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The effect of 8 weeks HIIT training on adiponectin gene expression in obese rats.

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Abstract

Background: Obesity is the result of accumulation of excess adipose tissue due to an imbalance between food intake and energy intake. Exercise can play an effective role in reducing adipose tissue due to increased energy consumption. The aim of this study was to investigate the effect of high intensity training (HIIT) on adiponectin levels in adipose tissue in rats on a high fat diet.

Methods: For this purpose, 32 rats with a lifespan of 8 weeks were randomly divided into 3 groups: obese and HIIT, obese without HIIT and normal weight. The HIIT training program was performed for eight weeks. At the end of the training period, the rats were anesthetized. The subcutaneous adipose tissue of the animal was also sampled and qRT-PCR analysis was performed to evaluate the expression of adiponectin gene.

Results: The results showed that adiponectin gene expression decreases under the influence of obesity. But with HIIT training and reduced obesity, its expression increases.

Conclusion: In general, the results of this study showed that HIIT training can reduce obesity, increase adiponectin gene expression in animals with high fat diet.

Keywords: High Intensity Interval Training (HIIT), Obesity, Adiponectin

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Introduction

Over the past two decades, obesity has tripled as one of the many problems in developed and developing countries. Obesity or the increase in body fat reserves is caused by an imbalance between energy intake and energy consumption and is associated with various diseases such as cardiovascular diseases, high blood pressure, type 2 diabetes and several types of cancer. Among the factors that influence the amount of weight gain, besides heredity, are changes in lifestyle, nutritional status, lack of physical activity, and especially the disruption of the energy balance in the body. Therefore, due to its importance in the field of health, regulating body weight leads researchers to identify interesting hormonal signals related to body homeostasis and metabolic-related diseases (1). Adipose tissue is the main organ for lipid storage and plays an important role in fatty acid metabolism and glucose homeostasis. For this reason, in recent years, the study of adipose tissue has been considered in many studies due to its role in the spread of obesity and its related complications. Today, fat tissue is no longer considered as a non-secretory tissue that protects the body against cold, but it is an active endocrine tissue that has the ability to secrete many regulatory factors. (2). Adipose tissue, especially white adipose tissue, acts as the largest endocrine organ by secreting hundreds of hormones, peptides and cytokines, collectively known as adipokines. These adipokines affect various processes in the peripheral and central nervous system. Cytokines derived from adipose tissue are called adipocytokines, one of the most important of which is adiponectin, which is responsible for increasing energy consumption and fat catabolism, as well as increasing fatty acid oxidation and insulin sensitivity (3) .

This protein, which contains 244 amino acids, is made up of 4 parts, one part is for the guidance of the hormone and its secretion outside the cell, a part that is different between different species, and the other part is a region of 65. It has collagen proteins, and the other is a globular (circular) domain that is very similar to TNF-a (4). Unlike some adipocytokines, the concentration of adiponectin decreases in obese people. Also, adiponectin is considered as a resistant factor in the pathogenesis of several processes related to cardiovascular diseases. It seems that adiponectin acts as an anti-atherosclerotic agent through direct effects on endothelial cells due to its beneficial effects on lipid and glucose metabolism and insulin sensitivity. It has been observed that the plasma concentration of adiponectin is lower in patients with diabetes and cardiovascular diseases (5). Adiponectin leads to increasing insulin sensitivity, anti-inflammatory and antioxidant effects, and these positive effects of

adiponectin can explain the inverse relationship between circulating adiponectin levels and diseases such as type 2 diabetes, cardiovascular disease, metabolic syndrome, and obesity. Adiponectin, by activating MAPK and AMPK and signaling related to them, has an effect on increasing insulin sensitivity and increasing gene expression of proteins involved in the transfer and oxidation of lipids, such as CD36 and acyl-CoA oxidase (6,7)

Research shows that exercise has an effect on the circulating levels of adipokines, so that swimming, running and cycling exercises have been shown to significantly decrease leptin levels and significantly increase serum adiponectin, so that this decrease is correlated with the decrease in BMI, and therefore, these two adipokines have been introduced as indicators sensitive to body composition (5). The decrease in body fat percentage has been reported in studies that used long-term exercises, and most of these studies have mentioned the decrease in body fat percentage as the reason for the decrease in leptin and the increase in adiponectin levels (6). Sports activity can be an effective factor in improving obesity, but the information about the effect of sports activity on adiponectin level is contradictory (8). According to the studies conducted in this field, it was shown that the studies that investigated the effect of exercise training on adiponectin reported contradictory results. In addition to these studies that investigated the effect of HIIT exercises on the expression of this important gene in samples, fat paid is very little. Despite the importance of intense interval training and its effect on increasing the concentration and expression of adiponectin, most of its transient effects, especially in adipose tissue, remain largely unknown. Therefore, in this research, we try to investigate the effects of high intensity interval training (HIIT) on the expression of the adiponectin gene and its plasma concentration, as well as the blood lipid profile.

Material and Methods

Due to the fact that this research was conducted on animal subjects and it was possible to fully control the variables, this research is experimental. Also, a post-test research design was implemented with three groups of subjects.

The present research was conducted in a laboratory manner, and for this purpose, 32 male mice rats from the Pasteur Institute of Iran with a weight range between 212-231 grams and an average weight of 223.37 ± 4.97 grams and ten weeks old were purchased and sent to the animal laboratory. They were transferred to Tehran University. 8 mice were

considered as healthy control group and the rest of the mice received high fat diet for 6 weeks. Then these obes mice were randomly divided into two groups of control (n=12) and HIIT training group (n=12).

Familiarization course

Transferring laboratory animals from one environment to another causes stress and changes in their physiological conditions, and animals need time to adapt to new conditions. In this research, after being transferred to the animal laboratory of the Faculty of Physical Education and Sports Sciences of Tehran University, the rats were familiarized with the living conditions in the animal house and how to run on the treadmill for 1 week.

The method of keeping animals

Animals were maintained according to the Helsinki Protocol for the Protection of Laboratory Animals Used for Scientific and Laboratory Purposes. The animals were placed in their groups and in cages made of transparent polyethylene, completely separated and marked. Three rats were kept in each cage. All groups used one type of food (standard pellet) and were kept in one place so that they had free access to food and water. The daily food intake of mice was recorded. The animals were kept under a sleep-wake cycle (12 hours of light and 12 hours of darkness) and at a temperature of 22±3 degrees Celsius and a humidity of 35 to 50%. To ensure proper environmental conditions and maintain proper humidity, temperature and ventilation (to adjust the level of pollution in the place and reduce the bad smell of the environment caused by the accumulation of ammonia from animal urine and reduce the possibility of respiratory diseases in animals) from the air conditioner and from the thermometer and hygrometer was used to monitor day and night temperature and humidity changes.

Prepare food rations

To induce obesity, a high-fat diet was used for 6 weeks. To prepare high-fat food, 1% of cholesterol powder and 1% of 100% pure corn oil were added to the standard rat food that was purchased from Parsdam Food Company.

The exercise protocol carried out in this research was:

-In the first week, 8 repetitions of 40 seconds were performed at a speed of 25 m/min

and active rest of 2 minutes between repetitions at a speed of 10 m/min (slope of 5%).

-In the second week, 10 repetitions of 40 seconds were performed at a speed of 25 m/min and active rest of 2 minutes between repetitions at a speed of 10 m/min (slope of 10%).

-In the third week, 10 repetitions of 40 seconds were performed at a speed of 28 m/min and active rest of 2 minutes between repetitions at a speed of 10 m/min (slope of 10%).

-In the fourth week, 10 repetitions of 40 seconds were performed at a speed of 32 m/min and active rest of 2 minutes between repetitions at a speed of 10 m/min (slope of 10%).

-In the fifth week, 10 repetitions of 40 seconds at a speed of 35 m/min and active rest of 2 minutes between repetitions at a speed of 10 m/min were performed (slope of 10%).

-In the sixth to eighth week, 10 repetitions of 40 seconds at a speed of 35 m/min and a 2-minute active rest between repetitions at a speed of 10 m/min were performed (slope of 10%).

Preparation of adipose tissue

Immediately after 48 hours of the last training session, the animals were anesthetized with a combination of Ketamine (30-50 mg/kg body weight) and Xylazine (3-5 mg/kg body weight). Then, the chest of the animal was split open and to ensure the least harm to the animal, a blood sample was taken directly from the animal's heart. Next, the subcutaneous fat tissue of the rats was sampled and the desired tissue was immediately frozen in liquid nitrogen (temperature -196 degrees) and while being transferred to the laboratory at a temperature of -70 degrees, it was kept until the implementation of the desired laboratory protocol. The target was used using immunoblotting method (tissue homogenization) and then qRT-PCR analysis method to identify the changes of the desired variables. During this period, these tissues were kept in the respective vials at -70 degrees. At the time of homogenization, 1 ml of PBS buffer with aprotinin was used and centrifuged at 3000 rpm for 15 minutes and the two parts of supernatant and sediment were separated. All steps of sample homogenization, centrifugation and analysis were done at the Pasteur Institute.

RNA extraction

RNA was extracted from subcutaneous fat tissue using the Rneasy protect mini kit (QIAGEN) according to the company's instructions. So that 20 mg of the tissue is crushed

using a scalper and inserted into a microtube, and then RNA was extracted using the RNeasy Protect kit according to the instructions of the German manufacturer. The amount of RNA was measured by the optical density (OD) method and absorbance at 260 nm by Nanodrop Nanodrop ND-1000 (NanodropTechnologies, Wilmington, DE)**.**

Statistical Analysis

After confirming the normality of the data distribution with the Kolmogorov-Smirnov test, one-way analysis of variance and Tukey's post hoc test were used. P > 0.05 was considered as a significant level**.**

Results

According to the significance level (P<0.05), it can be said that eight weeks of HIIT training has a significant effect on the level of adiponectin gene expression in obese rats. In other words, these results showed an increase in adiponectin gene expression in the HIIT training group compared to the obese and normal control groups (Tables 1 and 2)**.**

Table1. Changes in adiponectin expression in research groups

P	T	Mean differ	df	n	
0/007	3.166	0/674	14	16	**Adiponectin (pg/ml)**

Table 2. Tukey's post hoc test results

P-value	Groups	
0/001	Obes control group	**HIIT group**
0/06	Normal control group	
0/04	Normal control group	**Obes control group**

Discussion

The research results showed that there is a significant difference between the average expression of adiponectin in normal weight control and obese control rats, and the normal weight group expressed more adiponectin compared to the obese control group. Eight weeks of HIIT training has a significant effect on the level of adiponectin gene expression in obese mice; in other words, these results show an increase in the level of adiponectin gene expression in the HIIT training group compared to the obese control group.

The effect of exercise training on visceral adiponectin levels and subcutaneous adipose tissue is not completely clear. Some researchers have reported an increase, and others no change or decrease in adiponectin levels in response to sports activities. The difference in the results of different studies is probably due to the difference in the intensity, duration, type of sports activity, the presence or absence of metabolic diseases such as diabetes and cardiovascular diseases, weight, age, gender and also the type of subjects studied. (human and animal) (9, 10, 11,12).

Phillips and colleagues (2014) (13)investigated long-term high-intensity training on adiponectin expression and showed that during long-term training periods, the increase in adiponectin level is accompanied by the improvement of lipid profile or the reduction of body fat mass. Mohebi et al. (2009) (14) investigated the effect of 12 weeks of aerobic exercise on the expression of adigonectin gene in obese subjects and showed that these exercises improve body composition, body weight and adiponectin concentration. In their research, Tawfighi et al. (2009) (3) examined the effect of water exercise on serum adiponectin levels and insulin resistance in obese postmenopausal women. The findings of the research showed that the implementation of the exercise protocol significantly increases the amount of serum adiponectin in the experimental group. However, there was no significant difference between the post-test and pre-test findings of these values in the control group. Suri et al. (2014) (5) investigated the effect of high intensity interval training (HIIT) on the serum levels of adiponectin and leptin in obese children. The findings of this research showed that there is a significant difference in leptin and adiponectin levels as well as BMI before and after a period of HIIT training. It can be said that HIIT exercises probably decrease the serum levels of leptin and increase adiponectin through the reduction of fat mass. Therefore, performing HIIT exercises with the necessary precautions in obese people can have positive effects. In their research, Kazemi et al (2015) (2) examined the effect of intense interval training on the levels of adiponectin, insulin and plasma glucose in male rats. The results of their research showed that there was a significant increase in visceral fat adiponectin levels and a significant decrease in plasma insulin in the training group. But these changes in subcutaneous fat adiponectin and plasma glucose were not significant. The results of their research showed that HIIT exercises can increase adiponectin in visceral and subcutaneous fat tissue. The increase in insulin sensitivity in the present study is probably related to the increase in adiponectin levels in fat tissue and also to HIIT as an effective

factor in reducing fat tissue. Kramer et al. (2007) (6) in a review article, and by reviewing the studies that investigated the effect of sports activity on adiponectin concentration, propose the hypothesis that the amount of exercise seems to be an influencing factor on how adiponectin responds. In other words, long-term sports performance with high training volume (intensity, duration and frequency) has an effect on adiponectin concentration, and these factors can express the contradiction reported in the context of the inconsistency of some of these researches compared to the results of the current research (15). Considering that in the aforementioned studies the intensity of activity has been introduced as the main and effective factor involved in the response of adiponectin levels to sports activity, it seems that the high intensity of activity in the present study can be one of the main reasons for the significant increase in the levels of adiponectin of visceral adipose tissue in the experimental group.

Conclusion

The results of this research showed that HIIT exercises generally increase the amount of adiponectin in fat tissue. By activating AMP kinase in muscle, adiponectin stimulates glucose consumption and fatty acid oxidation and improves insulin action, and exercise improves glucose consumption and fatty acid oxidation by activating AMP kinase in muscle. Exercising plays a role in increasing the amount of serum adiponectin. As a result, in general, it can be said that HIIT activities increase the level of adiponectin and also increase the body's metabolism and regulate the oxidation of fatty acids and glucose consumption in the body, reducing the possibility of cardiovascular diseases and metabolic disorders.

Competing interests

There is no competing of interest to disclose

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