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Research Article

The effects of aerobic exercise and Pistacia vera hull ethanol extract on the expression of superoxide dismutase in rats on a high-fat diet

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Abstract

Background: During aerobic metabolism, skeletal muscle produces significant amounts of superoxide anion due to electron leakage from the mitochondrial electron transport chain. Superoxide dismutase (SOD) enzyme is one of the most critical enzymes for neutralizing reactive oxygen species. However, obesity weakens an antioxidant defense system, especially SOD, and obese people are more sensitive to oxidative damage. Aerobic exercise and medicinal plants can reduce obesity-induced oxidative stress in skeletal muscle tissue. Since the effect of aerobic exercise and pistachio peel extract on SOD gene expression has not been investigated, the present study intends to investigate the effect of aerobic exercise and Pistacia vera hull ethanol extract (PVHEE)on SOD gene expression in rats fed a high-fat diet.

Materials and Methods: In an experimental study, 30 young female Wistar rats aged 12 weeks and weighing 180-200 grams were selected as subjects and randomly divided into 5 groups (n=6 in each group), including control Normal diet, high-fat diet control, aerobic exercise, receiving PVHEE, aerobic exercise and receiving PVHEE were divided. The program of aerobic training was four weeks and five sessions of running on the treadmill for rodents in the range of 50-60% Vo2Max. Sixty milligrams per kilogram of body weight of ethanol extract of pistachio skin were fed to rats by gavage for four weeks and five times a week. Following completion of the interventions, soleus muscle tissue was removed to determine SOD gene expression by real-time PCR.

Results: SOD gene expression in the group fed high-fat diet was significantly lower than in the group fed normal diet (p=0.0001). Four weeks of aerobic exercise (p=0.04), PVHEE (p=0.02) and the combination of aerobic exercise - PVHEE (p=0.0001) caused a significant increase in SOD gene expression compared to the control group. The highest SOD expression was observed in the aerobic exercise and PVHEE group.

Conclusion: The results obtained from the present study showed that feeding with high-fat diet weakens the enzymatic antioxidant defense system of skeletal muscle by reducing SOD gene expression. Both aerobic exercise and PVHEE can prevent the weakening of the antioxidant defense system of muscle tissue. This will reduce skeletal muscle oxidative damage.

Keywords:
Pistacia vera hull ethanol extract, SOD, aerobic exercise , obesity

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1. Introduction

Obesity causes oxidative stress by creating an imbalance between oxidants and antioxidants (1). During aerobic metabolism, skeletal muscles produce significant amounts of superoxide anion due to electron leakage from the electron transport chain (2). Reactive oxygen species (ROS) produced by contraction up to physiological levels stimulate the expression of antioxidant genes and in higher amounts (pathological levels) weaken the enzymatic antioxidant defense system. In these conditions, oxidative stress is created, which can negatively affect both the structure and function of the tissue (3). One of the factors in oxidative stress in obesity is the increase in inflammatory mediators. Another mechanism is the increase in reactive oxygen species. In any case, obesity causes the depletion of antioxidant resources, including SOD, and obese people are more sensitive to oxidative damage (4). SOD acts as an intervention to prevent free radical chain reactions and plays an effective role in modulating oxidative stress. In fact, enzymes are the first line of defense against free radicals. As a defense strategy, skeletal muscle cells increase SOD enzyme gene expression to remove ROS (6). Performing regular aerobic exercise leads to an increase in antioxidant defense, a decrease in lipid and protein peroxidation (7). Green pistachio skin is a rich source of phenolic and antioxidant compounds and includes the major part of pistachio by-products (5). It has been reported that PVHEE consumption can increase plasma antioxidant capacity due to the presence of significant amounts of antioxidants such as β-pinene (8). Also, different PVHEE have anti-inflammatory activity and can be used as a potential source of new treatments (9,10).

Antibacterial activity of essential oils of some pistachios has also been investigated and its role in food safety and reduction of pathogenic risks has been realized (11). Examining this issue may be the solution to fighting obesity-induced oxidative stress. In obese rats, aerobic exercise and PVHEE can both increase antioxidant defense against oxidative stress, but their effects on oxidative stress markers have not been assessed. Thus, the present study examined the effects of aerobic exercise and PVHEE on superoxide dismutase gene expression in rats fed a high-fat diet.

2. Materials and Methods

Animals

In an experimental study, thirty 12-week-old female Wistar rats in the weight range of 180-200 grams were purchased from the Pasteur Institute in Tehran. Following this, the animals were transferred to the laboratory boarding house of the Tehran branch of the Islamic Azad University. In order to adapt to the environment and control disturbing factors, the rats were housed in the animal house environment for 2 weeks before the start of the implementation protocols. The rats were randomly divided into 5 groups of six. The groups included: normal diet control, high-fat diet control, aerobic exercise, receiving PVHEE, and aerobic exercise plus receiving PVHEE. The animal house and laboratory environment were regularly heated and humidified and the rats were placed in these conditions. Air ventilation was done with a silent fan to remove the unpleasant smell. The rats were placed in special bicarbonate cages 32x15, free access to city water and a special rat diet. All stages of the research were carried out according to the guidelines for working with laboratory animals of the Ministry of Health and Medical Education.

High fat diet

To induce high-fat diet, 20% palm oil, 1.5% cholesterol and 0.25% were added to the normal diet. The normal diet control group was also fed commercial pellets for rodents (12).

Aerobic exercise program

This study used treadmill running for rodents. First, the rats learned how to run on a treadmill for a week. After that, they ran for four weeks and five sessions per week on the treadmill with an intensity of 50-60% Vo2Max. Running speed in the first week was 16 meters per second, which increased to 26 meters per second after four weeks. In each session, the rats were first warmed up for 5 minutes and cooled down for 5 minutes after the end of the program (13).

Pistachio Soft Hull Extract

PVHEE was prepared by the Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR. The ethanolic extract was dissolved in distilled water and administered to the subjects at a dose of 60 mg per kilogram by the gavage method. This was done for four weeks and five times per week.

Tissue preparation

Forty-eight hours after the last intervention session and after 12 hours of fasting, the subjects were treated with a combination of 2.7 ml of xylazine (Rompun® 2%, Bayer, Puteaux, France) and 10 ml of ketamine (Imalgène® 1000, Merial, Lyon, France) at a dose of µL/100 g of body weight and became unconscious (14). After complete anesthesia, blood was drawn from the left ventricle. After ensuring the animal's death, the soleus muscle was quickly removed from the rats' body. After washing with PBS (phosphate buffered saline), it was placed inside coded 2ml microtubes. The microtube was transferred into a nitrogen tank and kept in a -70degree freezer until final execution and cell analysis.

SOD gene expression assay

In order to investigate SOD gene expression in soleus muscle, the qPCR method was applied Using GAPDH as a reference gene, we compared SOD gene expression to it. For this purpose, primer design was done first, and then total RNA was extracted from the tissues and converted into cDNA. Then cDNA was amplified by PCR method. In accordance with the existing standard method for TRIzol extraction, RNA was extracted manually using TRIzol material prepared by Kiazist.

Table 1: Sequence of primers

Gene	Gene sequence
SOD F	GCCCGGCGGATGAAG
SOD R	CCTTTCCAGCAGTCACATTGC
GAPDH F	AACCCATCACCATCTTCCAG
GAPDH R	CCAGTAGACTCCACGACATAC

3. Results

Statistical analysis

Shapiro-Wilks test was used to check the normality of the data distribution. The independent t-test was applied to examine the differences between the normal diet and the high-fat diet groups, and the one-way analysis of variances was applied to examine the effect of aerobic exercise and Pistachio Soft Hull Extract on SOD gene expression. Bonferroni's post-hoc test was also used to determine the difference between the groups. All analyses were done with SPSS software version 22 and at $p < 0.05$.

significantly lower than in the group fed normal diet ($p=0.001$) Figure 1

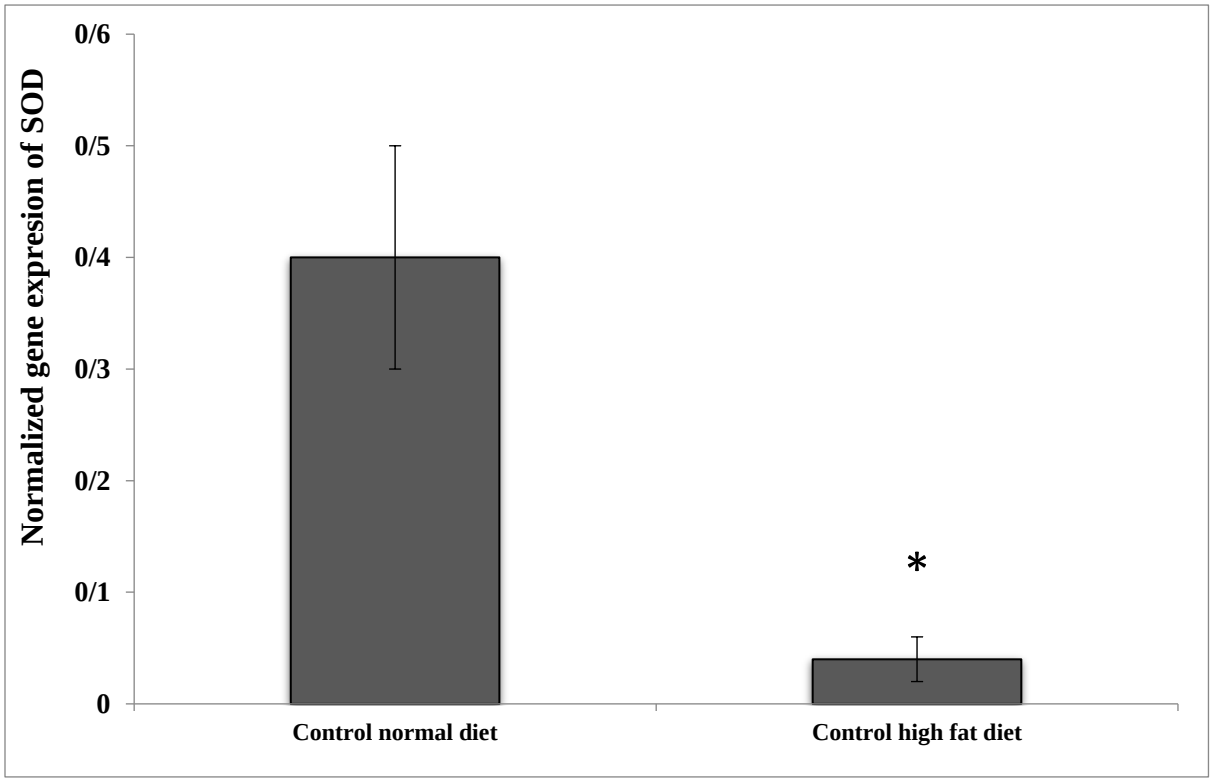

Figure 1- Comparison of SOD gene expression in the normal diet control group and the high-fat diet control group. * Sign of significant difference compared to the control normal diet group. Data are reported based on mean and standard deviation.

Four weeks of aerobic exercise (p=0.04), PVHEE (p=0.02) and the combination of aerobic exercise - PVHEE (p=0.0001) caused a significant increase in SOD gene expression compared to the control high-fat diet group. The highest rate of increase was observed in the combination of aerobic exercise and PVHEE.

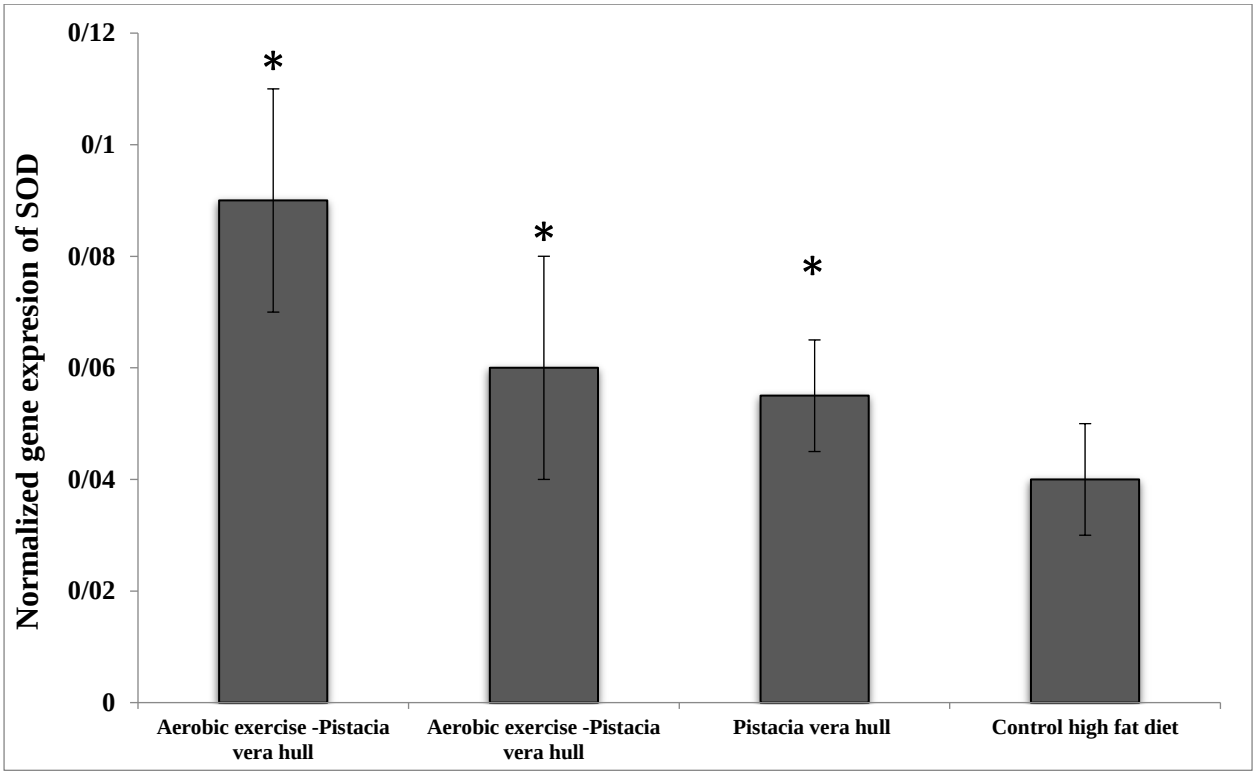

Figure 2- Comparison of SOD gene expression in the control groups of high-fat diet, aerobic exercise, PVHEE and aerobic exercise- PVHEE. * Sign of significant difference compared to the high-fat diet control group. Data are reported based on mean and standard deviation.

4. Discussion

The first finding of this study showed that feeding high-fat diet decreased the expression of the SOD antioxidant enzyme gene. It is well known that obesity is one of the main factors in oxidative stress. It has been reported that feeding high-fat diet reduces SOD in tissues (15,16). SOD forms the first line of defense against ROS and converts superoxide into oxygen and hydrogen peroxide. Then GPx converts hydrogen peroxide into water. As a result, it converts highly reactive oxygen species into water and oxygen and protects tissue from oxidative damage (17). It seems that the decrease in antioxidant enzyme activity, especially SOD and GPx, is a response to chronic systemic oxidative stress (18). A high-fat diet through several mechanisms can cause ROS and increase systemic oxidative stress and affect the enzyme antioxidant defense system, and as a result, antioxidant activity decreases, and then the systemic and tissue oxidative stress is created (19). SOD is one of the main antioxidant enzymes both in the bloodstream and inside the body tissues. SOD enzyme activity indicates intracellular antioxidant capacity. The imbalance between the production of superoxide anion (O2-) and the activity of SOD enzyme can cause the production and formation of more free radicals. It is well known that the decrease in the amount of SOD in the tissues is associated with the increase in the amount of reactive oxygen species and cell damage, which is more observed in obesity and there is a negative relationship between the activity of this enzyme and the amount of fat received (20).).When the level of activity and gene expression of antioxidant enzymes, especially SOD, decreases in the blood or tissues, the ability to neutralize reactive oxygen species decreases. In addition, free radical production increases.

In this condition, the increase of lipid peroxidation is increased. As a result, the level of MDA as the main indicator of lipid peroxidation increases. This indicates the development of oxidative stress and oxidative damage at the tissue level. According to previous studies, feeding high-fat diet causes a significant increase in ROS levels. Long-term exposure to excessive levels of ROS can lead to cell damage as well as dysfunction, which may ultimately lead to reduced SOD expression as a cellular adaptation. High-fat diets are associated with low-grade chronic inflammation. Increased levels of pro-inflammatory cytokines (such as TNF-α and IL-6) can suppress antioxidant enzymes, including SOD, through different signaling pathways (such as the NF-κB pathway). This inflammation can negatively affect muscle cells and reduce skeletal muscle antioxidant defense capacity. HFD often leads to insulin resistance, which alters metabolic signaling pathways. Insulin normally enhances antioxidant enzyme expression. Impaired insulin signaling due to a high-fat diet may decrease SOD expression. On the other hand, HFD can cause the accumulation of ceramide in skeletal muscle tissue and cause significant changes in skeletal muscle. These changes can affect gene expression through different transcription factors and thus reduce SOD expression. HFD can increase ROS production by disrupting mitochondrial function, which may reduce SOD expression. Another finding of the present study showed that aerobic exercise increased SOD gene expression in the soleus muscle. The evidence clearly shows that aerobic activities, especially with moderate intensity, can produce amounts of reactive oxygen species. As a result, reactive oxygen species can stimulate antioxidant enzyme gene expression, including SOD and CAT, at the cell level.

This is why researchers believe aerobic activity with moderate intensity is an antioxidant factor (21). In order to justify this finding, the effect of aerobic training on the ability of peripheral tissues, especially skeletal muscle cells and fat tissue, to absorb glucose can be mentioned. Researchers believe that glucose uptake by peripheral tissues as a result of aerobic exercise is due to the changes in the insulin signaling pathways in the tissues. This causes a decrease in insulin resistance and glucose uptake by the tissues and oxidative stress decreases (22). In the present study, the PVHEE significantly increased SOD expression. Pistachios' green shell contains high amounts of bioactive compounds, mainly phenolic compounds (23). These bioactive compounds have antimicrobial and antioxidant effects (24-28). Bioactive compounds in PVHEE ROS and reduce oxidative stress. This may stimulate the body's endogenous antioxidant defense mechanisms, including SOD expression. On the other hand, some phytochemicals found in the PVHEE can activate transcription factors such as Nrf2 (Erythroid nuclear factor 2). Nrf2 is a key regulator of antioxidant response genes including SOD. Once activated, Nrf2 translocates to the nucleus and binds to antioxidant response elements (AREs) in the promoter regions of target genes, increasing their expression. Another mechanism by which pistachio green skin increases SOD gene expression is to decrease inflammation. The anti-inflammatory properties of PVHEE may help reduce pro-inflammatory cytokines. Lower levels of inflammation can prepare the environment for increased gene expression of antioxidant enzymes. Another mechanism is the increase in mitochondrial function and biogenesis due to polyphenols in PVHEE. These polyphenols enhance the expression of antioxidant enzymes.

In the present study, the highest level of SOD gene expression was observed in the combination of aerobic exercise and PVHEE group. Due to their common mechanisms of reducing inflammation, decreasing insulin resistance, and increasing transcription factor activity, aerobic exercise and green pistachio skin extract seem to enhance each other's effects on the expression of SOD gene.
1)

Conclusion

The results of this study showed that feeding with high-fat diet decreased gene expression of one of the most key antioxidant enzymes in skeletal muscle. Aerobic exercise and PVHEE alone reduced the negative effect of a high-fat diet on antioxidant defense capacity by increasing SOD gene expression. The maximum effect was observed when these two interventions were combined. Based on this, since both aerobic exercise and PVHEE protected skeletal muscle tissue against the negative effects of eating high-fat diet, it is recommended that in the conditions of eating a high-fat diet, any of these interventions be used.

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

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