

Effect of aerobic training intensity on irisin in streptozotocin-induced diabetic rats

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Abstract

Aim: Irisin is a recently identified exercise-induced hormone that increases energy expenditure. The effect of chronic aerobic training intensity on irisin concentration is not well known. Thus, we examine the effect of aerobic training intensity on irisin in streptozotocin (STZ) -induced diabetic Wistar rats.

Material & Methods: Diabetes was induced by intraperitoneal injection of STZ. Animals were randomly divided into four groups (n=8 in each group): control group (CON), diabetes group (D), diabetes and moderate-intensity exercise group (D + ME; running speed was set at 10-17 m.min⁻¹), and diabetes and high-intensity exercise group (D + HE; running speed was set at 17-28 m.min⁻¹). The rats in the exercise groups

were made to run on the treadmill for 30 min per one day, 3 times a week, during 8 weeks.

Results: The results indicated that serum irisin concentration was higher in the D + HE group than CON, D and D + ME groups ($P < 0.05$) and no significant differences were observed between CON group and D + ME group.

Conclusions: Our findings suggest that that exercise intensity has an effect on exercise-induced irisin responses.

Key words: Exercise intensity, Irisin, Diabetes mellitus, Myokine

1. Introduction

Clinical studies have shown that skeletal muscle as a secretory organ, can produce physiological active substances that are referred to as myokines (1). Irisin is a novel glycosylated polypeptide hormone derived from muscle after shedding of the extracellular portion of the type I membrane protein fibronectin type III domain-containing protein 5 precursor gene (2). After its release, irisin signals adipose tissue to become more similar to brown-like adipocytes. The proposed beneficial effects include the browning of white adipose tissue and increased thermogenesis, which promotes insulin sensitivity, body weight, and glucose tolerance in mice (3). Irisin has been proposed to improve glucose homeostasis by increasing fatty acid oxidation and utilizing glucose via the AMPK signaling pathway in diabetic mice (4). Irisin is related more strongly to insulin resistance than other myokines (5) and recent studies have demonstrated that serum irisin levels were lower in patients with type 2 diabetes mellitus (DM) compared with nondiabetic patients (6,7).

Aerobic training is a useful therapy for improving DM and it is important to investigate whether or not these training improve DM by altering the irisin concentrations. Several studies exploring the effects of exercise on circulating irisin levels have resulted in inconsistent findings. Circulating irisin increased (8-10) not affected (11,12) or decreased (13) in response to exercise.

Little is known about how the intensity of aerobic training influences irisin concentration. Egan et al. (2010) reported that a single bout of endurance high-intensity running exercise caused greater increase in PGC-1 α (a primary factor for irisin secretion) compared with isocaloric low intensity exercise at 3 h after exercise (14). Tsuchiya et al. (2014) also indicated that a single bout of high-intensity exercise causes greater irisin response compared with low-intensity exercise under similar energy consumption (15). By our knowledge, there is no study that was performed to examine the effect of chronic aerobic training intensity on irisin concentration. Thus, we examine the effect of high-intensity exercise (HE) *vs.* moderate-intensity exercise (ME) on irisin in streptozotocin (STZ) -induced diabetic rats.

2. Material and methods

Animals

The present study was approved by the Ethics Committee on Animal Use of Marvdasht branch, Islamic Azad University, Marvdasht, Iran. Adult male Wistar rats weighing 174.5 ± 15.6 g (at the beginning of the study) were used in this experiment. The animals were kept in accordance with the Guide to the Care and Use of Experimental Animals (1993).

Rats were submitted to seven days of acclimatization in polypropylene boxes (dimensions 41 cm \times 34 cm \times 17.5 cm), containing wood shavings (for absorbing urine and water). Six animals were placed in each box. Throughout the experimental period, rats were housed under controlled temperature ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$), humidity ($45\% \pm 15\%$) and lighting conditions (7:00 a.m. to 19:00 p.m.) with food and water made available *ad libitum*. Animals were randomly divided into four groups (n=8 in each group): control group (CON), diabetes group (D), diabetes and moderate-intensity exercise group (D + ME), and diabetes and high-intensity exercise group (D + HE).

Induction of diabetes

At the end of the acclimatization period, all animals were submitted to type 2 DM induction protocol as described by Wang et al. (16). To

induce diabetes, a single intraperitoneal injection of STZ (50 mg/kg, dissolved in 0.01-M citrate buffer at pH 4.5; Sigma Chemical Co.) was given to each animal. Blood glucose levels were determined 3 days after STZ injection using a blood glucose tester (Arkray, Kyoto, Japan). Rats with blood glucose levels above 300 mg/dL were used in the diabetes groups (17). Blood samples were obtained from a cut at the tip of the animal's tail.

Treadmill exercise protocol

In the first week of the preliminary experiments, the rats were adapted to treadmill (Danesh Salar, Tehran, Iran). The adaptation consisted of 10 min of exercise at a speed of 8 m.min⁻¹ on a 0° incline. For the D + ME group, the running speed was set at 10 m.min⁻¹ with 0° incline during the first 4 weeks of training. For the next 4 weeks, the running speed was set at 17 m.min⁻¹ on a 0° incline. For the D + HE group, the running speed was set at 17 m.min⁻¹ with 0° incline during the first 4 weeks of training and running speed was increased for the next 4 weeks to 28 m.min⁻¹ on a 0° incline. Electric shocks were used sparingly to motivate the animals to run. Electrical shocks were applied to the metal grid behind the lane to stimulate rats that failed to run spontaneously. The rats in the exercise groups were made to run on the treadmill for 30 min per one day, 3 times a week, during 8 weeks. The non-exercise groups remained in their cages.

Biochemical measurements

At the end of the experimental period (eight weeks), the animals fasted for eight hours. Euthanasia was conducted by cardiac puncture under anesthesia (sodium thiopental 50 mg/kg ip). Serum irisin (Eastbiopharm Co., Ltd. Hangzhou, China) was quantified using Rat ELISA Kits following the manufacturer's instructions.

Statistical Analysis

Results were expressed as the mean \pm SD and distributions of all variables were assessed for normality. For the comparison among the groups, one-way analysis of variance (ANOVA) was performed followed by Bonferroni *post hoc* test. Pearson correlation and general linear

regression analysis were performed to calculate a correlation. Data analyses were performed using SPSS software for windows (version 17, SPSS, Inc., Chicago, IL) and statistically significant differences were established at $P < 0.05$.

4. Results

Changes in body weight

Body weight changes are presented in Table 1. Body weight in the CON group ($t = -2.3$, $P = 0.04$), D + ME group ($t = -2.5$, $P = 0.008$) and D + HE group ($t = -2.5$, $P = 0.03$) was increased after the intervention. Although the body weight in the D group was decreased after STZ injection, this did not achieve statistical significance.

Table 1. Changes of body weight during the intervention (mean \pm SD)

Groups	0 week (gr)	8 week (gr)
CON	176.1 \pm 54.2	188.8 \pm 58.6*
D	204.1 \pm 52.6	191.8 \pm 79.6
D + ME	140.6 \pm 23.4	167.7 \pm 41.4*
D + HE	144.0 \pm 17.9	166.6 \pm 29.9*

Changes in biochemical variables

One-way ANOVA test indicated that serum irisin concentration was higher in the D + HE group than CON, D and D + ME groups ($P < 0.05$). Serum irisin was lowest in the D group in compare with other groups and no significant differences were observed between CON group and D + ME group; however, serum irisin was higher in the D + ME group in compare with the D group ($P < 0.05$; see Figure 1).

5. Discussion

Exercise training may improve DM by altering the irisin concentrations. The effects of aerobic training intensity on irisin concentration are not well known; therefore, we examine the effects of different intensity of aerobic training on irisin concentration in STZ-induced diabetic rats.

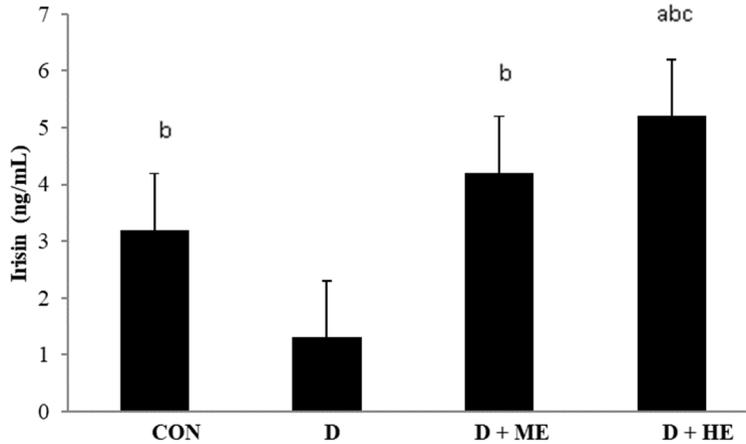


Figure 1. Mean (\pm SD) changes in irisin concentration
a. Significant differences with CON group
b. Significant differences with D group
c. Significant differences with D + ME group

Recently, it has been established that high serum irisin was independently associated with the development of DM and irisin is a useful predictor of DM (7). Irisin plays an important role in energy metabolism (22), glucose tolerance, increasing the expression of GLUT-4 and mitochondrial biogenesis (23) and, further, that irisin can change the browning of adipose tissue in exercise subjects (24).

The results showed that irisin concentration was increased after HE ($P < 0.05$) and although irisin level was higher in the D + ME group in compare to the D group, but there was no significant differences were observed between D + ME group in compare to the CON group. Thus it seems that chronic HE is more effective than ME for enhancement of irisin concentration in STZ-induced diabetic rats. Previously, it has been shown that a single bout of high-intensity endurance exercise caused greater increase in irisin concentration compared with isocaloric low intensity exercise (14,15,25). Tsuchiya et al. (2014) noted that HE caused significantly greater responses of blood lactate and serum LDH, indicating that metabolic and mechanical stimuli for skeletal muscles were higher during HE than low-intensity exercise (15). Skeletal muscle is an endocrine organ that can produces myokines (1), which are released into the circulation during exercise. Peroxisome proliferator-activated

receptor γ coactivator 1α or PGC- 1α is a molecule involved in the regulation of gene expression that plays a critical role in the maintenance of glucose, lipid, and energy homeostasis (26). In the original study, Boström et al. (2012) showed that murine skeletal muscles, upon increased levels of PGC- 1α , induce the expression of a protein called fibronectin type III domain containing 5 (FNDC5), which after cleavage is secreted into the blood stream as irisin (2). As per the molecular mechanism, exercise is the stimulus for release of PGC- 1α which is a coactivator of PPAR γ (involved in energy metabolism). This in turn stimulates expression of FNDC5 which in turn is proteolytically cleaved to release the active hormone, irisin (29). Irisin has cell surface receptors. It increases the expression of UCP1 and Cidea mRNA, which causes browning of primarily subcutaneous and also of visceral adipose tissue and thereby inducing thermogenesis (30). White adipose tissue, which is a storehouse of energy, is converted to brown adipose tissue which dissipates energy as heat. This in turn causes increase in total body energy expenditure (29).

Boström et al. (2012) noted that changes in Irisin were accompanied by an increase in total body energy expenditure, modest weight loss, and modest improvements in glucose intolerance. They also showed an increased FNDC5 expression after 10 weeks of endurance exercise in obese male type 2 DM patients (2). In summary, these findings suggest that irisin secretion after chronic aerobic exercise is affected by exercise intensity in STZ-induced diabetic rats.

6. Conclusion

Generally, we hypothesized that HE would cause greater irisin response than ME. The present results support our hypothesis, that exercise intensity has an effect on exercise-induced irisin responses.

Conflict of interests: No conflict of interests amongst authors

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