



ORIGINAL ARTICLE

An Investigation into Irisin Levels: Acute and Chronic Effects of Combined Training and Its Association with Anthropometric Variables in Overweight Men

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(Received: 5 March 2017 Accepted: 20 May 2017)

KEYWORDS

Combined training;
Irisin;
Myokine;
Overweight

ABSTRACT: Irisin, a novel myokine secreted from skeletal muscles, has an important relationship with physical activity and health. However, it has been reported as a therapeutic target for metabolic diseases. The purpose of this study was to measure both acute and chronic effects of combined training on serum Irisin levels and the relationship between Irisin and anthropometric variables such as weight, body mass index (BMI) and fat percentage in overweight young men. In 2016 in Gonbad City, Golestan Province, northern Iran, 20 overweight men aging 20-25 yr with BMI of 25-30 were randomly selected and equally divided into control and experimental groups. Fasting blood samples were collected three times, before the start of training, after the first session of training (acute), and 48 h after the end of the eight-week training (chronic). Combined training (endurance and strength) was done 3 times/week for eight weeks, as training in first session started at 50% of intensity and it was progressively increased 5% each week. Serum Irisin levels increased significantly when its levels were compared before training and after the completion of combined training ($P \leq 0.05$). However, no significant correlation was observed between the concentration of serum Irisin levels and anthropometric characteristics of the subjects both before and after the training. Eight weeks of combined training could be an efficient exercise type in increasing serum Irisin levels in overweight men. Irisin may causes metabolic and physiological changes within the body and play a protective role against overweightness and obesity associated with inactivity.

INTRODUCTION

Regular physical activities have many health benefits and prevent different diseases such as

cardiovascular disease, diabetes, cancer, obesity, and even cognitive diseases such as Alzheimer's

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disease [1-4]. Besides, physical activity is one of the most important factors affecting energy metabolism [5]. The balance between energy intake and energy expenditure causes the balancing of body weight. Signals sent by peripheral tissues such as fat, muscle, and digestive tract act at the central level. They are involved in controlling body weight [6, 7]. Nevertheless, existing knowledge about the cellular and molecular mechanisms involved in physical activity is limited.

Physical activity causes different cellular and molecular responses and changes in hormonal levels. Skeletal muscles, by releasing signals into the bloodstream, act as environmental endocrine glands and are involved in regulating several blood stream involved in the regulation of several physiological and metabolic pathways. These released hormones into the bloodstream are known as myokines. Myokine's secreted by muscle contraction play a protective role against many diseases associated with sedentary lifestyle [8]. This newly identified myokine, i.e., Irisin, being influenced by physical activity, is highly related to health. Irisin is secreted from muscles as the proteolytically cleaved product of FNDC5 and released into the plasma [9].

Irisin, is taken from the Greek Goddess Iris (messenger of the gods), was first discovered in 2012. Containing 112 amino acids (Amino acids 29-140) and having a 12 kDa molecular weight [9, 10], Irisin exists in different parts of the body including skeletal and heart muscles, adipose tissues, liver, brain, bone, pancreas, and kidney [11]. Moreover, causing an increase in energy expenditure and fat oxidation, it acts like physical activity. Irisin acts mainly on the subcutaneous "beige" fat and makes it "brown" by increasing the expression of UCP-1 and other thermogenic genes increasing energy expenditure [9, 12]. Obesity depends on the number or amount of white fat cells. White adipose tissues store the redundant energy as triglycerides, while brown

adipose tissues oxidize fuels, dissipate energy in the form of heat, and act against obesity and overweight [13].

Irisin has received considerable attention for its improving metabolic health and browning white adipose tissues, and, thus, the associations of Irisin and anthropometric variables have been probed by many researchers. Some studies have indicated that Irisin levels positively correlated with anthropometric variables [14, 15] and some reported negative correlations among them [16, 17].

Besides, Irisin is an exercise-responsive myokine, several studies have been carried out to determine changes in Irisin levels due to physical activity and inconsistent results have been reported. Some authors reported increased levels of Irisin [9, 18, 19]; however, in other studies, irisin level either did not change or decreased [20, 21]. The effects of aerobic and resistance training were compared on circulating Irisin level in overweight/obese adults and reported that Irisin level significantly increased after 8 wk of training only in resistance training group [18]. Moreover, circulating Irisin level was significantly increased in healthy individuals after 10 wk of endurance training [9]. However, no changes in Irisin level was reported after 26 wk of both aerobic endurance training and strength endurance training in middle-aged overweight subjects [21].

Regular physical activity is beneficial to health and prevents many diseases; however, using aerobic or resistance exercise programs alone is not sufficient to take advantage of physical activity. Thus, American college of sports medicine recommends both aerobic and resistance training (i.e., combined training). Although the beneficial role of Irisin in metabolic diseases and pathological conditions have been attested, changes of circulating Irisin levels following exercise training of different types and intensities have not been fully explored.

We investigated the acute and chronic effects of combined training on serum Irisin levels and the association between Irisin levels and anthropometric variables in overweight young men.

MATERIAL AND METHODS

Subjects

After the call for cooperation in 2016 in Gohbad-e-Kavoos, a city in Golestan Province in the north of Iran, volunteers filled out the special questionnaire to determine their level of physical activity and disease records. Among them, 20 qualified volunteers (overweight, aging 20-25 yr, and having a body mass index of 25–30) were selected randomly divided into control (n=10) and experimental (n=10) groups.

All stages of the investigation were in accordance with the Declaration of Helsinki and were approved with the Research Ethics Committee of Sports Sciences Research Institute with the code of IR.SSRI.REC.1395.109.

Anthropometric Measurements

Initially, the objectives and methodology of the study were explained to the participants and one week before the start of training in the gymnasium, the subjects were familiarized with the movements and devices. In addition, some measurements such as weight, height, body fat percentage, one-repetition maximum (1 RM) of various movements and maximum oxygen consumption of the subjects were recorded. The subjects' heights were measured while standing without shoes against the wall. Their weights, with minimal clothing, were also measured using a digital scale. In addition, body mass index of subjects were calculated by dividing weight (kg) by square of the height (m²).

To estimate body fat percentage, skin fold thickness was measured, according to the Jackson and Pollock methods, by a caliper at three sites

(chest, abdominal, and thigh) in the right side of body. Each person performed all measurements three times in each area and the average of three measurements was considered as the final record. For reliability and validity, all measurements were done at the same time, preferably morning (after the overnight fasting).

Training protocol

Combined training program (strength and endurance) was carried out progressively three times a week for eight weeks. In each session, having performed 10 min of warm up exercises including jogging and stretching movements, participants ran for 20 min by 50% to 85% of maximum heart rate (training started with 50% of intensity and it was increased 5% each week). It was followed by strength training which was for large muscles of the upper and lower body and included bench press, lat pull-down, barbell shoulder, leg press, and knee flexion with 50% to 80% of one repetition maximum (training started with 50% of intensity and it was increased 5% each week). Participants performed three sets with 10 repetitions (rest interval between sets was 1 min and between exercises was 2 min). The end of each training session included a 10-min cooldown though walking slowly [22].

Blood sampling

Blood samples were collected at three times: pre-training, after the acute training, and 48 h after the end of the training period (chronic). Before blood sampling, subjects were asked to be fasting, and after sitting on a chair for 15 min, 3 ml of blood were collected from antecubital vein by a laboratory expert. Then blood samples were kept at room temperature for one hour to be clotted. After clotting, blood samples were centrifuged at 3000 rpm for 10 min under 4 °C to separate serum. Then, serum samples were dispensed into a plain microtube and were stored at -80 °C until final

measurements. Serum Irisin levels were determined by Enzyme-Linked Immunosorbent Assay (ELISA) kits specific to human samples based on the manufacturer's instruction. The sensitivity of the assay was 0.78 ng/ml and detection range was 3.12 ng/ml–200 ng/ml.

STATISTICAL ANALYSIS

Having assured the normality of the distribution of data through Kolmogorov-Smirnov test, we ran ANOVA 2×3 with repeated measures and the Bonferroni post hoc test. In addition, independent t-test was used for comparing the differences

between the two groups at different times of measurements. The relationship between Irisin and anthropometric variables were probed through Pearson correlation tests. All data were analyzed using SPSS soft-were ver. 16 (Chicago, IL, USA) and the level of significance was set at $P \leq 0.05$.

RESULTS

Mean and standard deviation of physical and anthropometric characteristics (including age, weight, height, body fat percentage, and body mass index) of two groups are presented in Table 1.

Table 1. Mean and standard deviations of physical and anthropometric characteristics of the subjects

Group variable	Measuring	Control (n=10)(M±SD)	Experimental (n=10)(M±SD)
Age (yr)	Pre-training	22.3±1.5	22.7±1.5
Height (cm)	Pre-training	178.8±3.1	180±4
Weight (kg)	Pre-training	86.6±2.6	87.3±3.9
	Post-training(chronic)	86.7±2.3	86.4±3.4
BMI (kg/m ²)	Pre-training	27.1±0.8	26.9±0.7
	Post-training(chronic)	27.1±0.9	26.9±0.6
Body fat (%)	Pre-training	22.8±1.6	22.6±1.3
	Post-training(chronic)	22.7±1.5	21.5±1.5

Key: BMI; body mass index, (M±SD); mean± standard deviations

Paired t-tests probing differences in anthropometric characteristics of the subjects at different times of measurement, i.e., before and after the training period, revealed that only fat percent in experimental group was reduced significantly and there was no significant difference in the participants' other characteristics.

The average food intakes of the subjects, as recorded three days before the blood samplings and analyzed with food analyzer software (NUTRITION 4), are presented in Table 2. A copy of the subjects' food intake forms completed in pre-training was returned to them so that they could follow them before the final blood sampling.

Table 2. Mean and standard deviations of food intake analysis

Group variable	Stage	Control(n=10)	Experimental(n=10)
Energy (kca)	Primary	3187±143	3241±179
	Secondary	3169±252	3272±204
Carbohydrate (gr)	Primary	451.1±58	460±21.7
	Secondary	442.8±37	455±33.1
Protein (gr)	Primary	129±48	131.7±29
	Secondary	132±39	139.8±36
Fat (gr)	Primary	121.1±19	127.3±48
	Secondary	129.7±31	120.3±37

The differences between Irisin levels at different times of measurements was significant ($F=15.19$, $sig=0.001$). In addition, there was a significant interaction between groups and times of measurements ($F=13.29$, $sig=0.001$). Moreover, there was no significant difference between the variance of Irisin in the control group at different times of measurement. However, there was a significant difference between the Irisin levels of the experimental group at different measurement

times. To further probe the differences, Bonferroni post hoc test was run the results of which showed that there were no differences between the concentration of Irisin levels as measured in pre-training and immediately after the acute training. However, after the chronic training, Irisin levels increased significantly, as compared to the pre-training and immediately after the acute training ($P\leq 0.05$) (Figure 1).

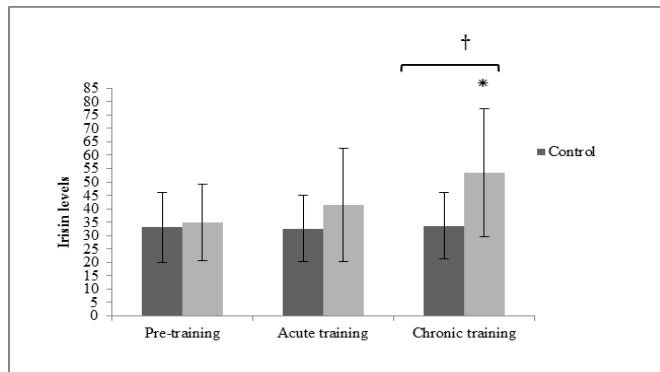


Figure 1. Irisin concentration at different times between the two groups
 *: significant within-group difference between pre-training and post-acute training
 †: significant between-group difference.

To compare the Irisin levels between the experimental and control groups at different times

of measurement, we ran independent t-test the results of shown in Table 3.

Table 3. The comparison between experimental and control groups at different times of measurement

Measurement	Leven tests		t	df	sig
	F	sig			
Pre-training	0.07	0.79	0.27	18	0.78
After acute training	10.82	0.004	1.13	14.53	0.27
After chronic training	13.45	0.002	2.34	13.48	0.03*

*significant difference ($P\leq 0.05$)

The differences between the concentrations of Irisin levels were showed between the two groups before and after the acute training were not significant. However, after the chronic training, a significant difference between the two groups was observed as the experimental group had the higher concentration of Irisin than the control group ($P \leq 0.05$). The relationship between Irisin concentration and anthropometric characteristics of the subjects before and after the training period were assessed using Pearson's correlation tests. Negative correlations were observed between Irisin levels and the subjects' anthropometric factors including weight, BMI, and body fat percentage. Although the relationship was not significant, with an increase in the weight, body mass index and body fat percentage of subjects, serum Irisin levels decreased.

DISCUSSION

The purpose of this study was to investigate the acute and chronic effects of combined training on serum Irisin levels. It also probed the relationship between serum Irisin levels and anthropometric variables in overweight young men. The results of this study showed that the combined exercise training (strength and endurance) after eight weeks causes a significant increase in Irisin levels. In addition, there was negative, but statistically non-significant, relationship between the concentrations of Irisin levels with anthropometric variables.

Having investigated the effects of aerobic and resistance training, after the completion of training, unlike our findings, there was no significant difference in the concentration of Irisin between the groups [21]. Moreover, the effect of combined training was investigated on Irisin level in middle-aged obese men. The combined training consisted of strength training followed by aerobic training, 3 times/week, for 24 wk. The exercise session consisted of resistance training (3 sets of 6-10 maximal repetitions) and endurance training

(running at 55%-85% of maximal oxygen consumption). Body composition and physical fitness markers at the end of combined training protocol improved; however, inconsistent with our results, Irisin level was not significantly changed [20].

Our findings were consistent with results indicating an increase in Irisin levels after high-intensity intermittent exercise and Pilates training in overweight young women [23]. The relationship was examined between Irisin levels and various factors such as obesity and physical activity among normal weight, overweight, obese, and athlete subjects found that obese subjects had significantly higher Irisin levels than subjects with overweight did and normal weight did. Besides, comparing the Irisin levels between athlete and healthy male groups of the same age, body mass index and fat percentage, they also reported that Irisin levels in athlete group were significantly higher [24]. Similarly, in our study, Irisin level was increased after the exercise and athlete healthy men had higher serum Irisin levels. This may reflect its protective roles against developing many diseases associated with sedentary lifestyle.

Irisin levels were compared in two groups including active athlete runners that ran more than 50 km per weeks and those running less than 60 min per weeks. The athlete group, despite having significantly lowers body weight, BMI, triglycerides, and white fat compared to the other group, had the same level of Irisin as the other group did. Unlike our findings, they also reported a significant positive correlation between the Irisin levels with BMI, cholesterol, triglycerides and body fat [14].

Some studies reported a positive correlation between Irisin levels and BMI [25,26]; nevertheless, some reported a negative correlation between Irisin levels and body mass index, waist circumference, hip circumference, and fat mass [16]. Age and muscle mass are the most important predictors for Irisin levels. Specifically, Irisin level

had a significant correlation with biceps muscle circumference and body mass index. It was also negatively correlated with age. Irisin levels in athletes and the young men were several times greater than in middle-aged women. Circulating Irisin level may depend on blood sampling time point. After the physical activity and muscle contraction, Irisin may have a short-term effect on restoring ATP homeostasis but may return to baseline soon after ATP levels are restored [25]. In another study, investigating the effects of one-year physical activity and lifestyle intervention on Irisin levels indicated that metabolic and anthropometric variables were improved at the end of the protocol. In line with the results of our study, despite having no correlation with gender, age, and BMI, Irisin levels increased significantly at the end of protocol [19].

Irisin has a physiologically protective role against obesity by browning the white adipose tissues. However, some studies reported a positive correlation between Irisin and obesity that is in conflict with the anti-obesity effect of Irisin. In pathological states of morbidity obesity, Irisin level cannot maintain the balance between the energy storage and energy expenditure; therefore, additional Irisin is secreted from peripheral tissues as a compensatory mechanism for dramatically increased fat storage [27]. The literature on Irisin suggests that decreased levels of Irisin are associated with the development of insulin resistance and metabolic diseases. Specifically, low levels of Irisin is related to various metabolic diseases including type 2 diabetes, chronic kidney disease, non-alcoholic fatty liver disease, obesity, and metabolic syndrome [28]. However, inconsistent results have also been reported which is possibly due to the differences in the subjects' status, gender, and age. In addition, differences in training programs, including the type, intensity, and frequency of exercise training protocols may yield different results.

CONCLUSIONS

Regular physical activity has positive effects on the body's metabolic health. Skeletal muscles, by increasing energy uptake, play an important role in energy homeostasis of body [29]. In addition, skeletal muscles have been identified as the main source of circulating Irisin level. Therefore, physical activity and muscle contraction release Irisin into blood stream. Consequently, increased Irisin levels because of exercise protect against obesity, overweight, and diseases associated with a sedentary lifestyle by increasing energy expenditure, fat oxidation, and browning adipose tissues.

ACKNOWLEDGEMENTS

This article has been taken from the first researchers' Ph.D. dissertation at Shahid Chamran University of Ahvaz. We hereby appreciate the faculties of Physical Education and Sports Science and subjects who participated in this study. The authors declare that there is no conflict of interest.

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