The Effect of a Regular Aerobic Exercise Combined with Consuming Garlic Extract on the Rest Levels of Plasma Irisin and FNDC5 of the Heart Tissues and Muscles of Aged Rats

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Abstract
The aim of this study is to assess the effect of a regular aerobic exercise program combined with consuming garlic on the rest levels of plasma irisin and FNDC5 of the heart tissue and muscle of aged rats.

Methodology: In this study, 42 male aged rat (age=40 to 50 weeks, initial weight= 250-300gr) were divided randomly into 6 groups with 7 rats in each group: 1) control group, 2) saline group, 3) sham group, 4) garlic group, 5) exercise group and 6) exercise-garlic group. The exercise-garlic group received 1 ml garlic supplement for one kilogram of body weight through oral gavage on daily basis. Swimming for 8 weeks (three days per week and 30 minutes in every session) was considered as the exercise plan of this group. After the last round of exercise session and following 10-12 hours of fasting the rats were anesthetized and their plasma as well as heart tissue and muscle were removed immediately and kept in a freezer at -70oc in order to measure plasma irisin and FNDC5 levels of heart and muscles. Rat Irisin Elisa Kit was used to measure plasma irisin through ELISA. Data was analyzed using independent t-test in order to determine the difference between groups. The significance level was set at P≤0.05.

Findings: According to the findings, plasma irisin and FNDC5 levels were increased after 8 weeks compared with control group. But this increase was not significant (P>0.05).

Conclusion: According to results, swimming exercise for 8 weeks, consuming garlic and a combined approach of exercise-garlic did not result in a significant increase in the plasma irisin and FNDC5 levels of heart tissue and muscle in normal aged rats.

Keywords: swimming exercise, plasma irisin, aged rat, garlic extract

Introduction
As aging proceeds, elders encounter an ever-increasing risk of chronic diseases due to a decreased level of physical activity and mobility originated from the nature of this period of life. This leads to overweight and obesity problems associated with cardiovascular diseases, diabetes, hypertension, chronic kidney diseases and other chronic diseases. Therefore, paying attention to the life style of elders and their nutrition are of high importance. This becomes more serious in developing countries due to lack of supporting programs or ineffective programs affecting elders’ quality of life [5]. According to recent reports, many hormones account for regulating energy cost and preventing overweight. The hormones are released from fat tissue and skeletal muscle. Some studies have concentrated on a number of proteins and hormones that are produced in skeletal muscle and regulate energy cost and can be measured both in skeletal muscle and blood [6]. Irisin is one of them. It is one of the newest discovered hormones associated with energy homeostasis and consequent obesity [22]. Irisin has significant and direct effects on fat tissue [21] and is considered as a potential indicator of myocardial infraction [17]. Irisin is secreted into blood through the contraction of skeletal
muscle and after releasing from type 4 phybronictoein protein membrane (including FNDC5 protein) [2]. Affecting white and brown fat tissue, this hormone increases energy consumption and consequently decreases weight [6]. Exercise triggers the production of irisin hormone and FNDC5. This makes body cells to burn their excessive energy [18]. The hormones convert white fat cells, accounted for saving energy, to brown fat cells. This is actually an approach by which exercise can control body weight. It seems, however, irisin and FNDC5 help the prevention of cell changes accounted for type 2 diabetes [16]. According to studies, regular exercise positively affects irisin and FNDC5 through increasing energy metabolism [7]. Moreover, it has been accepted that as a mediator the hormones transfers the beneficial effects of exercise to the fat tissues of human and rodents [9]. Sanchis-Gomar et al (2012) reported that irisin and FNDC5 are released from skeletal muscle. The extent of the release of both indicators can increase energy cost and maintain blood glucose at a normal level [10]. In contrast, it has been shown that irisin levels in blood flow, accounted for adjusting calorific cost, decreases in people who perform exercise [11]. In addition, the attention of many researchers has been attracted towards regular physical activity together with consuming herbs as factors improving quality of life and body composition, preventing overweight and preventing and treating cardiovascular, kidney, fatty liver, overweight, metabolic disorders, like diabetes, and hypercholesterolemia diseases [12]. Garlic is a medicinal herb with an increased consumption rate aimed at treating and preventing a large number of diseases. In some cases, it can be considered as a good substitution for drug therapy [20]. It is a plant that strengthens body immune system with positive effects on some types of cancer [19]. With its beneficial effects, it serves as an anti-spasm, anti-virus, antiseptic, blood pressure reducer, anti-malaria, appetizer and bile stimulating herb [3]. Seo et al [2014] showed that garlic and exercise did not affect the FNDC5 and plasma irisin levels of the skeletal muscle of rats that are under high-fat diet. They accepted that adjusted FNDC5 and irisin levels may not affect the inhibition of high-fat diet induced overweight and insulin resistance through garlic supplement and exercise [15].

Based on the studies carried out on irisin and FNDC5 levels on the one hand and considering the inattention of researchers to the effect of aerobic exercises on irisin and FNDC5 in elders on the other hand, this study aims to answer this question: Does an 8-week aerobic exercise along with consuming garlic have a significant effect on the plasma irisin and FNDC5 level of the heart tissue and muscle of aged rats?

**Methods**

This is an experimental study because its population consists of randomly selected Wistar male rats and many variables of the trials were under control in the laboratory. In this study, 42 aged male rats (age = 40 to 50 weeks; initial weight = 250-300 gr) were divided into 6 groups: 1) control group, 2) saline group, 3) sham group, 4) aerobic exercise group, 5) garlic group and 6) garlic-exercise group. During the study phases, the studied rats were kept in transparent polycarbonate cages (size = 30*15*15) made by Razi Rad Co. in the following condition: light/dark cycle ratio (LDR): 12h: 12h, temperature = 22 ± 2°C, humidity = 50% ±5%, and ventilation condition = acceptable. The food of trials was produced by Karaj Dam Behparvar Food Co. and was put in every cage based on the weight of rats that were measured once three days with standard weighing machine and considering the natural share of 10 gr per 100 gr of body weight. In all stages, water was supplied freely to rats within animal-specialized bottles with a volume of 500 ml. First of all, old garlic was supplied from market and was cleaned and ground. It was kept for 3 hours in room temperature and humidity and extraction process was performed through maceration method. To start extraction process, a total of 50 gr ground garlic was poured in a 1 liter balloon and methanol was added up with a ratio of 1:3. The solution, then, was filtered by Buchner funnel and methanol was added up to the remained slag. The slag was filtered again after 24 hours and was added to the first extract. The obtained solution, then, was distilled in a vacuum distillation device at a temperature of 50°C and at a speed of 70 rpm until the solution volume was decreased to 1/5 of its initial volume. At this point, the extract tank was separated from the device. When the solution was cooled it was...
decanted for three times with 50 ml chloroform. The remained volume was poured in a Petri dish with a given weight and was ovened within device at $50^\circ C$. When the extract was dried, it was weighted and every ¼ gram of the extract powder was mixed with 56 ml distilled water. The supplement group and supplement-exercise group were received 1 ml garlic extract for every kilogram of their body weight on daily basis for 8 weeks through oral gavage. Saline group, however, was received the same volume of saline supplement in the same manner [13]. Before the commencement of the main protocol, the trials of exercise group were trained for one week (five days per week and each time for five minutes) in order to learn swimming. The main exercise plan was as follows: swimming for 8 weeks (three days per week and 30 minutes for every session [13]) in water (temp=$32^\circ C \pm 2^\circ C$). A five minutes interval was considered before and after the main exercise in order to let the trials warm and cool. Blood sample collection and biopsy operations were conducted 48 hours after the last exercise session and after 10-12 hours of fasting. The rats were anesthetized by intra peritoneal injection of Ketamin (30-50 mg/kg) and Xylazine (3-5 mg/kg). Then, their blood, heart and soleus muscles were immediately removed and placed in a freezer at $-70^\circ C$ for measuring irisin and FNDC5 levels purposes. To avoid the influence of circadian heart rhythm rate, sampling operation was started at 8:00 a.m. and was completed at 11:30 a.m.

**Measurement of Variables**

A total of 0.5 gr of the collected tissue (heart and muscle) was placed in liquid nitrogen. The tissue, then, was homogenized in 1 ml phosphate buffered saline ix with 1mM PMSF in a homogenizer device at $0^\circ C$. The obtained solution was centrifuged for 10 minutes at $4^\circ C$ (speed=4000 rpm). In the next step, the outer layer of solution was filtered and used in ELISA test. The concentration of plasma irisin was measured through ELISA method using Rat irisin ELISA Kit, Sunlong Biotch, China with a sensitivity of 1 pg/ml. However, the concentration of FNDC5 of heart and soleus muscle was measured using the ELISA Kit of Biotech Rat Fibronectin Type III Domain Containing Protein 5 (FNDC5) ELISA Kit, China with a sensitivity of 1.5 pg/ml. Descriptive statistics was used to determine and classify the index of dispersion. Levene test and ANOVA were used to assess the equality of variables variance and the change between groups, respectively. Tukey test was used to determine differences between groups. In this assessment, sig. level of $P\leq0.05$ was considered as the measure for rejecting the null hypothesis. All statistical calculations were conducted in SPSS 16 and all graphs were drawn in Excel.

**Findings**

According to ANOVA analysis, there was a significant difference in plasma irisin between the studied groups after 8 weeks so that Tukey test results showed a significant increase of irisin in exercise group (6.73%), garlic group (5.97%) and exercise-garlic group (6.39%) compared with control group (see Fig. 1). However, no significant difference was seen in the serum levels of irisin between exercise-garlic and control groups ($P>0.05$).

Fig. 1: mean level of plasma irisin in the studied groups. * indicates a significant difference compared with control, saline and sham groups
According to ANOVA analysis, there was no significant difference in the FNDC5 level of heart tissue between the studied groups after 8 weeks. Although there was an increase in exercise group (11.46%), garlic group (7.90%) and exercise-garlic group (7.11%) compared with control group (see Fig.1), this difference was not statistically significant (P>0.05) (see Fig. 2)

Fig. 2: mean level of FNDC5 protein in the heart tissues of the studied groups

According to ANOVA analysis, there was no significant difference in the FNDC5 level of muscle tissue between the studied groups after 8 weeks. Although there was an increase in exercise group (19.86%), garlic group (17.21%) and exercise-garlic group (15.89%) compared with control group, this difference was not statistically significant (P>0.05) (see Fig. 3)

Fig. 3: mean level of FNDC5 in the muscle tissue of the studied groups
Discussion
As aging proceeds, elders encounter an ever-increasing risk of chronic diseases due to a decreased level of physical activity and mobility originated from the nature of this period of life. This leads to overweight and obesity problems associated with cardiovascular diseases, diabetes, hypertension, chronic kidney diseases and other chronic diseases. Therefore, it is very important to pay attention to one of the most important issues of this period of life, especially in developing countries [5]. The attention of researchers has been attracted to performing regular physical activities together with consuming herbs as factors improving quality of life and body composition, preventing overweight and preventing and treating cardiovascular, kidney, fat liver, overweight, metabolic disorders like diabetes and hypercholesterolemia [12]. The researchers stated that exercise is an important factor affecting the secretion of this hormone and the other effects of this hormone on energy metabolism. However, the type of exercise and the minimum duration of exercise required for physical health are not clear yet [8]. The type of this study and the specifications of the trials differentiate it from other studies. Roka et al (2013) showed that short-term resistance training stimulates the secretion of FNDC5 in hypodermic and visceral adipose tissue. They observed that white adipose tissue (WAT) decreases the secretion of FNDC5 in non-fed animals. The white fat tissue of fat animals creates a kind of resistance through the excessive secretion of this hormone as 72% of total secretion of FNDC5 and irisin is attributed to muscle-secretion [14]. Raeisi et al (2013) found that plasma irisin level increases significantly following a resistance training session. However, there was a significant increase in the relative expression of FNDC5 and UCP1 genes following exercise. They concluded that by secreting myokines like irisin, resistance training may improve body composition through increased conversion of white fats to brown fats [4]. It is likely, therefore, that the resistance training of this study could not apply the required pressure on muscles in order to stimulate the secretion of myokines like irisin and FNDC5. A brief look at the findings of this study reveals that consuming garlic has not a considerable effect on the studied indicators in mice.

Conclusion
The results of this study showed that following an 8-week aerobic swimming exercise, there is an increase in the levels of plasma irisin and FNDC5 of rats' heart tissue and muscle but this increase is not significant. Therefore, it is suggested to future studies to more concentrate on the effect of different types of exercise on plasma irisin variations.

References