levels in 26 overweight older men, although body fat mass decreased significantly (19). Bouchonville et al. (2013) also reported no change in triglyceride, adiponectin, and insulin resistance after 12 months of aerobic exercise in obese adults (20). The discrepancy in the findings regarding the response of type 2 diabetes markers to exercise in healthy and obese populations can be explained by differences in the type of exercise intervention, intensity, duration, repetition of exercise sessions, baseline level of fitness. On the other hand, a decrease in blood glucose can be partly attributed to an increase in insulin function at target tissue levels by regulating insulin receptor components, such as insulin receptor protein concentration, protein kinase B, increased IRS1, and glycogen synthesis as well as an increase in the number of glucose transporter proteins (21). Based on clinical evidence, a decrease in fasting glucose can be attributed in some ways to an increase in insulin function or a decrease in insulin resistance in response to interval exercise. In support of the findings of the present study regarding the reduction of insulin resistance in response to HIIT, a study by Ho et al. (2015) demonstrated that a 12-month weight loss program with diet restriction could significantly decrease insulin resistance and increase insulin sensitivity in overweight and obese individuals (22). The effectiveness of aerobic exercise on insulin resistance may be attributed to other exercise-induced changes. In this regard, Samjoo et al. (2013) reported that three months of moderate-intensity aerobic exercise improved oxidative stress markers as well as insulin resistance and inflammatory profile in obese individuals, independent of weight loss (23). The researchers noted that exercise has beneficial effects for reducing the risk factors associated with the pathogenesis of insulin resistance in obese populations. Some researchers have also attributed the decrease in blood glucose levels or glycemic profile to the improvement of inflammatory components in response to exercise. As mentioned earlier, improving insulin function at tissue levels plays a key role in improving the inflammatory profile. Steckling et al. (2016) demonstrated that glucose and HbA1C improvement after 12 weeks of HIIT at 70-90% of maximal heart rate is related to decrease in proinflammatory interleukins and increase in interleukin-10 levels (24).

Some recent studies have focused on the effect of genetic components on insulin signaling pathways. Others have considered the response of genetic components such as *FOXO1*, *IRS1*, *PPT1B*, and *FTO* that mediate insulin-dependent membrane glucose translocation in response to environmental stimuli, such as exercise. In this context, the eight-week HIIT increased *IRS1* expression in the gastrocnemius muscle of insulin-resistant obese rats. Consistent with this finding, Kirwan et al. (2000) reported that people with higher level of physical activity had higher IRS1 levels in the presence of hyperinsulinemia. In addition, the level of PI3K activity associated with maximal oxygen uptake was higher in this population than in inactive counterparts (25). The elevated levels of IRS1 are important in maintaining balance or reducing hyperglycemia in obese individuals. Other studies have also reported a 20-30% increase in membrane glucose uptake following the increase in IRS1 levels in response to exercise training. Activation of *IRS1* and *PI3K* is essential for glucose transporters activation in adipose and muscle tissues, and exercise activity is able to increase insulin receptor, IRS1, and MAP kinase activities in mammals (26). Moreover, continuous exercise activity could increase insulin sensitivity in target tissues of insulin (26). Another study also revealed that

increased *IRS1* expression may increase insulin sensitivity and decrease insulin resistance, thereby facilitating glucose transport into the cell membrane (27).

CONCLUSION

Our findings indicate that HIIT in form of treadmill running can increase insulin function in insulin-resistant obese rats. Given the important role of IRS1 in insulin signaling pathways, the decrease in insulin resistance and blood glucose levels may be attributed to the increased expression of *IRS1* in the gastrocnemius muscle of rats in response to HIIT. However, understanding the mechanisms involved in the effect of HIIT training on insulin function requires further studies.

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Ethics approvals and consent to participate

All procedures were carried out according to the ethical considerations in the care of laboratory animals. The study protocol was approved by the ethics committee of the Institute of Physical Education and Sport Sciences (ethical code: IR.SSRI.REC.1399.651).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this article.

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