Interactive effect of endurance exercise, resistance exercise, and cold weather on irisin changes in diabetic male rats

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Abstract

Introduction: The irisin hormone secreted by human and mouse muscles has positive effects on obesity and diabetes and can be a promising alternative in treatment of metabolic diseases. In the present study, we investigated the effect of eight weeks of endurance and resistance exercise activities and exposure to cold temperature on plasma irisin levels of diabetic Wistar rats.

Materials and Methods: Number of 46 obese male Wistar rats (12-week-old) weighing 325 ± 2 g with type 2 diabetes mellitus-induction of diabetes by STZ injection- were randomly divided into six groups. Two groups received the resistance exercise (8 weeks, three days a week, eight repetitions per day, fence length 1.35 m and slope 85 degrees) at normal (23 ± 2 °C) and cold (16 ± 2 °C) temperatures, two groups were allocated to endurance exercise (8 weeks, five days a week, at a speed of 20 meters per minute and a slope of 15%) at the normal and cold temperatures, the last two groups were control groups (without training intervention) at the ordinary and cold temperatures. 48 hours after the last training session, serum samples were collected while the rats were in fasting. Irisin levels were assessed by the ELISA method. Data were analyzed using SPSS software version 16 and one-way ANOVA and Tukey post hoc test ($P<0.05$).

Results: The results of one-way ANOVA showed a significant increase in serum irisin values in groups of endurance exercise and resistance exercise compared with control groups in both temperatures ($P<0.05$). There was a significant difference between fasting blood sugar levels in the endurance exercise and resistance exercise groups compared with the control groups at both temperatures ($P<0.05$). A significant decrease in the ratio of LDL to HDL was observed in the groups of endurance exercise and resistance exercise compared to the control groups at both temperatures ($P<0.05$).

Conclusion: Endurance and resistance exercises increase irisin protein and decrease fasting blood sugar and the ratio of LDL to HDL. It seems that irisin may be used as a possible treatment to improve diabetes, the change of lipid profile, and energy homeostasis.

Keywords: Cold temperature, Endurance exercise, Irisin, Ordinary temperature, Resistance exercise, Diabetic rat

Introduction

Diabetes and obesity-related diseases are among major healthcare challenges. Type 2 diabetes is the most common type of diabetes, characterized by a wide range of metabolic disorders such as increased hepatic

gluconeogenesis, impaired glucose uptake, metabolic inflexibility, and mitochondrial dysfunction (1). Many crucial studies have shown that exercise, alone as a treatment or in combination with lifestyle interventions such as dietary recommendations or weight loss, can prevent type 2 diabetes up to 50% (2). Interestingly, the effects of lifestyle changes are stronger than those obtained with metformin drug intervention (3). Thus, physical activity can play an important role in preventing and managing type 2 diabetes, and understanding the mechanisms by which physical activity improves insulin sensitivity may be effective in optimizing non-drug therapies. The discovery of myokines is a new way to understand the biology of exercise by providing evidence that muscles can communicate with other organs such as bone, liver, adipose tissue, brain, etc. (4,5). One of this myokine is irisin.

After proteolysis cleavage, glycolysis, and possibly dimerization of FNDC5, a new protein consisting of the fibronectin III domain is released. This protein, which is made up of 112 amino acids, is called irisin (5). Irisin leads to the expression of unpaired protein-1. An increase in irisin enhances oxygen consumption, improves glucose tolerance and insulin resistance, and facilitates weight loss compared to untrained individuals (5, 6).

New research indicates that irisin improves insulin resistance and type 2 diabetes (1). Irisin separation has been shown to be useful in explaining metabolic events, especially adipose tissue metabolism (7,8). Exercise promotes adaptations in skeletal muscle and white adipose tissue and protects against metabolic disorders, including obesity and type 2 diabetes. Exercise changes the profile of myokines (secreted by skeletal muscle) and adipokines (secreted by adipose tissue). These secreted proteins may act based on the endocrine method by facilitating tissue-to-tissue communication or cross-talk, possibly together to improve overall metabolic health. Some studies demonstrate that skeletal muscle contraction releases myokines that can cause white adipose tissue to beige so that beige fat marker genes and cells with small fat particles in white adipose tissue are increased (9). The physiological activity and regulation of irisin are not yet fully understood, and further studies are needed to identify the underlying mechanisms responsible for activating FNDC5 or irisin and its receptors. Numerous studies have examined the potential factors affecting irisin secretion. The majority of research literature on the effect of exercise on glycemic parameters in type 2 diabetes is related to aerobic exercise interventions. Aerobic exercise such as walking, jogging, and cycling involves the continuous and rhythmic movements of large muscle groups. The latest American Diabetes Association (ADA) guidelines state that individual sessions of aerobic exercise should ideally be performed for at least 30 minutes a day, three to seven days a week (10). Based on the growing evidence about the role of resistance training in glycemic control, ADA and the American College of Sports Medicine (ACSM) have recently updated their exercise guidelines for the prevention and treatment of type 2 diabetes to include resistance training (11).

Exercise can stimulate the secretion of irisin from skeletal muscle through a variety of mechanisms (12). Several studies have reported that an endurance exercise session enhances irisin secretion, but the results of these studies are not conclusive. Data from human studies have revealed that irisin concentrations increase significantly during (13) and immediately after the endurance exercise (14-17). In contrast, Pekala et al. (18) reported that irisin concentrations did not change the acute endurance activity in middle-aged men. Irisin's response to resistance training has also been investigated (19,20). Pekala et al. (18) indicated that in

resistance exercise, mRAN FNDC5 increased approximately 1.4-fold in muscle compared to baseline values, whereas plasma irisin concentrations did not change within 30 minutes after the exercise. In contrast, Huh et al. (17) recently found that resistance exercise (after 45 minutes) produced more irisin responses (measured immediately after exercise) than endurance exercise (after 36 minutes).

Some studies have revealed a significant reduction in irisin after the endurance and resistance training (21), and some have indicated a significant increase in irisin (22) or no significant change (23,24). Also, some studies have suggested that irisin secretion increases more in resistance training than in combination and endurance training (25). The results of some studies also show that as the temperature decreases and the amount of muscle vibration activity increases, the amount of irisin secretion also increases (26, 27).

Previous research has reported only the effect of exercise or exposure on the cold temperature conditions (28) and only a few studies have examined the simultaneous effect of exercise activity and cold temperature (27, 29-31). The intensity of exercise seems to be an effective factor on irisin secretion because changes in serum irisin levels after low-intensity exercise has not been observed. Some studies have shown that aerobic exercise, like other resistance exercises or strenuous strength training, stimulates an increase in circulating irisin levels. Finally, exposure to cold increases human irisin secretion, possibly through tremor-dependent muscle contraction. When interpreting the results of the exercise activity-based studies, it must be remembered that there is a high degree of heterogeneity between research projects that make valid and generalizable conclusions somehow difficult (32,33).

Overall, differences in the results of previous research, as well as ambiguity in the effect of temperature on irisin secretion, as well as the determination of a more efficient training protocol, confirm the need for such investigations.

Therefore, this study aimed to investigate the interactive effect of endurance exercise, resistance exercise, and cold weather on irisin changes, blood sugar, and index of lipid in diabetic male rats.

Materials and Methods

In this experimental study, forty-six obese male Wistar rats (12-week-old, weighing 325 ± 2 gr) were used. The rats were purchased from the Animal Breeding Center of Hamadan University of Medical Sciences and they were placed in the animal laboratory of Hamadan University of Medical Sciences. The rats were kept for 2 weeks in a cage made of plexiglass under the controlled conditions, i.e., average ambient temperature 23 ± 2 °C, humidity 45 ± 2 %, and 12-h light/12-h dark cycles with free access to food and water. After two weeks of familiarity with the laboratory environment, the animals were randomly divided into six groups: Three groups of endurance exercise (n=8), resistance exercise (n=8) and control group (n=7) at the ordinary temperature (23 ± 2 °C); and three groups of endurance exercise (n=8), resistance exercise (n=8) and control group (n=7) at the cool temperature (16 ± 2 °C). A total of four training groups performed endurance and resistance exercise protocols at normal temperatures. For induction of diabetes, streptozotocin (Sigma Aldridge, made in the USA) (60 mg per kilogram of body weight) was dissolved in sodium citrate buffer (4.5 pH) (1 ml per kilogram of body weight) and it was injected intraperitoneally. 48 hours after the injection, blood samples were taken from the tail vein of rats to check their fasting blood sugar. A blood glucose level of 250 and above was considered as a

criterion of diabetes (35). All stages of keeping and slaughtering rats were carried out following the rules of the Animal Ethics Committee of the Institute of Physical Education and Sports Sciences with the ethics code of IR.SSRC.REC.1398.142.

Endurance Exercise Protocol

During one week, five days/a week with a speed of 15 meters per minute, for five minutes a day, on the treadmill with a slope of zero (2016 model, made in Iran), the rats were familiarized with endurance exercise. After the familiarity, they performed an endurance exercise program for eight weeks. The daily time and weekly speed increased so that in the fourth week the animals practiced at a speed of 20 meters per minute with a slope of 15% for 60 minutes per day. For the remaining four weeks, fixed time, speed, and slope, were maintained. At the beginning and end of the training, the rats warmed up and cool-down on the treadmill for five minutes at zero incline and 10 meters per minute. If the rats stopped at the end of the treadmill and did not run, the tail of the rats would be touched to continue running (36) (Table 1).

Table 1. Changes in the duration and speed of endurance protocol.

Exercise parameter	First week	Second week	Third week	Fourth week
Exercise duration (min)	5-17	20-32	35-47	50-60
Speed (m/min)	15	17	19	20
Slope	15	15	15	15

Resistance Exercise Protocol

Rats were familiarized with resistance exercise for one week, three days a week with four repetitions per day. They were placed below a ladder (135 cm length; two centimeters' distance, 85 slope; ladder had been made according to reference article standards) without attaching weights to their tails. The rats were encouraged to climb the stairs by touching the tail. After each ascent to the top of the ladder, they rested for a minute in a special place. After one week of adaptation, the first session of the main exercise began with an intensity of 30% of rats' body weight. When a rat with attaching weights to its tail reached the resting place on the ladder, it could rest for one minute. In the subsequent climbs, 15 grams were added to the initial load. Each training session had eight repetitions, and the exercise ended when the rat completed eight repetitions. To warm up and cool down at the beginning and end of the training, the rats, climbed the ladder twice without weights. At the end of eight weeks of resistance training, the rats carried a load equal to 100% of their body weight or the maximum load that they could carry (30).

Biochemical Assays

The rats were anesthetized 48 hours after the last practice session in both exercise protocols (in the fasting state nightly) using ketamine and xylazine (60 to 80 mg of ketamine per kilogram of body weight and eight milligrams of xylazine per kilogram of body weight) and the samples were isolated. The rats were destroyed by taking blood from the left ventricle and cutting off the head. Blood samples were kept at room temperature for one hour and then placed in a centrifuge for 15 minutes at 15,000 rpm. The serum samples were injected into micro-tubes by pipette and stored at -80C for subsequent use. Serum irisin samples were evaluated by the Kite-ELISA manufactured by the German company ZelBio (ZB-16281C-R9648, the diagnostic sensitivity of 0.05ng / ml). Also, the amount of glucose, LDL, and HDL in serum samples was assessed by the ELISA method (using Pars

Azmoun kit, made in Iran) in training and control groups.

Statistical Analysis

The normal distribution of the data was assessed using the Kolmogorov-Smirnov test and homogeneity of variances was checked by Levene's test. Then, the one-way ANOVA and Tukey post hoc tests were used to examine the between-group differences. The statistical significance level was P< 0.05.

Results

The average weight, blood glucose, the ratio of LDL to HDL, and irisin of rats after eight weeks in exercise and control groups at both ordinary and cold temperatures are shown in Table 2. According to Table 2, there were no significant differences in the weight changes of rats in the training and control groups at both temperatures (P<0.05).

Table 2. Changes in irisin, weight, fasting blood glucose, and the ratio of LDL to HDL after eight weeks in exercise and control groups at both ordinary and cold temperatures.

Groups	Irisin (ng/ml)	LDL/HDL (mg/dl)	FBS (mg/dl)	Weight (g)
Endurance group OT	0.38 ± 0.02*	0.44 ± 0.19*	478.00 ± 6.24*	238.41 ± 5.03
Resistance group OT	0.35 ± 0.05*	0.35 ± 0.09*	504.00 ± 8.46*	239.83 ± 6.41
Control group OT	0.26 ± 0.02	0.90 ± 0.19	558.00 ± 8.73	240.00 ± 1.22
Endurance group CT	0.39 ± 0.03*	0.48 ± 0.10*	472.00 ± 73*	238.64 ± 4.33
Resistance group CT	0.37 ±0.03*	0.41 ± 0.03*	478.33 ± 9.07*	240.00 ± 2.70
Control group CT	0.26 ± 0.05	0.99 ± 0.24	542.67 ± 6.42	240.04 ± 1.21

* Significant difference with control groups (P<0.05).
OT: ordinary temperature; CT: cold temperature; FBS: fast blood sugar.

According to Table 2, the means of values of irisin in exercise groups in both temperatures increased. One-way analysis of variance showed a significant difference between groups in serum irisin levels. Based on the results of Tukey's post hoc test, there was a significant difference between the endurance exercise group (p=0.002) and resistance exercise (p=0.008) on one hand and the control group at the ordinary temperature on the other hand. Moreover, significant differences were located between the endurance exercise group (P=0.005), resistance exercise (P=0.014), and the control group at cold temperature. Also, according to Table 2, the ratio LDL/HDL decreased in exercise groups. Based on the results of Tukey's post hoc test, there was a significant difference between the endurance exercise group and the control group (P=0.009, P=0.003), and between the resistance exercise group and the control group (P=0.002, P=0.001) in ordinary and cold temperatures, respectively. One-way analysis of variance revealed a significant difference between groups in fasting blood sugar (FBS) levels. The results of the Tukey post hoc test indicated that there was a significant difference between the endurance exercise group (p=0.001) and resistance exercise (p=0.007) with the control group at ordinary temperature, and endurance exercise group (P=0.016) and resistance exercise (P=0.028) with the control group at cold temperature. The difference between the type of exercise and temperature was not significant.

Discussion

The present study investigated the effect of two types of endurance and resistance exercise and simultaneously it also examined the effect of temperature on the level of irisin secretion, blood sugar, and lipid profile in diabetic rats. In this study, both endurance and resistance exercise increased irisin secretion compared to the control group, but there was no significant difference between

endurance and resistance training in irisin secretion. There was no statistically significant difference between the irisin values in the training and control groups at two temperatures, although the irisin values of the groups at cold temperatures were slightly higher than ordinary temperatures. Furthermore, both endurance and resistance exercises reduced fasting blood sugar and there was no difference between the type of exercise and temperature in the rate of fasting hypoglycemia. In the study of lipid profile changes, the reduction of the LDL fat, and an increase in the HDL have always been considered. In this study, the ratio of LDL to HDL was used to compare the simultaneous effect of exercise on the lipid profile. In the present study, both endurance and resistance exercise significantly reduced this ratio.

In addition to the structure of the exercise activity, metabolic conditions can also affect irisin responses. Irisin is a molecule that plays a major role in maintaining metabolic homeostasis. Irisin levels - as a metabolic regulator - can vary depending on metabolic states (needs). Therefore, it can be said that in addition to the structure of the exercise activity, metabolic conditions can play a role in irisin responses to the type of exercise activity (32).

In Kucukkaraca's study (23), in contrast to the current research, after four weeks of endurance training, there was no significant difference between the amount of irisin and fasting blood sugar in the control and exercise group in diabetic rats. The reasons for the difference between the results of these two studies may be associated with the length of the training period and the duration of the exercise training per day. Their study lasted four weeks and 30 minutes a day; but, in the present study, the duration of training per day was one hour and the duration of the training period was eight weeks. However, in the present study, in accordance with Kucukkaraca 's findings, endurance exercise caused a significant change in the lipid profile.

The metabolic condition is one of the factors influencing the irisin (38). Shabani and Izaddoust (24) did not find a significant change in the amount of irisin in resistance and endurance training groups compared to the control group, which was inconsistent with the results of the present study. The reasons for this difference between the results of the two studies can be justified based on the metabolic status and age of the subjects. Circulating irisin levels decrease with age (34,39), whereas circulating irisin and muscle FNDC5 expression increases with endurance training in older adults (5). In Shabani and Izaddoust's research, the subjects were healthy and recreationally active. Young and healthy people need more intense exercise for the secretion of irisin. Their study subjects participated in a more intense combination exercise. In the current study, the rats were diabetic and physically inactive and were 12 weeks old and the intensity of endurance exercise and resistance was sufficient for subjects to secrete irisin.

Contrary to the current research, Tsuchiya et al. (25) showed that resistance training increased irisin secretion more than endurance training and combinational (aerobic and resistance) exercise, when exercises had equal time. In their study, both exercises were performed for one hour reporting that resistance exercise was more effective in healthy and active men. In the present study, the duration of both exercises during the day was not the same. Endurance training reached an hour and its speed was increased to 20 meters per minute during the 4 weeks. In one resistance training session, the number of repetitions was the criterion. In each session, eight repetitions had to be completed, and in each ascent, 15 grams were added to the maximum weight of the previous session (37). This factor may have caused the difference between the results of the current

research and the findings of Tsuchiya et al. Also, the healthy and active research samples of Tsuchiya et al. in comparison with the diabetic and obese samples of this study can be another reason for the difference in the results of the two studies. In a study by Bubak et al. (31), there was no significant difference in the amount of irisin at different temperatures, which corroborates with the results of this study. Although in Bubak et al.'s study the exercise was performed in environments with different temperatures, in the present study three groups of rats (endurance, resistance, and control) were at 16°C for eight weeks, but all training groups practiced at normal training temperatures. In Chung et al.'s (28) study, the effect of the synergistic between temperature and exercise was not observed which was inconsistent with the results of the present study. The results of their research showed that separate and independent exercise from cold causes changes to the biomarkers. In another study, Chung et al.'s concluded that the effect of cold and exercise on various tissues on various biomarkers was different. Contrary to current research, Lee et al. (26) found that cold, like exercise, increased irisin secretion, and at lower temperatures, irisin was produced more. Lee et al.'s research was conducted in only one session and with healthy individuals, and the temperature was reduced intermittently over a period of time until the temperature reached 12 degrees. In the present study, the duration of the study was eight weeks and the rats were diabetic and the temperature remained constant at 16 degrees for eight weeks. It is possible that the body's response to cold, in the long run, may be different from one session. Also, people's metabolic status may affect this response. In contradiction of the present study, the results of the study done by Naresh et al. (27) showed that mild temperatures of 16 degrees, such as four degrees, affect biomarkers. But in our study,16 degrees did not cause more changes than the normal temperature and there was not any statistically significant difference between the two control groups, although the changes in the cold group were slightly greater than the other control group. The difference between the present study and Naresh's study was in the metabolic status of the subjects and the study duration.

Conclusion

In general, the results of this study support the role of resistance and endurance exercise in increasing plasma irisin and simultaneously lowering fasting plasma glucose after eight weeks in obese male rats with diabetes. In this study, there was no difference between the effects of endurance and resistance exercise on the expression of this protein. Besides, increasing this protein significantly reduced blood glucose levels among diabetic rats. This conclusion can be drawn that increased expression of these proteins is associated with lowering blood glucose and homeostasis blood glucose. Likewise, the results of this study support the role of endurance and resistance exercise in reducing the ratio of LDL to HDL in male rats with diabetes. Also, the cold temperature has not synergistic or independent effects on irisin, lipid profile, and fasting blood glucose.

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Conflict of interest

The authors of the article did not declare any conflict of interest.

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