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Short-Term Effect of Aerobic Exercise and High-Fat Diet and Consumption of Curcumin Extract on Leptin Gene Expression in Liver Tissue in Male Rats

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ABSTRACT

Introduction: Leptin is a protein hormone with a spiral structure similar to cytokines, which is mainly synthesized and released by subcutaneous fat cells in a steady pulsating manner with a peak secretion near midnight. The aim of this study was to compare the short-term effects of aerobic exercise and high-fat diet and curcumin extract on leptin gene expression in the liver tissue of male rats.

Methods: Fifty male rat with two-month-old were prepared and divided into five groups: control (G1), high-fat diet (G2), curcumin and high-fat diet (G3), exercise and high-fat diet (G4), curcumin+exercise+high-fat diet (G5). Data analysis was compared using one-way analysis of variance. Bonferroni's post hoc test was used to accurately determine the differences between groups. The mean of intra-group differences was also compared using the t-test of the sample.

Results: The results showed a significant difference in the average leptin gene expression of subjects among 5 groups. The results of Bonferroni's post hoc test also showed an increase in the high-fat diet group compared to the control group(P=0.0001), and a decrease in the high-fat diet + curcumin group(P=0.0001), the high-fat diet + exercise group(P=0.0001), and the high-fat diet + exercise + curcumin group compared to the control group(P=0.0001).

Conclusion: Leptin gene is expressed under the influence of short-term exercises and the consumption of curcumin, which is an antioxidant. So that the use of each agent alone causes a decrease or increase in the leptin gene expression in the liver, and the simultaneous use of both factors causes a decrease in the leptin gene expression of these cells in the muscle tissue.

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Introduction

Leptin was discovered in 1994 by isolating the obesity gene. This substance is a protein hormone with a spiral structure similar to cytokines (Larijani and Ghodsi, 2004), which is mainly synthesized and released by subcutaneous fat cells in a steady pulsating manner with a peak secretion near midnight. According to the family to which it belongs, it can be an internal regulator on the thymus gland and the factors secreted during the reaction such as interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF-α). Since the increase in plasma concentration of leptin is proportional to the fat content of fat cells and the degree of obesity is influenced by dietary interventions or daily exercise activity, leptin can report the long-term state of body fat tissue accumulation to the brain. Obesity in mice and in some people is caused by leptin gene mutation, which results in defective protein synthesis. Obesity in mice is a consequence of mis-transcriptional splicing of leptin mRNA, which produces a short leptin receptor (Furukawa et al., 2017). As mentioned, the level of leptin hormone can be affected by the nutritional status, neuroendocrine and immune function of the body. In addition, hormones such as sex hormones, catecholamines and thyroid hormones play a role in leptin regulation. These hormones are effective on leptin production by regulating the gene responsible for obesity. In addition, cortisol and growth hormones are the most important hormones that help to increase the level of leptin secretion (Baek et al., 2018). There is a relationship between leptin changes with negative energy balance, sympathetic activity and some metabolites. Among the potential regulators of leptin secretion are

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pressure caused by exercise, changes in fuel displacement, concentration of systemic hormones and the effect of the amount of energy consumed. The reduction of fat mass is one of the reasons for which leptin levels change (Milan et al., 2015).

Among the plant and natural factors effective on leptin, polyphenols and flavonoids are very effective (Perl et al., 2015). Among these polyphenols, curcumin, or the yellow extract from the turmeric plant, is a major biological polyphenol that is widely used as a spice, food additive, and as a herbal medicine in Asia. ¬Gird (Martins et al, 2016). Research has shown that curcumin has anti-inflammatory, cell protection, apoptosis and antioxidant effects. By activating AMPK, it increases the expression of PGC-1α and further inhibits Adipo-R2 (Relling et al., 2006). In addition, curcumin has other cardiovascular protective effects that can improve heart health in patients and people at risk of obesity from high-fat diets (Kandula et al., 2016). Although the beneficial effects of curcumin have been well demonstrated; however, based on the searches, there are still not enough studies to prove the effect of curcumin on leptin in the liver of obese rats and inhibiting the protein breakdown pathway. In his study, Jin (2018) stated that many natural products in nature are usually consumed by healthy or sick people to prevent or treat chronic diseases. Among them, several dietary polyphenols, including the compound curcumin, have 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 a defined receptor. In addition, the available bioavailability of these polyphenols is usually very low due to their poor intestinal absorption. These recently discussed properties have created enormous problems for drug manufacturers. This review does not discuss how curcumin, other dietary polyphenols or their derivatives are made into pharmaceuticals. Instead, it discusses how curcumin and dietary polyphenol research has enriched our knowledge of insulin signaling, including providing researchers' perspectives on how these studies have added to the understanding of the well-known hepatic insulin function. On the other hand, the extent of permanence of leptin changes after performing sports activity is a subject that has received less attention and the findings of existing studies are contradictory (Jin et al., 2016). The effect of exercise training in preventing the occurrence of non-alcoholic fatty liver disease has shown that 16 weeks of optional running exercise with an intensity of 50 to 75% of the maximum oxygen consumption on a treadmill can be used in the expression of non-alcoholic fatty liver leptin gene in rats. In addition, the effect of regular daily exercise on increasing the oxidation of fatty acids, in order to prevent fatty liver disease, has been studied on laboratory rats, and along with these regular exercise exercises, feeding with fatty foods was also done in the control and experimental groups. Investigated and studied. Researchers' findings of aerobic activities can have a significant effect on non-alcoholic fatty liver disease. In their experimental studies, many other researchers and researchers have investigated the effect of regular daily exercise activities on the oxidation of fatty acids in preventing non-alcoholic fatty liver disease in laboratory rats. Undoubtedly, the relationship between energy consumption and physical and sports activities is one of the strongest reasons for the benefits of exercise for the treatment of fatty liver disease (Sonoli et al., 2016). Sirico et al. (2020) in a study titled "Effects of Physical Exercise on Leptin and Markers of Obesity in Children: A Systematic Review and Meta-Analysis" stated that new findings on adipose tissue physiology and obesity-related inflammatory status suggest that modifying levels Adiponectin may be relevant for long-term prevention of obesity-related chronic disease. They conducted a systematic review with meta-analysis of electronically identified randomized controlled trials. A database search was conducted to investigate the effect of physical exercise without concurrent dietary intervention on leptin or other inflammatory markers in children up to 18 years of age with a body mass index greater than the 95th percentile for age and sex. Seven trials were included in the meta-analysis, with a total of 250 participants. The findings showed that compared to the control group without any lifestyle modification, physical exercise led to a decrease in leptin. They concluded that whether the effects of physical exercise improve inflammatory status in obese children is still unclear (Sirico et al., 2016). According to the mentioned materials regarding the effect of exercise, diet and curcumin extract

supplementation, the present study investigated the short-term effect of aerobic exercise, high-fat diet and curcumin extract on leptin gene expression in liver tissue in male rats.

Material & Methods

The current research is fundamental and experimental. This research was conducted in a laboratory and controlled manner (Code of Ethics No. IR.IAU.SHAHROOD.REC.1400.059 from Islamic Azad University). Considering that it was not possible to access human subjects due to space, ethical and time constraints, therefore, animal subjects (male rats) were used. At first, the necessary permits were obtained and then according to the instructions of the Iranian Society for the Protection of Laboratory Animals, they were kept in separate cages. The statistical population of female rats and sampling method is random and its volume includes fifty male rat with two-month-old. The sample size was determined using G POWER software based on the statistical method of analysis of variance and alpha error level of 0.05 and power of 0.85 equal to 50 mice, which were randomly divided into five groups: control group, high-fat diet, curcumin and high-fat diet. Exercise and high-fat diet, curcumin + exercise + high-fat diet were divided.

In the present study, rats kept in controlled conditions for two weeks in order to familiarize and adapt to the living environment, nutritional and training conditions; they were divided into five groups. In order to avoid stress and changing physiological conditions, the samples were kept for two weeks under new conditions (temperature (22±2°C), ambient humidity (50±5%) and light-dark cycle of 12:12 hours). During this period, all subjects freely consumed standard food and water. During these two weeks, the samples were subjected to the familiarization program on how to operate on the animal electric treadmill (ST008, manufactured by Tabriz University). This smart animal treadmill had five separate channels, all related factors such as the amount of 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 familiarization period, the incline of the treadmill was 0%, the speed was 10-15 meters per minute, and the training duration was 5-10 minutes per day. Chips and wood chips were used to absorb the urine and feces of animals and also for their comfort. Once every two days, the wood chips were replaced and the cages were washed and cleaned once a week. In the present study and during this period (compatibility with the environment), five rats were kept in each cage. These mice are sensitive to respiratory diseases, so dust or ammonia from animal urine should not accumulate in the breeding and maintenance hall. To prevent such a situation, it is necessary to change the air flow in the hall 10-15 times per hour. In this research, an ordinary device was used to ventilate the air flow of the animal house. This device was on all day and night. At the end of this period, rats were randomly replaced in five groups after weight matching.

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

Aerobic exercise protocol	Weeks of training					
	1	2	3	4	5	6
Training duration (minutes per day)	10	20	30	40	45	50
Reel speed (m/min)	25	26	27	28	29	30
Slope of the turntable (percentage)	15	15	15	15	15	15

Table 2. Research protocol

group	First and second week	Day 14	3rd to 8th week (six weeks)	Day +2
G1	Keeping in controlled conditions with the aim of getting to know and adapting to the living environment, nutritional and training conditions	Weight measurement	----------	Measurement of research variables
G2			High-fat diet gavage	
G3			Gavage of high-fat diet and curcumin	
G4			High-fat diet gavage and aerobic exercise	
G5			Gavage of high-fat diet and curcumin and performing aerobic exercise	

High-fat diet

All the groups receiving high-fat food used a high-fat food emulsion containing the compounds of the following table in the amount of 1.5 mg per kilogram of body weight daily for six weeks, which, in addition to the regular rodent food, in the composition The diet of mice was intended (Mirghani et al., 2019).

Aerobic Exercise

The exercise group participated in the aerobic exercise program on the animal smart electronic treadmill for five days a week (Sunday, Monday, Tuesday, Thursday and Friday) and for six weeks.

RNA extraction, cDNA synthesis and gene expression

Real Time-PCR method was used to check the gene or mRNA expression of the desired proteins. To prepare the PCR master mix to prepare cDNA, first all the following items are poured into a microtube using a sampler in the same proportion as mentioned in the kit protocol, and then we add 0.5 microliters of the desired RNA to the master mix. The ingredients were mixed in an ice pool. cDNA was used according to the recommended protocol to perform PCR by Viragen's Mix Red. The primers were received in lyophilized vials and then diluted with Sina Gene's TE buffer according to the ratio indicated on the vial. After that, 180 microliters of TE buffer, 10 microliters of forward primer (F) and 10 microliters of reverse primer (R) were poured into the designated tubes. The sequence, length and type of primer designed for leptin gene are as follows:

Table 3. Specifications of the primers used in the Real Time PCR process

Gene name	Primer sequence	Gene access code	Product length (open pair)
leptin	F 5′-CCTGTGGCTTTGGTCCTATC-3′ R 5′-ATACCGACTGCGTGTGTGAA-3′	NM_013076.3	138

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

Results

The results of the Shapiro-Wilk test show that the distribution of data in all variables is normal. Therefore, one-way analysis of variance test was used to analyze the data. The findings related to the descriptive characteristics of the subjects including pre-test and post-test weight and leptin gene expression of mice in the groups are shown in Table 4.

According to the results of the present study, there is a significant difference between the short-term effect of aerobic exercise and high-fat diet and curcumin extract on leptin gene expression in liver tissue in male rats.

Table 4. Findings related to the descriptive characteristics of the subjects and Average difference between the groups and the control group related to leptin gene expression

group	Pre-test weight		Post-test weight		Leptin gene expression		t	P Value
	Mean	S.D	Mean	S.D	Mean	S.D		
G1	2.06	3.02	2.66	3.65	-	-	-	-
G2	2.06	3.50	2.93	4.32	2.38	0.050	87.12	0.001
G3	2.06	2.91	2.83	2.87	1.79	0.049	30.11	0.001
G4	2.06	3.02	2.47	6.25	0.57	0.044	50.24	0.001
G5	2.06	3.29	2.40	3.7	0.48	0.048	33.72	0.001

Table 5. Results of analysis of variance between groups of leptin gene expression in five groups

group	P	F
Control **high-fat diet** **Curcumin and high-fat diet** **Aerobic exercise and high-fat diet** **Aerobic exercise and curcumin and high-fat diet**	***<0.0001**	**8.11**

As can be seen in Table 4, the difference in means between the control group and other groups shows that leptin gene expression has increased in the high-fat diet group compared to the control

group. But in the three groups of high-fat diet + curcumin, high-fat diet group + exercise and high-fat diet + exercise + curcumin group, it decreased, which is significant at the error level of 0.

As can be seen in Table 5, the results of analysis of variance showed that the average leptin gene expression of the subjects showed a significant difference among the 5 groups (Table 6).

Table 6. Bonferroni post hoc test results

Variable	group	High-fat diet group	High-fat diet and exercise group	High-fat diet group and curcumin	High-fat diet group, exercise and curcumin
Leptin	Control	M=-1.614*, P=0.0001	M=0.099*, P=0.0001	M=-0.32*, P=0.0001	M=0.095*, P=0.0001
	high-fat diet	----------	M=1.713*, P=0.0001	M=1.294*, P=0.0001	M=1.709*, P=0.0001
	Aerobic exercise and high-fat diet	----------	----------	M=0.419*, P=0.0001	M=-0.004*, P=0.0001
	Curcumin and high-fat diet	----------	----------	----------	M=0.415*, P=0.0001

The results of the Bonferroni post hoc test also showed a significant difference between the groups, but no significant difference was observed between the high-fat diet and exercise group and the high-fat diet, exercise and curcumin group (Table 6).

Discussion

The findings of the research indicated a difference in the means between the control group and other groups, so that the leptin gene expression increased in the high-fat diet group compared to the control group. But it decreased in the three groups of high-fat diet + curcumin, high-fat diet + exercise group, and high-fat diet + exercise + curcumin group. Many previous researches have reported a significant increase in leptin following the consumption of high-fat meals, which have been expressed in different amounts depending on the type of diet and dosage. This significant increase in leptin levels can indicate the presence of inflammatory conditions following the consumption of a high-fat diet. The exact mechanism of leptin response to high-fat diet is not yet known. A possible mechanism is the recruitment of neutrophils following the consumption of high-fat food, which causes the production of leptin (Babalwa et al., 2019). It has been suggested that cytokines are the bridge between obesity metabolism disorder and inflammation. It has also been suggested that one third of leptin production originates from fat tissue. Therefore, it is likely that the level of leptin change in subjects with similar amount of fat tissue is close to each other (Singh et al., 2016). On the other hand, short-term aerobic exercise caused a significant decrease in leptin levels. Gu et al observed that short-term exercise did not significantly alter leptin levels. Another study showed that 60 minutes of exercise with an intensity of 75% of the maximum oxygen consumption significantly increased leptin levels. They stated that leptin is probably released during exercise from active muscles and immune organs. On the other hand, some researches have shown that leptin levels increase quickly and immediately after intense and acute exercise and return to their resting levels 24 hours later (Frayn et al., 1996). . Several possible explanations for the different results of studies on the effect of exercise on cytokines have been stated. First, the type, intensity and duration of physical activity can have a different effect on the profile of cytokines. For example, a greater increase in cytokines has been seen after extroverted exercises compared to introverted exercises. Also, the range of cytokines increase depends on the duration of training. In addition; It should be kept in mind that the activity of cytokines after training is not limited to one of them, for example, leptin is after training. In the present study, short-term aerobic training significantly reduced leptin levels compared to the control group. However, in the groups with high-fat diet and no exercise, the levels of leptin increased significantly compared to the control group. Significant changes in leptin levels following exercise and a normal diet can be related to the exercise program and duration of exercise. It is also possible that the sampling of the subjects caused leptin levels to return to resting levels. The findings obtained from the Copenhagen Marathon studies (1996, 1997 and 1998) suggest that there is a negative relationship between increasing running time and increasing leptin levels. Therefore, runners showed lower levels of leptin (Frayn, 2002),

which results were in line with the present research. In addition, the significant decrease seen in leptin levels after intense exercise in subjects on a high-fat diet indicates the beneficial effect of exercise on the reduction of inflammatory factors following a high-fat diet. Physical activity protects the body against diseases related to low-grade chronic general inflammation. This long-term effect of physical activity may be caused by the response of short-term physical activity, which is partly modulated by muscle-derived leptin. It has been shown that physiological concentrations of leptin stimulate the release of anti-inflammatory cytokines that accept the leptin antagonist in circulation and inhibit the production of pro-inflammatory cytokines. In addition; Leptin stimulates lipolysis. The anti-inflammatory effects of physical activity may protect against TNF-a-induced insulin resistance (Park et al., 2005). Dabidi Roshan and Barzegarzadeh (2011) observed that 8 weeks of high-fat diet increased leptin levels in rats. However, 8 weeks of aerobic exercise decreased leptin in subjects, which is in line with the results of the present study (Dabidi Roshan and Barzegarzadeh, 2011).

Conclusion

Finally, according to the results, the leptin gene is expressed under the influence of short-term exercises and the consumption of curcumin, which is an antioxidant. So that the use of each agent alone causes a decrease or increase in the leptin gene expression in the liver, and the simultaneous use of both factors causes a decrease in the leptin gene expression of these cells in the muscle tissue.

Conflict of Interest

The authors declare that there is no conflict of interest in the present study and the present study was carried out at the expense of the authors.

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