Original Article

The effect of 8-week resistance training on IRS-1 gene expression in gastrocnemius muscle and glycemic profile in diabetes rats

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Abstract

Background: The insulin receptor substrate-1 (IRS-1) has an important role in insulin signaling pathways in the target tissue of obese or insulin-resistant individuals. This study aimed to assess the effect of resistance training on fasting glucose, insulin resistance, and IRS-1 gene expression in gastrocnemius muscle in male Wister rats with type 2 diabetes (T2D). **Materials and Methods:** For this purpose, T2D induced by high- fat diet (8 weeks) and STZ in fourteen male Wistar rats (220 ± 10 g) and then assigned into exercise (resistance training, 8 weeks, 5 days/weekly, n = 7) and control (no-training, n = 7) by randomly. Fasting blood samples were obtained for measuring glucose, insulin, and calculating insulin resistance (HOMA-IR). Also, the IRS-1 gene expression in gastrocnemius muscle was measured 48 hours after the last training session of both cases and controls. **Results:** Compared to control, IRS-1 gene expression in gastrocnemius muscle increased significantly by resistance training in exercise groups ($p = 0.001$). Fasting glucose ($p < 0.001$) and insulin resistance ($p = 0.007$) were reduced in the exercise rats compared to the control group. **Conclusion:** Based on the results, improved fasting glucose and insulin function after resistance training in T2D diabetes could be attributed to enhancing IRS-1 expression in gastrocnemius muscle by training.

Keywords: Insulin receptor substrate-1, Insulin resistance, Gene expression, Glucose

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Introduction

The prevalence of diabetes has increased dramatically in the last decade [1],and it will increase to 592 million by 2035 worldwide[2]. Diabetes has been recognized as one of the well-known causes of mortality in the world[3]. Increased insulin resistance in target tissues such as muscle tissue and adipose tissue is the most prominent cause of the disease[4]. This type of diabetes includes all individuals with insulin resistance who usually has relative (rather than absolute) insulin deficiency, and accounts for over 90% of all types of diabetes[4]. Although the underlying causes of this type of diabetes are not yet fully specified, scientific sources have confirmed that insulin resistance, rather than beta-cell dysfunction, is one of the primary causes of this type of diabetes[5].

Although inadequate diet and physical inactivity along with obesity have been linked to increased insulin resistance and the increased incidence rate of type 2 diabetes, clinical studies have specified genetics and heredity as the major contributors to the incidence of the mentioned disease. Genetic abnormalities in the pathways leading to the synthesis and secretion of insulin from the pancreas as well as genetic abnormalities affecting the insulin signaling pathways in target tissues (such as the liver

tissue, skeletal muscle, and adipose tissues) provide the basis for the incidence of this disease in susceptible individuals[6]. Impairments in the expression of genes or proteins such as Forkhead box-O1 (FOXO1), PPARγ, and fat mass and obesity-associated protein (FTO) have an influence on the energy homeostasis as well as the glucose and fat metabolism in target tissues such by affecting the lipolysis or insulin function[6, 7]. The associations between protein levels and their expression with obesity, lipid profile, and insulin resistance have been frequently reported[7, 8]. Among various types of proteins, insulin receptor substrate family (IRS-1, IRS-2) is of particular importance in glucose metabolism. Insulin receptor substrate-1 (IRS-1) is a cytoplasmic insulin receptor substrate and insulin-like growth factor receptor[9] and is a mediator of glucose homeostasis[10].

IRS-1 and IRS-2 are both involved in glucose metabolism; however, each affects different tissues. IRS-1 plays a major role in glucose metabolism and insulin function in the skeletal muscle, while IRS-2 is involved in insulin resistance and glucose metabolism in the liver and adipose tissues[11]. IRS-1 is a cytoplasmic substrate for insulin and an IGF1 receptor component that plays an important role in insulin signaling pathways. Also, some genetic association studies have revealed that alterations in protein levels and IRS-1 expression in insulin signaling pathways, especially along with the PI3K kinase pathway, cause a defect that partly affects the insulin resistance[12, 13]. Moreover, some studies have suggested the decreased IRS-1 gene expression in diabetic patients and have indicated its association with impaired insulin signaling pathways and increased insulin resistance in these patients[12]. The mentioned effects ultimately resulted in an increased blood glucose level. Thus, increased IRS-1 expression in target tissues such as the skeletal muscle by intrinsic or extrinsic stimuli or interventions appears to be associated with decreased insulin resistance and improved glycemic profile in diabetic patients. These interventions include dietary care, medication management, and regular physical activity[14]. Clinical studies have indicated that regular physical activity or exercise training along with dietary modifications plays a crucial role in the

prevention[15] and control[16] of type 2 diabetes. However, the mechanism responsible for type 2 diabetes is not well-recognized.

In this regard, Hamada et al. (2006) have suggested that a single exercise session leads to increased glucose uptake in skeletal muscle cell membranes through insulin-dependent mechanisms, and this effect can be maintained for several hours after the exercise[17]. Some of the benefits of exercise training appear to be related to its effect on Jun N-terminal kinases (JNK) and IRS changes[18]. In this respect, Ruppel et al. (2006) found that a single session of exercise associated with a decrease in the high-fat diet-induced insulin resistance in laboratory rats. The mentioned study has attributed the observed decrease to the increased JNK activity and increased IRS-1 expression[19].

Regarding the effect of exercise training on the expression of genes affecting the synthesis and secretion of insulin from the pancreas or insulin function in the target tissue, the studies by Eizadi (2016) and Soori (2017) showed that 12 weeks resistance training resulted in a significant decrease in TCF7L2 and MTNR1B expression in pancreas tissue and increase in serum insulin in male rats with type 2 diabetes[20, 21]. On the other hand, decreased PTP1B expression and increased GLUT4 expression as genes involved in insulin function in skeletal muscle or adipose tissue in response to aerobic and resistance training have been reported by several studies[22, 23]. However, the effect of long-term exercise training, especially resistance training on IRS-1 expression in the skeletal muscle in type 2 diabetic rats has not been reported so far. Therefore, given the effective role of IRS-1 in insulin function of muscle cells, the present study aimed at determining the effect of eight weeks of resistance training on IRS-1 expression in gastrocnemius muscle, insulin resistance, and blood glucose levels in type 2 diabetic rats.

Methods

Here, forty 10-weeks-old male Wistar rats (220 ± 10 g), procured from the institutional animal house facility were used for all the experiments and after induction of T2D were randomly divided into exercise (resistance training, 8 weeks, 5 days/weekly, n = 7) and control (no training, n = 7) groups aimed to

determine the effect of resistance training on IRS-1 expression in gastrocnemius muscle, fasting glucose and insulin resistance. Animals were maintained under standardized conditions (12-h light/dark cycle, 25 ± 2 °C & humidity 45-55 %). The rats were left for 1 week for acclimatization before the commencement of the experiment. The study was approved by the Department of Exercise physiology of Islamic Azad University, Saveh Branch, Iran and carried out under CPCSEA (Committee for Control and Supervision of Experiment on Animal) guidelines.

Induction of type 2 diabetes. T2D was induced by 8 weeks high-fat diet (HFD)[24] and followed by a single intraperitoneal (i.p.) injection of 25 mg/kg streptozotocin (dissolved in citrate buffer, pH 4.5). It should be noted that the high- fat diet was continued to the end of the study for both groups. Hyperglycemia was confirmed by elevated blood glucose levels on day 7 after diabetes induction and only animals with fasting blood glucose levels between 150- 400 mg/dl were selected for were served as T2D rats and used in the study [20].

Resistance training protocol. The exercise group was climbed on a stepladder a 26-step, 1-meter vertical ladder with a gradient of 80% without any resistance 6 times in 3 training sessions to learn how to exercise. Then they completed a resistance training lasted 8 weeks for 5 days in weeks. To warm up and cool down, the rats before and after the workout, they were climbed and descended the ladder 2 times without any resistance.

Each exercise session was performed in the form of 5 set with 4 repetitions on each set, and the resistance was increased through attaching a weight to rats' tails. The rest between sets and between repetitions was 3 min and 45 sec, respectively. The resistance was increased gradually during training intervention (see Table 1). Finally, all rats were dissected 48 hours after the last training session following 10 to 12 hours overnight fasting. It should be noted that diabetic control rats were not included in the training program during this period.

Table1. Distribution pattern of exercise intensity in the resistance training group based on a percentage of body weight

Time (weeks)	Resistance (bodyweight %)
1	30
2	40
3	50
4	60
5	70
6	80
7	90
8	100

Sample Collection and Biochemical Assays. The rats in each group were anesthetized by intraperitoneal injection of 10% ketamine (50 mg/kg) and xylazine 2% (10 mg/kg) 48 hours after the last training session (10 to 12 hours fasting). Then, rats were anesthetized and blood samples were collected through cardiac puncture. After that, gastrocnemius muscles were removed and immersed in RNAlater Solution until gene analysis was performed to determine IRS-1 expression. The blood samples were used to analyze glucose and serum insulin. The serum was separated by centrifugation (5 min, 3,000 rpm) and was analyzed for glucose using a Cobas 6000 Analyzer (Roche, Germany). Glucose was measured by enzymatic colorimetric assay using glucose oxidase technology (Pars Azmoon kit, Tehran). The intra-assay and inter-assay coefficient of variation of the method were 1.74% and 1.19 respectively for glucose. Insulin was measured by the ELISA method (Demeditec, Germany). The intra-assay and inter-assay coefficient of variation of the method were 2.6% and 2.88 respectively for insulin. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated by the formula fasting blood glucose (mg/dl) x insulin (uIU/ml)/405[25].

Real-time – PCR. The RNA was extracted by RNeasy protect mini kit (QIAGEN) from gastrocnemius muscle according to manufactures instructions[26]. Real-time PCR quantification of IRS-1 mRNA was performed with the Rotor gene 6000

system using One-Step SYBR Prime Script RT PCR kit (Takara co.) according to manufactures

instructions. We used RNA polymerase II as a control gene.

Table2. The primer sequences of the studied gene.

Genes	Primer sequence	Product size	Tm	Gene Bank
IRS-1	For: GGCCATGAGCGATGAGTTTC Rev: GGCGGAGGATTGTTGAGATG	159bp	60	NM_001191052.1
RNA Polymerase II	For: ACTTTGATGACGTGGAGGAGGAC Rev: GTTGGCCTGCGGTCGTTC	164 bp	60	XM_008759265.1

Statistical analysis. All statistical analyses were performed through the use of a statistical software package (SPSS, Version 15.0, SPSS Inc., IL, USA). Data were tested for normal distribution by the Kolmogorov-Smirnov test. Comparisons between the means of each group were done using the independent t-test. The differences between the groups were considered to be significant at a p-value of ≤ 0.05.

Results

Pre and post-training of body weight for 2 groups are shown in Table 3. Data represented by Mean and standard deviation. Based on the independent t-test, no significant difference was found between 2 groups at pre-training. On the other hand, despite a significant increase in body weight at the end of the training program compared to baseline for 2 groups, no significant difference was found between 2 groups at post-training.

Table3. Pre and post-training of body weight of 2 groups (Mean ± SD).

Group	Pre-training	Post-training	sig
Control	302 ± 11	377 ± 13	< 0.001
Resistance	297 ± 18	384 ± 9	< 0.001
sig	0.518	0.260	-

The effect of resistance training on IRS-1 gene expression in gastrocnemius muscle was the main aim of the present study. Based on analysis data, IRS-1 expression was significantly higher in the exercise group than in control rats. In other words, 8-weeks resistance training resulted in a significant increase in IRS-1 expression in gastrocnemius muscle compared with control subjects (Table 4, Fig 1).

Table4. Fasting glucose and insulin resistance after training intervention of exercise and control groups (Mean ± SD).

Variable	Control group	Exercise group	sig
Fasting glucose (mg/dl)	301 ± 23	190 ± 15	< 0.001
Insulin resistance (HOMA-IR)	3.82 ± 0.48	3.04 ± 0.41	0.007
IRS-1 expression	1	1.61 ± 0.37	0.001
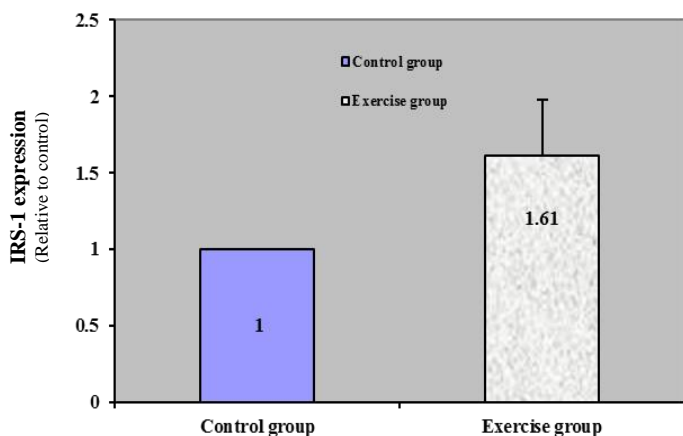
Fig 1: IRS-1 expressions in gastrocnemius muscle in exercise rats compare to control group.

Fig 3: Insulin resistance after resistance training in exercise and control groups.

Significant differences were also observed between 2 groups concerning fasting glucose and insulin resistance. On the other hand, resistance training resulted in a significant decrease in fasting glucose (Table 4, Fig 2) and insulin resistance (Table 4, Fig 3) compared with control subjects (Table 4, Fig 3).
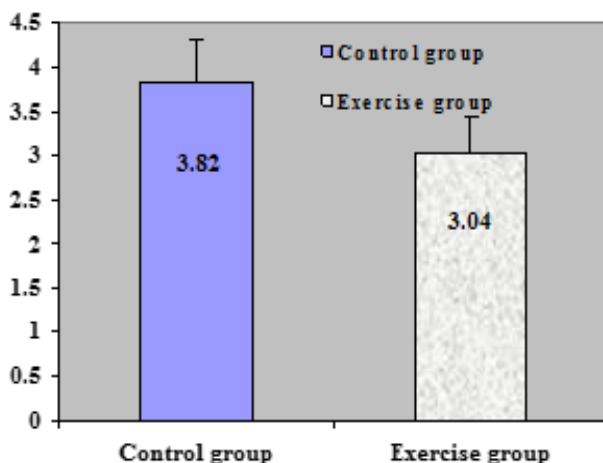
To assess the effect of vitamin D3 on TLR2 and TLR4 expression, human monocytes were exposed to 10^{-9} M of vitamin D3. We found that vitamin D3 suppresses the mRNA expression of TLR2 and TLR4 in patients with type II diabetes. TLR2 and TLR4 expression in the monocytes exposed to vitamin D3 are significantly decreased in comparison with monocytes not treated with vitamin D3 ($P<0.05$) (Figure 2).
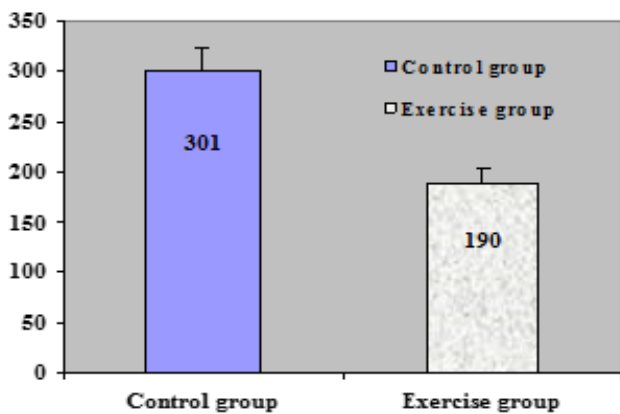

Fig 2: Fasting glucose after resistance training in exercise and control groups.

Discussion

The increased IRS-1 expression in response to resistance training was the main finding of the present study. In other words, eight weeks of resistance training over five sessions per week significantly increased the relative IRS-1 expression in the gastrocnemius muscle of type 2 diabetic rats as compared to the control group that did not participate in the training program.

However, Kirwan et al (2000) have revealed that individuals with higher levels of physical activity have higher IRS-1 levels in the presence of hyperinsulinemia, and the activity level of PI3K that is associated with the improvement of the maximum

oxygen uptake is higher in the mentioned individuals as compared with inactive ones[27]. Theses researchers have declared that increasing IRS1 levels are important in maintaining balance or reducing hyperglycemia. Also, the results of the study conducted by Hawley et al. (2008) indicated that the effects of the exercise training on IRS-1 and IRS-2 varied widely due to differences in the exercise stimuli (type, intensity, and duration of exercise), past diets, the level of training, and the muscles or the type of the muscle fibers[28]. Furthermore, some studies have also indicated that the expression level of IRS-1 protein increases after one day of exercise training, while it is reduced by 50% 16 hours after the chronic training (five days of swimming)[29].

In addition to increased IRS-1 expression, a decrease in glucose levels following the resistance training was also found in the present study. There are sometimes contradictory findings regarding the response or adaptation of glucose to different exercise methods. Vancea (2009) and Maltais (2016) have reported no changes in the glucose level after 20 weeks of aerobic training and four months of resistance pieces of training respectively [30, 31] However, in line with the findings of the present study, Glans et al. (2009) reported a decrease in the plasma glucose level after six months of aerobic and resistance training in diabetic patients [32]. In another study, 12 weeks of exercise training for three 60-minute walking sessions per week resulted in a significant decrease in the level of fasting glucose [33]. Sheu et al. (2004) also reported a significant decrease in the blood glucose levels after 12 weeks of aerobic training in combination with a diet intervention in obese non-diabetic women [34]. Some studies have attributed the improvement in the blood glucose to changes in the insulin function in the target tissues or, in other words, a decrease in insulin resistance. It should be noted that the decrease in insulin resistance in response to resistance training is another finding of the present study. In line with the findings of our study, Ho et al (2015) have ascribed the improvements in the blood glucose levels following a 12-month weight-loss program involving a diet management program to the reduced insulin

resistance along with an increased level of insulin sensitivity in overweight and obese individuals [35]. Furthermore, Samjoo et al (2013) reported a significant decrease in blood glucose along with improvements in oxidative stress markers, insulin resistance, and inflammatory profile after 3 months of aerobic training [36]. In another study, 12 weeks of moderate-intensity aerobic training (Abd El-Kader, 2013) resulted in not only a decrease in the hemoglobin A1c and glucose but also a decrease in insulin resistance in type 2 diabetic men and women[37].

Moreover, based on in vitro evidence, a decrease in insulin resistance or, in other words, an increase in insulin function in the target tissues such as fatty or muscle tissues can be attributed to exercise-induced hormonal or genetic changes. Some studies have reported a 20-30% increase in the membrane glucose uptake due to increased IRS-1 levels in response to exercise training. Researchers in this study have suggested that the activation of IRS-1 and PI3K is essential for GLUT4 activation in fatty and muscle tissues, and the exercise training can increase insulin receptor, IRS-1, and MAP kinase in mammals [38]. Also, Delpasand et al (2018) also revealed an increased IRS-1 expression after a two-month low-calorie diet combined with aerobic exercise in obese rats [39]. Laboratory studies have revealed that insulin initiates a wide range of growth and metabolic effects by binding to its receptor and activating tyrosine kinase. These events lead to the phosphorylation of tyrosine kinase residues at the level of anchor proteins of insulin receptor substrate proteins (IRS) [40]. In this regard, Carvalho et al (1999) suggested an inverse relationship between IRS-1 expression and insulin resistance and declared that the transcriptional degradation of this gene plays a key role in the insulin resistance and the pathogenesis of diabetes and reducing IRS-1 protein levels and decreasing its expression in fatty tissues increase the likelihood of type 2 diabetes in susceptible individuals [41]. Moreover, clinical studies have also revealed that proper dietary patterns and regular exercise training are associated with decreased insulin resistance in obese or type 2 diabetic patients [42]. Regular exercise training has

been proven to increase insulin sensitivity in the fatty and muscle tissues by accelerating insulin signals, especially at the cascade level dependent on IRS-1 and GLUT4 at post-transcriptional stages [38]. Besides, other studies have revealed that an increased IRS-1 expression level in response to some stimuli can increase insulin sensitivity and decrease insulin resistance, thereby facilitating the entry of glucose into the cell membrane [43].

Conclusion

Collectively, it seems that eight-weeks of resistance training leads to decreased glucose and insulin resistance in type 2 diabetic rats. Based on our findings as well as previous evidence, this improvement may be attributed to an improved insulin function induced by IRS-1. IRS-1 expression was significantly increased in the gastrocnemius muscle of rats following resistance training. Further studies are required to provide an understanding of the mechanisms responsible for the role of exercise-dependent IRS-1 alterations on insulin signaling pathways at the target tissue.

Conflicts of Interest

None declared.

Acknowledgment

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