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The effect of selenium consumption on liver damage index during exercise in a hot environment in rats

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Abstract

Introduction: Exercise in a hot environment can lead to an increase in core body temperature and heat stress, which is associated with dysfunction of the liver. The purpose of this study was to investigate the effect of exercise in a hot environment combined with selenium consumption on liver indices in rats.

Methods: In this experimental study, 32 adults male Wistar rats weighing between 200 and 250 grams were divided into four groups: control, exercise in a normal environment, exercise in a warm environment, and exercise in a warm environment with selenium supplementation. Exercise was performed for eight weeks in a normal and warm environment. Selenium was administered as an oral supplement at a specific dose. At the end of the period, liver tissue was sampled and ALT and AST gene expression were measured.

Results: AST gene expression showed a significant increase in the warm environment training groups compared to the control group (p=0.001) and a significant decrease in the warm environment + selenium training group (p=0.001), while the difference between the control group and the normal environment training group (p=0.725) was not significant. ALT gene expression showed a significant increase in the warm environment training group compared to the control (p=0.001). Also, the warm environment + selenium training group had a decrease in ALT gene expression compared to the warm environment training group (p=0.04).

Conclusions: Training in a hot environment can lead to liver damage, but selenium supplementation plays an effective role in reducing these damages and improving physiological responses.

Key Words: Liver damage indices, selenium, exercise in hot environments.

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1. Introduction

In recent years, extensive research has been conducted on the effects of hot environmental conditions on the health and physiological performance of living organisms. One of the major challenges arising under such conditions is an increase in body temperature and heat stress, which may result in a combination of hypoxia and direct thermal injury, ultimately leading to impaired liver function. Exposure to a hot environment can have several adverse effects on liver health, including increased inflammation, metabolic disturbances, and an overall decline in hepatic function (1).

Furthermore, exercise under hot and humid conditions increases the production of reactive oxygen species (ROS) and induces oxidative stress in hepatic tissue, which may result in cellular damage and elevated levels of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Hepatic dysfunction under these conditions may be attributed to the accumulation of free radicals and lipid peroxidation-induced damage, which can impair the liver's detoxification and metabolic functions. At high environmental temperatures, the body becomes highly activated to maintain thermal homeostasis, and this sustained physiological effort places additional stress on the hepatic system. Studies have shown that elevated environmental temperature may disrupt normal hepatic metabolism by reducing hepatic blood flow and activating inflammatory pathways (2). On the other hand, moderate to severe heat stress can cause damage to the liver and kidneys and may potentially result in death (3). Therefore, attention to preventive strategies may play an important role in protecting liver health.

Selenium (Se) may exert an antioxidant effect on the liver, at least partly by enhancing glutathione peroxidase activity, the reduction of which contributes to the pathogenesis of hepatic inflammation and fibrosis. Se may influence metalloproteinases, cytokines, and growth factors involved in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Both selenium status and dietary selenium intake are negatively associated with hepatitis, cirrhosis, and liver cancer, while selenium levels in patients with NAFLD are lower than those in healthy individuals. Selenium supplementation may effectively alleviate metabolic disorders by reducing oxidative stress and regulating the KEAP1/NRF2 pathway, potentially through increased synthesis of Selenoprotein P (SEPP1) (4, 5). Selenium may protect hepatic tissues by attenuating inflammatory immune responses and modulating elevated reactive oxygen species (ROS) levels, thereby promoting tumor-cell apoptosis. Higher blood selenium concentrations have been significantly and inversely associated with advanced hepatic fibrosis. As the severity of liver injury increases, serum selenium levels decrease significantly. Therefore, selenium supplementation may have therapeutic potential in the management of chronic liver diseases (6).

Physical inactivity is strongly associated with fatty liver disease, irrespective of body weight. Increasing physical activity through exercise can improve fatty liver disease (7, 8). Exercise affects fatty liver disease through multiple pathways. In the liver, exercise increases fatty acid oxidation, decreases fatty acid synthesis, and protects against mitochondrial and hepatocellular damage by reducing the release of damage-associated molecular patterns. Consequently, physical exercise is an established therapeutic strategy for improving fatty liver disease (9, 10).

Hot environments can induce heat stress, which has significant adverse effects on liver function. Exercise under such conditions is associated with increased production of reactive oxygen species (ROS) and increased oxidative stress in hepatocytes, potentially resulting in lipid peroxidation, mitochondrial dysfunction, and elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (2, 3, 11). A detailed investigation of these effects provides a foundation for a better understanding of the mechanisms underlying heat-induced hepatic injury and may contribute to the development of more effective management strategies. Lee et al. (2014) investigated the effects of strenuous exercise in a hot environment on hepatic tissue and demonstrated that exercise under hot and humid conditions induced greater tissue damage and cellular fragmentation in hepatocytes. Moreover, serum ALT and AST concentrations increased significantly in both exercise groups, which was associated with inflammation, oxidative stress, and apoptosis (2). Chen et al. (2022) investigated the protective effects of selenium supplementation against heat stress and exercise-induced fatigue-related hepatic injury, as

well as its potential underlying mechanisms. Their findings showed that selenium significantly increased antioxidant levels, inhibited lipid peroxidation, and reduced the levels of hepatic enzymes. In addition, selenium increased hepatic glutathione and glutathione peroxidase levels and exerted a hepatoprotective effect through regulation of the NF-κB/IκB pathway, thereby inhibiting the release of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) in the liver. Therefore, selenium may be considered a promising supplement for the development of heatstroke-prevention products or nutritional supplements (12).

Based on the available evidence, elevated environmental temperature and exercise under hot conditions can disrupt thermal homeostasis, induce oxidative stress, and cause cellular damage in hepatic tissue. On the other hand, selenium, as an essential micronutrient with antioxidant and anti-inflammatory properties, may protect hepatic tissue against oxidative stress-induced injury by enhancing glutathione peroxidase activity and attenuating inflammatory responses. Accordingly, the present study aims to investigate the effects of selenium supplementation on markers of exercise-induced hepatic injury under hot environmental conditions in rats. Therefore, the primary research question is: Can selenium supplementation reduce the markers of hepatic injury induced by exercise under hot environmental conditions in rats?

2. Methods

2.1. Participants

The present study employed an experimental, laboratory-based research design. The study population consisted of male Wistar rats, which were selected because of their biological characteristics, appropriate resistance to laboratory conditions, and widespread use in physiological research. The rats were obtained from Semnan University of Medical Sciences and weighed between 200 and 250 g. Among the available animals, 32 adult male rats were randomly selected and assigned to four equal groups, with eight rats in each group.

The present study was approved by the Ethics Committee of Shahrood University of Technology under ethics code ETHICS-2410-1110. All ethical principles and regulations governing the use of laboratory animals were strictly followed in accordance with the guidelines approved by the Ethics Committee for Medical Research.

2.2. Study design

Exercise Protocol

The exercise intervention was performed for eight weeks, six days per week. The ambient temperature during exercise was controlled according to the experimental condition as follows:

- Normal-temperature environment: 22-24°C
- Hot environment: 36±1°C

The exercise sessions were performed on a motorized treadmill at a 0° incline. The training program was progressively designed according to the protocol presented in Table 1.

Table 1. Exercise Training Protocol

Week Exercise Duration (min) Treadmill Speed (m/min)

Week	Exercise Duration (min)	Treadmill Speed (m/min)
1	10	10
2	20	15
3	30	20
4	40	25
5-8	To exhaustion	Starting at 15 m/min; increased by 5 m/min every 20 min

During weeks 5–8, the animals performed exercise to exhaustion. The initial treadmill speed was set at 15 m/min and was increased by 5 m/min every 20 min until the animal was unable to continue exercising (13).

Animal Diet and Water Intake

Throughout the study, the rats were fed a standard laboratory diet provided as commercially prepared pellets obtained from Semnan University of Medical Sciences. The diet contained a balanced combination of protein, fat, and carbohydrates and was provided ad libitum. Based on standard nutritional requirements, the rats required approximately 14 g of food per 100 g of body weight per day. Water intake was approximately 12–16 mL per 100 g of body weight per day.

In the groups receiving selenium supplementation, the daily diet was enriched with selenium powder. First, the standard animal pellets were completely ground. Subsequently, 45 mg of selenium powder was added per 100 g of feed. The resulting mixture was prepared as a soft paste, molded, and dried again to produce standardized nutritional pellets. This

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procedure ensured uniform distribution of the supplement and improved its acceptance by the animals (14).

Tissue Sampling

To assess the effects of the independent variable on potential changes in the dependent variables according to the predetermined study protocol, the animals were anesthetized 48 h after completion of the exercise intervention using diethyl ether. Cotton wool was saturated with ether and placed inside a glass chamber. Each rat was exposed to the ether-soaked cotton for several minutes until anesthesia was achieved. After confirming adequate anesthesia, the rats were dissected, and liver tissue samples were collected.

Following excision, the liver tissue was rinsed with physiological saline and weighed using a digital balance with a precision of 0.0001 g. The tissue samples were then immediately placed in microtubes and transferred to a liquid nitrogen tank for subsequent analyses. Finally, the samples were transferred to a −80°C freezer and subsequently sent to the laboratory for analysis.

Gene Expression Analysis

The expression levels of the ALT and AST genes in liver tissue were assessed using quantitative real-time polymerase chain reaction (qRT-PCR) with SYBR Green chemistry. The final reaction volume was 20 µL, and each reaction was performed in duplicate. Primers were designed based on information obtained from the National Center for Biotechnology Information (NCBI) Gene database and synthesized by Pishgam Company. GAPDH was used as the reference gene.

The thermal cycling protocol for real-time PCR consisted of an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Melt-curve analysis was performed to verify the specificity and validity of the amplification products, and a standard curve was generated to optimize the experimental conditions. Relative gene expression was calculated by normalizing the expression levels of ALT and AST to that of the reference gene. The expression levels of the target genes were determined using the comparative 2^−ΔΔCT method.

2.8. Statistical analysis

After data collection, descriptive statistics were used to calculate the mean and standard deviation. The normality of data distribution was assessed using the Shapiro–Wilk test, while the homogeneity of variances was evaluated using Levene's test. Following confirmation of the normality of the data distribution, differences among the study groups were analyzed using one-way analysis of variance (one-way ANOVA). When appropriate, the Bonferroni post hoc test was used for pairwise comparisons. Statistical significance was set at $p<0.05$ and all analyses were performed using SPSS version 27.

3. Results

The expression of the AST gene was significantly higher in the exercise-in-hot-environment group compared with the control group (p = 0.001), whereas AST gene expression was significantly lower in the exercise-in-hot-environment + selenium group compared with the exercise-in-hot-environment group (p = 0.001). No significant difference was observed between the control group and the exercise-in-normal-environment group (p = 0/725).

The expression of the ALT gene was significantly higher in the exercise-in-hot-environment group compared with the control group (p = 0.001). Furthermore, the exercise-in-hot-environment + selenium group showed a significant reduction in ALT gene expression compared with the exercise-in-hot-environment group (p =0.004) (Table 2).

Table 2. Bonferroni Post Hoc Test Results for ALT and AST Among the Study Groups

Variable	Group Comparison		Mean Difference	Standard Error	p-value
AST (U/L)	Control	Exercise in normal environment	2.414	0.201	0.725
		Exercise in hot environment	2.174	0.201	0.001*
		Exercise in hot environment + selenium	1.390	0.201	0.001*
ALT (U/L)	Control	Exercise in normal environment	1.099	0.205	0.205
		Exercise in hot environment	1.636	0.205	0.001*

		Exercise in hot environment + selenium	1.263	0.205	0.001*

*Statistical significance was set at $p<0.05$.

4. Discussion

The aim of this study was to investigate the effects of selenium supplementation on markers of hepatic injury in rats performing exercise under hot environmental conditions.

The findings of the present study showed that AST gene expression increased significantly in the exercise groups, particularly in the exercise-in-hot-environment group, compared with the control group. In contrast, selenium supplementation reduced and modulated the expression of this gene. These findings indicate the adverse effects of heat stress and strenuous physical activity on hepatocytes, as well as the protective role of antioxidant supplementation in attenuating such injury.

The increase in AST gene expression during exercise in a hot environment may be attributed to increased oxidative stress and mitochondrial membrane damage. AST is a marker of cellular injury and is predominantly present in the mitochondria and cytosol of hepatocytes; therefore, increased expression or release of AST generally indicates necrosis or increased cellular membrane permeability (2). In this regard, Lee et al. (2014) reported that exercise in a hot and humid environment significantly increased ALT, AST, and other oxidative stress and inflammatory markers. From a physiological perspective, heat stress stimulates the NF-κB and MAPK pathways and increases the production of proinflammatory cytokines, including IL-6 and TNF-α, ultimately leading to cellular injury and increased expression of genes associated with hepatic enzymes (2).

In contrast, selenium supplementation resulted in a significant reduction in AST gene expression. The potential mechanism underlying this protective effect may be attributed to the role of selenium in the synthesis and activity of glutathione peroxidase (GPx) and other antioxidant enzymes, which neutralize reactive oxygen species and prevent lipid peroxidation (15). By enhancing the activities of superoxide dismutase (SOD), catalase (CAT), and GPx, selenium increases the cellular antioxidant defense capacity and may prevent cellular membrane damage and subsequent release of AST [8]. Furthermore, selenium may play a role in inhibiting the NF-κB/IκB pathway and reducing the secretion of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α. This process may attenuate inflammation and preserve cellular integrity in hepatic tissue (16).

The findings of the present study are consistent with those reported by Ghasemi et al. (2022), who found that selenium supplementation significantly reduced AST and ALP levels and attenuated hepatic tissue damage (17). Similarly, the present findings are in agreement with those of Movahedinia et al. (2011), who reported that selenium supplementation in overweight individuals reduced lipid peroxidation products following exercise, although its effects were less pronounced under resting conditions (18).

In contrast, the present findings are inconsistent with those reported by Chen et al. (2022). The lack of a significant reduction in hepatic injury following selenium supplementation in their study may be attributed to the form of selenium supplementation used. Selenium-enriched soy peptides exhibited greater antioxidant and anti-inflammatory activities in vivo than selenium-enriched soy protein and selenomethionine. These compounds are organic forms of selenium in which selenium replaces sulfur in sulfur-containing amino acids (12).

From a physiological perspective, physical activity under hot environmental conditions increases oxygen consumption, stimulates mitochondrial metabolism, and promotes the production of reactive oxygen species (ROS). In the absence of an adequate balance between ROS production and clearance, this process may lead to oxidative damage in hepatic tissue. Under such conditions, selenium may prevent the upregulation of AST gene expression by scavenging ROS, stabilizing cellular membranes, enhancing endogenous antioxidant defenses such as GPx and CAT, and attenuating inflammatory responses [8]. Other studies have also addressed the role of selenium in regulating heat-response mechanisms and reducing inflammatory effects, reporting that selenium supplementation increases HSP70 expression and decreases proinflammatory cytokines (19). This response is consistent with the effects observed following selenium supplementation in the present study.

Overall, the increased expression of the AST gene in the exercise-in-hot-environment group may indicate cellular injury associated with heat and oxidative stress, whereas the reduction in AST expression observed in the selenium-supplemented groups

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supports the potential protective, anti-inflammatory, and antioxidant roles of selenium in mitigating hepatic injury. These findings highlight the potential importance of antioxidant interventions under conditions of exercise-induced heat stress and physiological strain.

On the other hand, the findings of the present study showed that ALT gene expression increased significantly in the exercise groups, particularly in the exercise-in-hot-environment group, compared with the control group. This finding suggests that physical activity under heat stress may induce alterations in hepatic cellular responses through increased core body temperature and oxidative stress. These changes may be associated with increased hepatocellular stress and altered ALT expression. Although ALT is widely recognized as a sensitive biochemical marker of hepatic injury, increased ALT gene expression alone does not necessarily indicate hepatocellular membrane damage or leakage of the enzyme into the bloodstream.

The findings of the present study are consistent with those reported by Mousavi et al. (2020), who found that strenuous endurance exercise increased ALT and AST levels in rats and attributed these changes to increased ROS production and exercise-induced hepatic inflammation (20). Similarly, the findings are in agreement with those of Rezaei et al. (2022), who reported that exercise under heat stress increased thermal stress and ROS production, resulting in greater hepatocellular stress and higher ALT expression (21).

In contrast, the present study showed that selenium supplementation significantly reduced ALT gene expression. This finding is consistent with the results reported by Ahmadi and Rostami (2021), who demonstrated that selenium improved hepatic health and reduced ALT levels through enhancement of glutathione peroxidase (GPx) activity and attenuation of lipid peroxidation. In addition, the antioxidant properties of selenium may exert protective effects on hepatic cells by inhibiting inflammatory pathways such as NF-κB and enhancing endogenous antioxidant capacity (22).

In a study with findings inconsistent with those of the present study, Lin et al. (2022) investigated the effects of different selenium doses on hepatic injury. They reported that low doses of selenium did not exert a beneficial effect on hepatic injury, whereas higher selenium exposure was significantly associated with hepatic injury. Interestingly, in their study, both selenium status and selenium intake were inversely associated with liver injury (4). These findings suggest that the effects of selenium on hepatic health may depend on its dose, form, and physiological context.

Overall, the findings suggest that exercise under hot environmental conditions may alter ALT gene expression through oxidative stress and inflammatory responses. Selenium supplementation, potentially through attenuation of ROS production and enhancement of antioxidant defenses, may modulate ALT gene expression and contribute to the maintenance of hepatic cellular homeostasis under heat stress. However, because ALT gene expression is distinct from circulating ALT activity and histological evidence of liver injury, further studies incorporating serum biochemical markers and histopathological assessments are warranted to confirm the hepatoprotective effects of selenium.

5. Conclusion

Based on the findings of the present study, the expression of both the AST and ALT genes was significantly altered in the exercise groups compared with the control group, with the most pronounced changes observed in the group performing exercise under hot environmental conditions. These findings suggest that exercise, particularly under heat stress, may induce molecular alterations in hepatic cells associated with metabolic and oxidative stress. In contrast, selenium supplementation significantly attenuated the expression of both AST and ALT genes. The lowest expression levels of both genes were observed in the hot-exercise plus selenium group, suggesting that selenium may modulate hepatic molecular responses to exercise-induced heat stress, potentially through its antioxidant properties.

Overall, the findings indicate that strenuous exercise under hot environmental conditions may alter the expression of genes related to hepatic function and cellular stress, whereas selenium supplementation may attenuate these molecular responses. However, because gene expression does not directly reflect circulating enzyme activity or structural liver injury, further studies incorporating serum ALT and AST activities, histopathological assessments, and additional oxidative stress and inflammatory biomarkers are warranted to confirm the hepatoprotective effects of selenium. Considering the limitations of the present study, future research should investigate different forms and doses of selenium supplementation to determine the optimal dose and formulation for mitigating exercise- and heat-induced hepatic stress.

6. Highlights

According to the findings of the present study, Selenium supplementation may be considered as a potential nutritional strategy during strenuous exercise in hot environmental conditions, given its possible role in modulating hepatic enzyme-related gene expression and antioxidant responses. Further controlled studies are needed to establish its optimal dose, form, and clinical relevance.

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Conflicts of Interest
The authors declare that they have no conflicts of interest related to the publication of this article.

Authors' Contributions
Conceptualization: E.V., A.D.; Methodology: E.V., A.D.; Formal analysis: E.V., A.D.; Investigation: M.Gh., E.V.; Writing - original draft: E.V., M.Gh.; Writing review & editing: E.V., M.Gh.; Supervision: E.V.

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