

Research Article

The effect of aerobic training and curcumin supplementation on the expression of IGF-1 gene in muscle rat

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Abstract

Background: The purpose of this research was to investigate the effect of four weeks of moderate aerobic training and nanocurcumin supplementation on IGF-1 gene expression and its receptor in the soleus muscle of rats.

Materials and Methods: 32 male Wistar rats, approximately eight weeks old, with a weight range of 200 ± 10 grams, were prepared and kept for one week under standard conditions of light, temperature, and humidity. Rats were randomly divided into 4 groups of 8 series: healthy control group, aerobic training group, nanocurcumin group and aerobic training + nanocurcumin group. The training protocol was for 4 weeks, 5 days a week, in the first week, 20 minutes of activity with a speed of 18 meters per minute, and finally in the 4th week, it reached 35 minutes of activity with a speed of 20 meters per minute. Nanocurcumin supplement was also used for each animal at 80 mg / kilogram of body weight. One-way analysis of variance and Bonferroni's post hoc test was used at the significance level ($P < 0.05$).

Results: The results showed an increase in IGF-1 and IGF-1R gene expression levels in the soleus muscle in all groups compared to the control group.

Conclusion: The combination of training and nanocurcumin supplementation probably increases the antioxidant capacity of the body and causes a greater increase in the expression of the IGF-1 gene and IGF-1R in skeletal muscles, which indicates more anabolic effects.

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1. Introduction

Inactivity due to industrial life or injury can be associated with an increase in the loss of muscle mass and increase the level of disability in people. Two important factors in improving body composition are daily sports activity and nutritional interventions. The best and biggest changes in the body happen when proper nutrition programs are implemented at the same time as regular sports activities (1). Improving body composition, which means increasing muscle mass and reducing unnecessary fat mass, is one of the most important elements of physical fitness, which improves sports performance and prevents people from suffering from diseases related to chronic inflammation caused by obesity and overweight (2). The production of free radicals and oxidants is an inevitable necessity of running sports programs, and many sports adaptations are the result of the release of these destructive molecules that make the body stronger against them (3). All kinds of sports activities increase the secretion of insulin-like growth factor (IGF-1) and increase the size of muscles as well as the strength and power of skeletal muscles. Insulin-like growth factor-1 is one of the best growth factors and has many beneficial results of physical activity. One of the most important functions of IGF-1 is regulating protein synthesis in skeletal muscles and promoting body growth (4). IGF-1 after binding to the IGF-1R receptor, phosphorylates an insulin receptor substrate intracellular adapter protein (IRS-1), which phosphorylates Phosphoinositide 3-kinase (PI3K), followed by Akt phosphorylation (5). Mammalian target of rapamycin (mTOR) is a target Downstream of Akt, mTOR activity is closely related to anabolic/catabolic balance. The IGF-1/Akt/mTOR pathway is essential in promoting muscle hypertrophy (6).

Skeletal muscle cell size is determined by the balance between the synthesis of new proteins and the degradation of old proteins. Under physiological conditions, the rate of protein synthesis and degradation is balanced and myofiber size is maintained. Under physiological conditions, the rate of protein synthesis and degradation is balanced and myofiber size is maintained. In the condition of weight loss, on the contrary, myofiber protein degradation is accelerated and the rate of protein synthesis is suppressed, resulting in muscle weakness and fatigue (7). IGF-1 can regulate protein synthesis and degradation pathways (6). In a study conducted on young healthy subjects, high levels of circulating IGF-1 were negatively associated with body fat, body mass index, and total cholesterol, and positively associated with aerobic fitness and muscular endurance, In contrast, lower levels of IGF-1 were associated with various pathological conditions including chronic diseases, inflammation and malnutrition (3).

Recently, the complementary role of curcumin in the anti-atrophy effects of skeletal muscles has been shown. Taking this supplement by increasing the antioxidant capacity and reducing the oxidants leads to the development of muscle hypertrophy processes. This polyphenol has anti-inflammatory, antioxidant and anti-tumor effects and inhibits aging processes in skeletal muscles by directly removing ROS. Curcumin interacts with various molecular targets including cytokines, growth factors, proteins, enzymes and receptors (8). The results of a study showed that curcumin improved hypoglycemia in rats by increasing IGF-1 gene regulation and improving oxidative stress caused by diabetes (9).

Although curcumin has been investigated in various clinical conditions, studies to evaluate its effect on muscle changes caused by exercise training are rare.

Considering that so far, there is no study on the simultaneous role of curcumin and aerobic training with moderate intensity and has not investigated the changes in the expression of the IGF-1 gene and its receptor in skeletal muscle, and considering the existence of contradictions in the results of previous researches, in this study, the effect of moderate aerobic training and nanocurcumin supplementation on the expression of the IGF-1 gene and its receptor IGF- 1R was investigated in soleus muscle of rats.

2. Materials and Methods

In this experimental and fundamental research, 32 Wistar male rats, approximately eight weeks old and weighing 200 ± 10 grams, were obtained from the Pasteur Laboratory Animal Breeding and Reproduction Center (Tehran, Iran) and then transferred to the Animal Sports Physiology Laboratory. Animals had free access to standard laboratory food and water. Rats were kept for one week under standard conditions in terms of proper ventilation and 12-hour dark and light period, temperature of 22 ± 3 degrees Celsius and humidity of $50 \pm 3\%$. Then the rats were randomly divided into 4 groups of 8 series. Healthy control group, aerobic training group, nanocurcumin group, aerobic training + nanocurcumin group.

Estimation of maximum running speed

For the test to determine the maximum speed, the animals performed the warm-up program at a speed of 5 meters per minute for 5 minutes. Then they started the test with a speed of 9 meters per minute (5 minutes for each of the speeds). Next, the speed of the conveyor belt was increased by 2 meters per minute until the animal reached a stop. The criterion of the animal's disability was the inability to return to running on the treadmill within 10 seconds (10,11,12).

Exercise protocol

First, for one week, the rats were prepared to perform aerobic activities and familiarize themselves with a special treadmill. The training protocol for 4 weeks, 5 days a week, in the first week was 20 minutes of activity at a speed of 18 meters per minute and finally in the 4th week, 35 minutes of activity at a speed of 20 meters per minute (Table 1) (13).

Table 1: Aerobic training protocol

type of training	week	intensity	duration	Frequency
	1	18 m/min	20 min/day	5 day/week
	2	18 m/min	25 min/day	5 day/week
	3	20 m/min	30 min/day	5 day/week
	4	20 m/min	35 min/day	5 day/week

Nanocurcumin supplementation

Chitosan (500 mg) was dissolved in 2% acetic acid solution (50 mL) and mixed with curcumin in ethanol (1 mg/mL). 15 ml of 1% TPP solution was added drop by drop under constant magnetic stirring. Then the solution was stirred for 1 hour and centrifuged at 10,000 rpm for 30 minutes. The obtained pellet was resuspended in water and further lyophilized to obtain chitosan nanoparticles encapsulated in curcumin. The size and morphology of formed nanoparticles were analyzed using scanning electron microscope (SEM), particle size with Zeta sizer device and product stability with DLS device. Commercially manufactured nano curcumin by Exir Nano Sina Company (Tehran, Iran) was used as a comparative sample of product quality. After preparing the solution, 80 mg per kilogram of body weight was gavage for each animal (14).

Gene expression evaluation method

All stages of keeping and slaughtering rats were done according to the criteria of Pasteur Laboratory Animal Ethics Committee (Tehran, Iran). 48 hours after the end of the research and supplementation protocol, all rats were anesthetized by intraperitoneal injection of a combination of ketamine (70 mg/kg) and xylazine (3 mg/kg) and the soleus muscle was removed with a surgical kit by a specialist. It was taken out and after washing, it was frozen in a saline solution and stored in a refrigerator at -80. The expression of genes was measured by designing the primers of the studied genes whose sequence is in Table 2.

Table 2: Sequence of genes

	Gene	Sequence
1	IGF1	Forward: GGTAGGGTAGGTTGGAAATG
		Reverse: GCGAAGGTCTTGGTCACATC
2	IGF-1R	Forward: AAGTSGGTAATGGCATGAGA
		Reverse: GTTTGGTTTCCCACGGCTTC

Statistical analysis

In order to analyze the data, descriptive and inferential statistics were used. At the level of descriptive statistics, indicators such as mean, standard deviation, and in the inferential statistics section were used to analyze the data, one-way Anova and Bonferroni's post hoc test were used at the significance level ($P < 0.05$).

3. Results

According to the results of the Kolmogorov-Smirnov test, the distribution of the data related to the research variables is normal. Moderate aerobic training and nano curcumin supplementation had a significant effect on IGF1 gene expression in rat muscle cells. The results of Bonferroni's post hoc test showed that there was a significant difference between the control group and the experimental groups and the training group and the nanocurcumin group with nanocurcumin + training group ($p = 0.000$). The figure1 shows the comparison of IGF1 values.

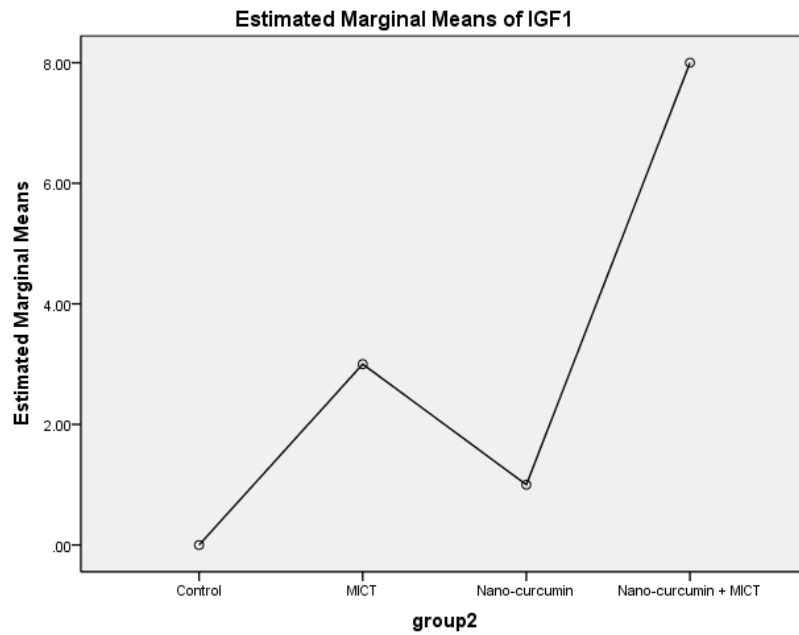


Figure 1: Comparison of IGF1 expression in four groups: control, training, nanocurcumin, nanocurcumin + training

The result of moderate aerobic training and nano curcumin supplementation has a significant effect on the expression of the IGF-1R receptor gene in rat muscle cells.

The results of Bonferroni's post hoc test showed that there is a significant difference between the control group and all the groups. The figure2 shows the comparison of IGF-1R values.

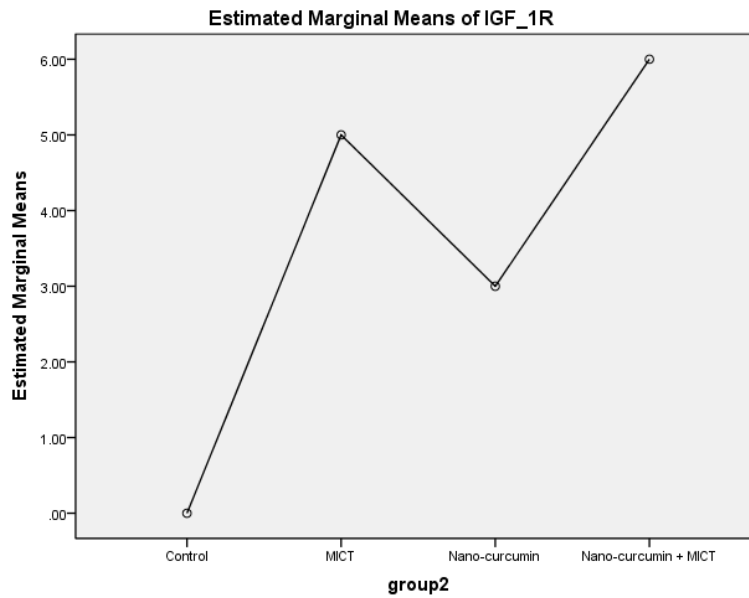


Figure 2: Comparison of IGF-1R expression in four groups: control, training, nanocurcumin, nanocurcumin + training

4. Discussion

In the present research, the IGF-1 gene expression values of soleus muscle increased in all groups compared to the control group

Sports training and nanocurcumin supplement alone are a suitable and sufficient intervention to increase muscle IGF-1 gene expression. In the case of lack of training, the level of IGF-1 gene expression in the soleus muscle will decrease, which can be prevented to a significant extent only by taking nanocurcumin supplements, and as a result, muscle wasting can be prevented (15). There is evidence that curcumin increases the serum activities of antioxidants such as superoxide dismutase (SOD). Curcumin can destroy various forms of free radicals such as reactive oxygen and nitrogen species (ROS and RNS, respectively) and regulate the activity of GSH, catalase and SOD enzymes in neutralizing free radicals. Also, it can inhibit ROS-producing enzymes such as lipoxygenase/cyclooxygenase and xanthine hydrogenase/oxidase (15). In this research, it seems that nanocurcumin supplementation alone has prevented the reduction of IGF-1 by developing the antioxidant capacity of the body and especially skeletal muscles, caused by injuries or skeletal problems, it has useful and significant effects that should be further investigated in other researches. When nanocurcumin supplementation was combined with moderate aerobic training, IGF-1 gene expression reached the highest level and showed an increase of 8.17 times compared to the control group.

Although all kinds of sports activities can increase the expression levels of the growth hormone gene as well as IGF-1, studies have shown that strength training increases the levels of IGF-1 more than other sports training (16).

It seems that due to the high production of oxidants, aerobic training moderates IGF-1 gene expression to some extent compared to resistance training. For this reason, resistance training creates a greater increase in muscle mass. Maybe combining an effective substance in reducing oxides and free radicals along with sports training with the development of antioxidant defense will reduce the modulatory effects of IGF-1, which seems to have played such a role in the present research. In the present study, the increase in IGF-1R gene expression in the soleus muscle of Wistar rats was significantly increased in all three intervention groups compared to the control group. All of the effects of IGF-1 are induced by binding to its specific cell surface receptor, the IGF-1R. If the IGF-1 receptor is not expressed or upregulated, none of the anti-apoptotic or anti-proteolysis pathways will be established and the breakdown of cellular proteins will begin (17). The amount of proteins in the body is the result of a homeostasis between the analytical and anabolic regulatory genes, and one of the most important and key pathways is IGF-1, which without its receptor will not have any effect on the cell and subsequent processes. . So binding of IGF-1 to its receptor is one of the most necessary and important cellular pathways to maintain muscle mass (17).

In chronic diseases such as metabolic syndrome or diabetes mellitus, the IGF-1 receptor expression levels decrease on the cell surface, which in the long run leads to the loss of a large amount of muscle tissue in sick people. Sports activity of any kind has the ability to increase the expression of IGF-1R on the cell surface. In fact, the increase of cellular receptors is one of the most important effects of training, especially aerobic training (18).

Conclusion

The obtained results indicate that aerobic training and nanocurcumin consumption alone increased the expression of IGF-1R and IGF-1 genes in muscle, but the combination of training and nanocurcumin supplementation probably caused a greater increase in IGF-1R and IGF-1 genes expression by increasing the body's antioxidant capacity, which indicates more anabolic effects. According to the positive effect of aerobic training and the consumption of nano curcumin in the subjects of this research and based on the similar results of past human research, it is suggested to implement the aerobic training method used in this research to increase the anabolic power and for synergistic effects, nano curcumin supplement be consumed in the amounts used in this research.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: H.V., M.H.; Methodology: H.V., M.H.; Software: H.V., M.H.; Validation: H.V., M.H.; Formal analysis: H.V., M.H.; Investigation: H.V., M.H.; Resources: H.V., M.H.; Data curation: H.V., M.H.; Writing - original draft: H.V., M.H.; Writing - review & editing: H.V., M.H.; Visualization: H.V., M.H.; Supervision: H.V., M.H.; Project administration: H.V., M.H.; Funding acquisition: H.V., M.H.;

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