The effect of high-intensity interval training (HIIT) on resistin gene expression in visceral adipose tissue in obese male rats

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ABSTRACT: It has been reported that obesity can result in the accumulation of various proinflammatory factors such as resistin in adipose tissue. This study aimed to investigate the effect of HIIT on resistin gene expression in visceral adipose tissues in diet-induced obese rats. Nineteen male rats were used in the present study. 6 rats (control group) were fed with standard diet for 8 weeks and then dissected and their visceral adipose tissues were sampled. At the same time, 13 rats were fed with a high-fat diet for 8 weeks. From these obese rats, 6 animals (fat group) were fed a high-fat diet for another 8 weeks and 7 animals (training group), underwent HIIT for 8 weeks. Training included running on a treadmill at 90% VO2max with 5 repetitions of 30 seconds in each session at 29 to 36 meters per minute with one-minute active rest intervals. The latter two groups were also dissected and their visceral adipose tissues were sampled. Measurement of resistin gene expression in tissue samples was done using real-time polymerase chain reaction. Data were analysed with one-way ANOVA. Obesity increased resistin gene expression in rats 2-fold but it was not statistically significant (P= 0.618). HIIT reduced resistin gene expression 8-fold compared to the fat group, but this reduction was not significant, too (P= 0.349). In conclusion, HIIT could reduce resistin gene expression as a proinflammatory factor in the visceral adipose tissue of obese rat. Despite the non-significance of differences, resistin gene expression was reduced several folds, so further studies are needed in this field.

Keywords: high intense interval training (HIIT), resistin, gene expression, visceral fat, rat

INTRODUCTION

Nowadays, living is associated with a sedentary lifestyle, since urbanization does not allow enough time for people to engage in sport and physical activity. According to the report of World Health Organization, insufficient physical activity is the fourth leading risk factor for mortality. Physical inactivity is a major factor on the accumulation of fat in the body (obesity) since physical activity is a key determinant of energy expenditure and thus fundamental to energy balance and weight control [1]. In this connection, there are a lot of ongoing studies which investigate the effects of physical activity on body weight, visceral fat, and pro-inflammatory factors. Recent studies
have shown that lack of physical activity in women and middle-aged men, even for a short-term, causes the accumulation of stored fat in the body, especially in skeletal muscles [2]. In contrast, physical exercise makes changes in mitochondrial oxidative capacity and as a result fat metabolism increases especially in skeletal muscle. It has been also stated that regular physical activity, along with reduction of visceral fat and free fatty acids in muscle, improves insulin function, too [2].

Recent studies suggest that adipose tissue is a highly differentiated tissue and numerous genes are expression in it [3]. Nowadays, adipose tissue is regarded as an endocrine organ, which can produce and secret more than 50 hormones or hormone-like compounds [4]. This tissue contains extracellular matrix, nerve tissue, blood vessels and a number of cells, including pre-adipocytes, fibroblasts, stem cells and immune cells such as macrophages and lymphocytes [5].

Among compounds that are secreted from cells, cytokines can be noted which are released by a variety of cells such as macrophages, endothelial cells and fat cells and muscles. These secretory products are considered main parts of inflammation or pro-inflammatory factors. Several studies suggest that obesity and sedentary lifestyle are among factors that stimulate the secretion of pro-inflammatory factors [6]. A cytokine that is secreted from white adipose tissue often called adipocytokine or adipokine (Figure 1). Some cytokines such as interleukins and tumor necrosis factor- alpha, apelin and omentin are also secreted from other tissues [7].

![Adipocytokines diagram](image)

Figure 1. Adipocytokines and their relationship with variety of diseases (adopted from Lago et al, 2011 [8])
Adipocytokines play a crucial role in body metabolism, including the intake and management of energy consumption. In addition, their role in carbohydrate metabolism and insulin sensitivity has been shown well [9]. Studies on the role of cytokines which are involved in the metabolism of carbohydrates and lipids have shown that visceral adipose tissues are more important than subcutaneous fats in terms of adipokine secretion because they are exposed to portal vein blood directly [7].

One of the important adipocytokines involved in the metabolism of lipids and carbohydrates is resistin which has a particular role in the development of insulin resistance [10]. Resistin, a cysteine-rich protein, was named due to production of insulin resistance in rat. The term is derived from two words "resistance" and "insulin", and it means a compound which causes insulin resistance. Resistin is found in adipocytes, macrophages, and some other cells. Resistin is secreted constitutively by mononuclear cells and has been reported to be involved in the development of inflammatory conditions in humans [5]. Resistin is expressed and secreted in human adipose tissues to some extent but mostly it is expressed in macrophages, liver, spleen, lungs, and placenta. However, in rat it is expressed largely in adipose tissue [10].

Due to the existence of a relationship between resistin levels and various diseases, health communities are looking for the ways to reduce the serum levels of resistin and other inflammatory factors. Scientific data and clinical or epidemiologic evidence suggest that physical activity reduces the production of inflammatory cytokines in adipose tissue directly and indirectly by improvement of vascular endothelial function and reduction in fat mass. For this reason, it has got a lot of interest in medical associations [11].

However, due to conflicting results of studies on the effects of regular exercise on serum resistin index, the mechanism of action is not well recognized and it is currently unclear whether these changes are caused by the direct effects of the exercise or by changes that occur in anthropometric indices and its variables [12].

It has been shown that HIIT can be a suitable approach in the treatment of patients with cardiovascular problems, obesity, diabetes, or even as a way to prevent the development of these disorders [13]. HIIT is defined as a exercise with a very high intensity, close to the peak to above peak at 90% of maximum oxygen consumption with very-short active rest (continued exercise with low-intensity) or passive rest (full rest) intervals [14].

It has been demonstrated that HIIT can improve and increase (7 to 10%) maximal oxygen uptake and aerobic metabolism as well as muscular function in athletes. At the same time, it can reduce carbohydrates consumption as a fuel but can increase lipid consumption [15]. HIIT improves insulin sensitivity significantly [16] and lowers blood pressure in obese people, as well. On the other hand, in patients with cardiovascular disorders, HIIT has improved the heart function, cardiovascular fitness and insulin function as well as markers of inflammation and atherosclerosis [17].

With reference to the results of above-mentioned studies, it was hypothesized that physical exercise could reduce the resistin gene expression in obese animals. Therefore, this project was conducted to study the effect of HIIT on resistin gene expression in visceral adipose tissue in diet-induced obese male rats.

**METHODS**

Nineteen male rats, 35 days old, with an average weight of 110 grams purchased from Pasteur Institute, Karaj-Iran and transported to the laboratory. This study was approved by the ethics committee of University of Tehran. Animal were kept at 12-hour cycle of light/darkness with 65% humidity at 24±2 °C under controlled condition. 6 rats as control group were fed with standard diet for 8 weeks.
Concurrently the remaining 13 rats were fed high-fat diet. The composition of diets adopted from Zhang et al., 2008 [18]. Standard diet consisting of 5% fat, 53% carbohydrate, 23% protein, and high-fat diet consisting of 22% fat, 48% carbohydrate and 20% protein provided by Pasteur Institute, Karaj-Iran. At the end of eight weeks, these rats were divided into two groups: fat group and training group. Training group underwent HIIT protocols for 8 weeks and fat group just continued to feed a high fat diet without exercise for another 8 weeks (Fig 2).

HIIT protocol: The HIIT protocol adopted from Kim et al., 2014 and included running of rat on an animal treadmill 5 days a week, between 8 am to 10 am, for 8 weeks. The exercise intensity was 90% VO2max. At the beginning, each practice session started with 5 repetitions of 30 seconds at 29 meters per minute along with one minute active rest intervals. The active rest was the continuation of running on treadmill but at 13 meters per minute. Then each week, 1 meter per minute to treadmill speed and one repetition per session were increased, so at week 8, it increased to 12 repetitions and the speed reached 36 meters per minute. In addition, this protocol included 5 minutes to warm up and

**Fig 2.** Experimental timeline of the study representing the type of diet, the duration of feeding or training course and the time points in which rats were anesthetized and samples of visceral adipose tissue were taken from each experimental group.

Obesity of rats was checked by Lee index. According to the following formula, rats with Lee index more than 310, known as obese rats [19].

\[
\text{Lee index} = \sqrt[3]{\frac{\text{Body Weight}}{\text{Body Length}}} \times 1000
\]

In the formula, the rat body weight is expressed in grams and length (Naso- Anal length) in centimeters.
5 minutes to cool down at 10 meters per minute [20].

**Sampling from adipose tissue:** 24 hours after the last session in training group or at the end of eight weeks in the control group and 16 weeks in the fat group, with 8 hours of fasting, all rats in order to dissect and tissue sampling, were weighed and made ready to be anesthetized. Rats were anesthetized by intravenous anesthesia using ketamine + xylazine at a dose of 75 mg ketamine per kg and 5 mg xylazine per kg body weight. When animal was anesthetized, abdominal cavity opened, blood taken from heart and its deaths ensured, it followed by collection of samples from visceral fat tissue with special forceps. Samples immediately after harvesting were washed with saline, placed in microtubes, transferred into nitrogen tank in order to be frozen quickly and then stored in a freezer at -80 °C until analysis.

Visceral adipose tissue samples were sent to genetic laboratory to measure the mRNA copies of resistin gene with Real-time PCR using Cybr Green and hereby the method of analysis is briefly described.

**Extraction of RNA:** The RNA extraction was done according to the kit instructions manufactured by Stratech, Germany. 25 mg of fat tissue was taken from micro-tubes following it was defrosted, crushed using a razor (Ultratodox T25) and homogenized using a shaker. The extraction procedure was continued according to the kit until the preparation of purified RNA was complete.

**Preparation of cDNA and Real-Time PCR process:** Due to the long strand of RNA sequence, to make cDNA from the total RNA, Random Hexane was used as a primer. Following binding of the primers to RNA chain, reverse transcription of the RNA was performed using the enzyme, reverse transcriptase. HPRT gene was used as housekeeping gene to control gene expression and compare with target gene (resistin).

The sequence of primers used was as following:

Resistin Forward: 5TCATGCCCAGAACCAGTTG3
Resistin Reverse: 5CAGCCCCAGGACAAGGAAGA3

In order to construct cDNA, directions of the American THERMO kit were followed and the Real Time-PCR was performed using Cybr Green. The Ct, Livak, method was used to calculate the changes in the relative expression of resistin gene in the experimental groups by applying following equations [21].

\[ \Delta Ct = Ct \text{ target gene} - Ct \text{ housekeeping gene} \]
\[ \Delta \Delta Ct = \Delta Ct \text{ test sample} - \Delta Ct \text{ control sample} \]

Relative fold change in gene expression = \( 2^{\Delta \Delta Ct} \)

**Statistical analysis:** Using descriptive statistics, data were presented as mean ± SD. First, Kolmogorov-Smirnov test was used to study the normal distribution of data. One-way analysis of variance was used for primary comparison between experimental groups followed by Tukey test to show statistical differences between groups. P<0.05 was considered as significant level for differences. All statistical analyzes were performed using SPSS software, version 19 and diagrams were drawn using EXCEL software, version 2012.

**STATISTICAL RESULTS**

Body weight of rats in different groups at the end of the experiment was shown in Figure 3. The results of analysis of variance test for body weight showed a significant difference between groups (P = 0.001). Tukey test showed that the weight of obese rats fed with fat diet for 16 weeks had significantly increased compared to control group (P = 0.001). Moreover, training group compared to fat body weight also showed a significant reduction (P = 0.001).
Regarding the effect of obesity on resistin gene expression, data showed that obese animals fed a high fat diet led to a two-fold increase in resistin gene expression in visceral adipose tissue compared with the control group (Figure 4), but this increase was not statistically significant (P = 0.618).

HIIT decreased resistin gene expression 8-fold compared to fat group (Figure 4), although this reduction was not statistically significant, too (P = 0.349).

Figure 3. The body weight of rats at the end of experimental period in different groups (mean± SD)

Figure 4. The relative expression of resistin gene in visceral adipose tissue of the experimental groups (mean± SD) in comparison to control group.
DISCUSSION

One of the main achievements of physical exercise is the prevention of heart diseases. Nowadays, due to urbanization and industrialization of human communities, most people are faced with a lack of time for exercise. Sports science experts are going to design more effective training models which need less time. Therefore, this research based on available data, was carried out for the first time in order to clarify the effect of HIIT on resistin gene expression, one of the pro-inflammatory factors, in visceral adipose tissue in rats. The results showed that, resistin gene expression in visceral adipose tissue of rats fed a high fat diet for 16 weeks, increased 2-fold along with weight gain and obesity in animals. This result is in line with findings of other researchers. Almost, in most studies, increases in resistin gene expression in tissue or serum have been reported in subjects with fat accumulation and obesity [22-25].

Jones and co-workers (in 2009) who studied the resistin gene expression in subcutaneous fat of human subjects, reported that resistin gene expression had been increased in obesity and fat accumulation and decreased significantly in subjects with weight loss due to exercise or calorie-restricted diets [23].

Studies in humans have shown an inverse relationship of serum resistin levels with waist-to-hip ratio but a direct proportion to the body fat mass. These findings are in accordance with the role of resistin in insulin resistance, i.e., the more accumulation of fat in the body, the higher serum resistin have been reported [26-28].

The results of present study demonstrated that HIIT reduced resistin gene expression 8-folds compared with fat group, however, this reduction was not a significant, possibly due to low number of animals and wide dispersion of data in fat group.

Kadoghlou and colleagues in 2007 showed that regular aerobic exercise is effective in reducing resistin serum levels. In addition, physical activity reduced IL-6 and C-reactive protein as well. In that study which carried out on 60 diabetic obese men and women, HIIT with 50-85% of VO2max for 16 weeks showed a significant reduction in resistin serum levels compared to control group [29].

In this regard, Balducci in 2010 in a 12-month study on the effect of exercise with different intensities on body weight and certain serum parameters, including cytokines in obese and diabetic subjects. It was shown that, HIIT significantly decreased pro-inflammatory agents in serum such as resistin, IL-6, C-reactive protein, interferon-γ and tumor necrosis factor-α [30].

The most similar study to the present research conducted in 2013 by Rashid Lamir et al. in which they studied the effect of aerobic exercise on resistin gene expression in lymphocytes of active young women. 20 active young women randomly divided into two experimental and control groups. The experimental group exposed to exercise 4 sessions of 60 minutes with 70 to 80% of their maximum heart rate for 8 weeks. Regular aerobic exercise in these subjects for 8 weeks led to weight loss and reduction in body mass index and blood lipids, including LDL, cholesterol. However, an increase in resistin gene expression in lymphocytes of subjects were reported. The researchers suggested that the significant increase in resistin gene expression in lymphocytes of subjects is related to the role of resistin in the body's antioxidant defense. Because in the process of inflammation in the body, lymphocytes secrete resistin. In fact, regular aerobic exercise combined with weight loss and lowered fat content, increase the body's antioxidant defense by stimulating the synthesis of resistin in lymphocytes [31]. The results of the present study is not consistent with findings of Rashid Lamir et al. Even though the reduction of resistin gene expression in visceral adipose tissue in rats was not significant, the present study did not show any increase in gene
expression with HIIT but there was several-fold reduction compared to the control and fat groups.

In fact, due to contradictory results, the mechanism for the effect of exercise on resistin expression is not clear yet. Increased serum levels after exercise in the findings of some researchers, has been justified by antioxidant effect of resistin [31, 32]. However, the reason for insulin sensitivity reported in some subjects in spite of high levels of serum resistin may be related to the mechanism(s) of resistin which is not yet well known, and more studies are needed to explore this subject.

In conclusion, this study showed that HIIT could reduce resistin gene expression as a proinflammatory factor in the visceral adipose tissue of obese rat. Despite the non-significance of differences, resistin gene expression was reduced several folds, so further studies are needed in this field.

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