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ORIGINAL ARTICLE

Short-term Effect of Aerobic Exercise, High-fat Diet and Curcumin Extract on Interleukin-six Gene Expression in Muscle Tissue of Rats

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KEYWORDS

Short-term aerobic exercise;
High-fat diet;
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ABSTRACT: Interleukin plays six major roles in various types of host defense mechanisms, including immune reactions and acute phase reactions. Therefore, the aim of the present study was to compare the short-term effects of aerobic exercise and high-fat diet and curcumin extract on the expression of interleukin-six gene in muscle tissues in male Wistar rats. For this purpose, 50 two-month-old male rats were prepared and divided into five groups: control group, high-fat diet, curcumin and high-fat diet, exercise and high-fat diet, curcumin + exercise + high-fat diet. Data analysis was compared using analysis test of one-way variance. Bonferroni supplementary test was used to accurately determine the differences between groups. The mean of group differences were also compared using one-sample t-test. The results showed that the mean expression of interleukin gene of 6 subjects showed a significant difference between the 5 groups. The results of Bonferroni post hoc test also showed a significant difference between the control group and other groups. Also, the gene expression of interleukin 6 in the two groups of high-fat diet and high-fat diet + curcumin increased compared to the control group. But in the two groups of high-fat diet + exercise and the group of high-fat diet + exercise + curcumin decreased, which was significant at the error level of 0.05.

INTRODUCTION

Adiponectin circulates in plasma in three forms: polymer, hexamer, and multimer. It should be noted that all forms have different biological activities and multimer seems to be its more active form [1]. In a study of 661 Japanese men, low plasma concentrations of adiponectin in the liver were associated with abdominal obesity, hypertriglyceridemia, low HDL, hypertension, high fasting glucose and metabolic syndrome, and on the other hand it is said that high levels of adiponectin in healthy individuals is associated with anti-inflammatory, anti-atherogenic and anti-diabetic effects. Adiponectin reduces the production and activity of TNF-α, and on the other hand, the anti-inflammatory activity of this hormone is associated with the inhibition of interleukin 6 production along with the patterns of production of anti-inflammatory cytokines such as interleukin 10 [2]. Interleukin plays six major roles in various types of host defense mechanisms, including immune reactions and acute phase reactions. When tissue is damaged and inflamed, or when infection occurs, interleukin-six is made and transported by the bloodstream to the liver, causing the rapid production of a wide range of acute-phase proteins and inflammatory biomarkers [3].

On the other hand, interleukin-six reduces the production of some other proteins, which results in progressive inflammatory disorders in organs and tissues. Excessive secretion of interleukin-6 causes the production of acute-

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phase reactive protein in the liver, which is an independent and important risk factor for cardiovascular disease and indicates an acute inflammation. The expression level of interleukin six is the same in adipose tissue and muscle but is higher in liver tissue [4]. In addition, curcumin has other cardiovascular protective effects that can promote heart health in patients and people at risk of obesity from (high-fat) diets. Although the beneficial effects of curcumin have been well demonstrated; however, according to research, there are still not enough studies to prove the effect of curcumin on interleukin 6 in the liver of obese rats and inhibit the pathway of protein breakdown. stated in her study that many natural products found in nature are usually consumed by healthy or sick people to prevent or treat chronic diseases[4]. conducted a study entitled "The effect of eight weeks of curcumin supplementation on the expression of some genes that regulate atrophic processes in the liver tissue of obese rats with a high-fat diet"[5].

The results showed that curcumin consumption significantly reduced the expression of FoxO3a, MAFbx, MuRF1 genes and significantly increased the expression of PGC1 gene [5]. Researchers conducted a study entitled " Gene expression of interleukin 6 of liver tissue as a possible therapeutic target by exercise and polyphenols: curcumin extract and short-term exercise as anti-obesity nutrients". They concluded that lifestyle modifications such as energy restriction and increased physical activity are among the therapeutic strategies used in response to adiponectin. Short-term exercise along with supplementation usage could be effective in expressing the liver adiponectin gene [6]. The composition of curcumin has attracted the most attention of biomedical researchers and drug developers. Unlike many so-called "good drug candidates", curcumin and several dietary polyphenols do not have a known therapeutic target or defined receptor. In addition, the bioavailability of these polyphenols is usually very low due to their poor absorption in the intestine. On the other hand, physical activity and exercise in the long term by reducing visceral fat mass and subsequently creating an anti-inflammatory environment, including increased secretion of interleukin-6 and adiponectin, play a role in controlling inflammatory diseases. Examining the background of existing research, it is clear that most of the aerobic exercises considered by researchers have been of low intensity and duration due to functional limitations in obese subjects. [7]. Therefore, according to the above-mentioned information about the effect of exercise, diet and consumption of curcumin extract, the present study investigates the short-term effect of aerobic exercise, high-fat diet and curcumin extract on the expression of interleukin six gene in muscle tissues in male rats.

MATERIALS AND METHODS

Grouping: In the present study, 50 two-month-old male rats were prepared and after two weeks of keeping in controlled conditions with the aim of familiarity and adaptation to living environment, nutritional and training conditions; divided into five groups: control group, high fat diet, curcumin and high fat diet, exercise and high fat diet, curcumin + exercise + high fat diet.

Methods

Preparation and keeping of the rats

In the present study, 50 two-month-old male rats were prepared and after two weeks of keeping in controlled conditions with the aim of familiarity and adaptation to the living environment, nutritional and training conditions; were divided into five groups. To prevent stress and change of the physiological conditions, the samples were kept for two weeks under new conditions {temperature 22 ± 2°C), ambient humidity (50 ± 5%) and light-dark cycle (12:12 h). During this period, all subjects freely consumed standard food and water.

During these two weeks, the samples were subjected to an introductory program on how to work on animal electrical treadmill (ST008, made by the University of Tabriz). This smart animal treadmill had five separate channels that all related factors such as slope (positive and negative), speed and time were controlled by the smart program. During this period, the amount of electric shock was constant at 0.1 mV. During the introductory period, the slope of the

treadmill was 0%, the speed was 10-15 m/min and the training duration was 5-10 minutes per day. The rats tested in this study were kept in polycarbonate cages made by Razi Rad Company with an approximate size of 54 × 34 × 21 cm.

Wood chips were used to absorb the urine and feces of the animals as well as their comfort. Once every two days, the wood chips were replaced and once a week, the cages were washed and cleaned. In the present study, during this period (adaptation to the environment), five rats were kept in each cage. These rats are susceptible to respiratory diseases, so dust or ammonia from the animal's urine should not accumulate in the breeding hall. To prevent such a situation, it is necessary to change the air flow in the hall 10-15 times per hour. In this study, a conventional device was used to ventilate the airflow. This device was on all day and night.

At the end of this period, the rats were randomly substituted into five groups after weight matching.

High-fat diet: All groups receiving high-fat diet, used 1.5 mg per kg of body weight daily for six weeks, a high-fat diet emulsion containing the following table ingredients, which in addition to the normal diet of rodents, it was included in the diet of the rats.

Aerobic exercise: The exercise group participated in an aerobic exercise program on an intelligent electronic treadmill for five days a week (Sunday, Monday, Tuesday, Thursday and Friday) and for six weeks.

Table 1. 6-week aerobic exercise program with an intensity equivalent to 75-70% of maximum oxygen consumption

Aerobic exercise protocol	Weeks of practice					
	1	2	3	4	5	6
Training duration (minutes per day)	10	20	30	40	45	50
Treadmill speed (meters per minute)	25	26	27	28	29	30
Treadmill slope (percentage)	15	15	15	15	15	15

Data analysis

Data analysis was compared using analysis test of one-way variance. Bonferroni supplementary test was used to accurately determine the differences between the groups. The mean of group differences were also compared using one-sample t-test.

Table 2. Research protocol

Group	first and second week	Day 14	Third to eighth week (six weeks)	Day 2+
Control	Keeping in controlled conditions with the aim of familiarity and adaptation to the living environment, nutritional and exercise conditions	Weight measurement	----------	Measuring research variables
High fat diet			High fat diet gavage	
Curcumin and high-fat diet			High-fat diet gavage and curcumin	
Aerobic exercise and high-fat diet			High-fat diet gavage and aerobic exercise	
High fat diet, exercise and curcumin			High-fat diet gavage and curcumin and aerobic exercise	

RESULTS

Descriptive findings

Table 3. Findings related to the descriptive characteristics of the subjects

	Pre-test weight		Post-test weight		Interleukin 6 gene expression	
Groups	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**
Control	3.02	2.06	3.65	2.66	-	**-**
High fat diet	3.50	2.06	4.32	2.93	0.050	**2.38**
Aerobic exercise and high-fat diet	3.02	2.06	6.25	2.47	0.044	**0.57**
Curcumin and high-fat diet	2.91	2.06	2.87	2.83	0.049	**1.79**
High fat diet, exercise and curcumin	3.29	2.06	3.77	2.40	0.048	**0.48**

Inferential findings

There is no significant difference among the short-term effect of aerobic exercise and high-fat diet and curcumin extract on the expression of interleukin 6 in muscle tissue in male Wistar rats.

Table 4. Levin test for examining the presumption of equality of variances to evaluate the interleukin 6 gene expression among the groups

Variable	F	Degree of freedom 1	Degree of freedom 2	Significance level
Interleukin 6 gene expression	23.902	4	48	**0.16**

As can be seen in Table 4, there is no significant difference among the groups studied in terms of interleukin 6 gene expression variable. Therefore, the assumption of equality of variances is confirmed and parametric test can be used.

Table 5. the mean difference among the groups with control group related to interleukin 6 gene expression

Groups	Mean difference	t value	P Value
Control group and high fat diet group	1.38	87.12	**0.001**
Control group and high fat diet group + exercise	-0.42	30.11	**0.001**
Control group and high fat diet group + curcumin	0.79	50.24	**0.001**
Control group and high fat diet group + exercise + curcumin	-0.52	33.72	**0.001**

As can be seen in Table 5, the difference of the means among the control group and other groups shows that the expression of interleukin 6 gene in the two groups of high-fat diet and high-fat diet + curcumin increased compared to the control group. But in the two groups of high-fat diet + exercise and high-fat diet + exercise + curcumin decreased, which was significant at the error level of 0.05

Table 6. The results of the intergroup analysis of variance of interleukin 6 gene expression in five groups

	F	P
Control		
High fat diet	3.59	***<0.001**
High fat diet and exercise		
high-fat diet + curcumin		
high-fat diet + exercise + curcumin		

As can be seen in Table 6, the results of analysis of variance showed that the mean gene expression of interleukin 6 of the subjects showed a significant difference among the 5 groups. The results of Bonferroni post hoc test also showed a significant difference among the control group and the high-fat diet group, the control group and the diet + exercise group, the high-fat diet group + curcumin, the control group and the high-fat diet group + exercise + curcumin. Therefore, the researcher null hypothesis is rejected.

DISCUSSION

The difference of the means among the control group and other groups showed that the gene expression of interleukin 6 in the two groups of high-fat diet and high-fat diet + curcumin increased compared to the control group. But it decreased in the two groups of high-fat diet + exercise and high-fat diet + exercise + curcumin. Many previous studies have reported a significant increase in interleukin-6 following the consumption of high-fat diets, which varies depending on the type of diet and the dose consumed.

This significant increase in interleukin-6 levels could indicate inflammatory conditions following a high-fat diet. The exact mechanism of response of interleukin 6 to a high-fat diet is not yet known. One possible mechanism is the induction of neutrophils following the consumption of high-fat diets that produce interleukin-6 [8]. Cytokines have been suggested to be a bridge between obesity metabolism disorders and inflammation. It has also been suggested that one-third of interleukin-6 production comes from adipose tissue. Therefore, it is likely that the rate of change in interleukin-6 levels in the subjects with the same amount of adipose tissue is close to each other [9]. On the other hand, short-term aerobic exercise significantly reduced interleukin-6 levels. Goe et al. observed that short-term exercise did not significantly change interleukin-6 levels, while it did increase interleukin-1 levels. Another study showed that 60 minutes of exercise with an intensity of 75% of maximal oxygen consumption significantly increased interleukin-6 levels. They suggested that interleukin-6 may be released during exercise from active muscles and immune organs. On the other hand, some studies have shown that interleukin 6 levels increase rapidly and immediately after strenuous exercise and after 24 hours return to resting levels [10]. Finally, the results of this study showed that the muscle gene is expressed under the influence of short-term exercise and the consumption of curcumin, which is an antioxidant. As the consumption of each alone reduces or increases the gene expression of interleukin 6 in the muscle and the simultaneous use of both factors reduces the expression of interleukin 6 gene of these cells in muscle tissue. Considering that the amount of curcumin consumption was the same in all research groups, it can be concluded that the effect of short-term aerobic exercise depends on the intensity of exercise and with increasing intensity of exercise, the amount of gene expression in these cells changes. The results of these findings are consistent with the results of Huang (2015) research [11].

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Conflict of interests

The author declares no conflict of interest.

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