

Kermanshah University of
Medical Sciences

Journal of Kermanshah University of Medical Science

Journal homepage: [Http://journals.kums.ac.ir/ojs/index.php/jkums](http://journals.kums.ac.ir/ojs/index.php/jkums)

Original Article

Muscle injury and oxidative stress following the use of selenium supplements and exhaustive aerobic exercise in young physically-active females

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Article Info

Keywords: Selenium, Muscle injury, Malondialdehyde, Creatine kinase, Lactate dehydrogenase

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Received: 08 November, 2015
Accepted: 05 April, 2016

J Kermanshah Univ Med Sci.
2016; 20(1): 1-5

Abstract

Introduction: The use of antioxidants before high-intensity training, which leads to the release of free radicals and muscle injuries, can result in reduced damage during exercise. Accordingly, in this study, we aimed to evaluate the effect of selenium supplement intake on oxidative stress, following exhaustive aerobic exercise among young physically-active females.

Methods: In this quasi-experimental study, 20 healthy girls (age: 23.6 ± 1.5 years, height: 1.61 ± 0.0126 m, and weight: 60.2 ± 7.13 kg) were randomly divided into exercise ($n=10$) and supplement + exercise ($n=10$) groups. The participants were asked to consume selenium supplements ($200 \mu\text{g/day}$) for a period of 14 days. The Bruce protocol stress test was conducted 24 h after the final intake of supplements and primary blood collection (in a fasting state). Also, immediately after performing the Bruce test, the second blood samples were drawn from the subjects. Oxidative stress markers (i.e., creatine kinase, lactate dehydrogenase, and malondialdehyde levels) were measured at each stage of blood sampling and were compared between the groups using paired t-test. Data analysis was performed by SPSS (version 18). P-value less than 0.05 was considered statistically significant.

Results: The results indicated that exhaustive aerobic exercise could cause a significant increase in creatine kinase, lactate dehydrogenase, and malondialdehyde levels. The comparison between the control and intervention groups suggested the significant effect of selenium supplementation on declining of lactate dehydrogenase level ($P < 0.05$).

Conclusion: The results of the present study demonstrated that selenium supplements could reduce oxidative stress, induced by exhaustive physical exercise.

Introduction

The impact of nutrition on sports activities has been the subject of scientific research for years. It is well established that optimum nutrition is a prerequisite for performing high-intensity physical activities and achieving the best possible outcomes. Today, nutrition studies mainly focus on the prevention of oxidative stress, induced by physical activity, and enhancement of recovery after exercise (1, 2).

Oxidative stress reflects an imbalance between reactive oxygen species (ROS) formation and cellular antioxidant capacity. According to the literature, antioxidant supplementation can reduce the signs or markers of exercise-induced oxidative stress (3, 4). In fact, antioxidants, even at low concentrations, can effectively prevent or delay the oxidation process in oxidizing components (e.g., DNA, proteins, and lipids) (5).

Long-term, high-intensity exercise might result in damage to muscles and tissues, induced by free radicals

and oxygenated compounds (6-8). Free radicals are chemical compounds with unpaired electrons. Consequently, they are highly active and try to steal electrons from other molecules, resulting in a chain of reactions (9). Muscle injuries during intense exercise might lead to the production of free radicals and further cellular damage, triggered by lipid peroxidation and protein oxidation.

Free radicals are measured in an individual based on the markers of free radical damage such as plasma concentrations of malondialdehyde (MDA) and lipid hydroperoxide (10, 11). Adverse changes might occur in oxidative stress and inflammatory markers such as MDA (indicator of oxidative cell injury), superoxide, protein carbonyl, and leukocytes in the serum and body fluids as a consequence of oxidative stress induced by intense exercise (12, 13).

MDA is the modified form of hydrogen peroxide, which lead to tissue damage through reactions with different tissues (14). Overall, various studies have confirmed the increased level of these markers following

intense exhaustive exercise (15). In fact, exhaustive physical activity provides a suitable model for evaluating the impact of oxidative stress on the human body (16).

According to previous studies, lipid peroxidation and muscle injury occur after intense exercise rather than increased antioxidant capacity. Therefore, intake of antioxidants could enhance the antioxidant status in the plasma and diminish the damaging effects of free radicals during intense exercise. However, it seems that these supplements have no beneficial impacts on the physical performance of an individual (17, 18).

Creatine kinase (CK) and lactate dehydrogenase (LDH) are enzymes responsible for adenosine triphosphate (ATP) formation in the anaerobic pathway and are identified as oxidative stress markers. Nevertheless, it should be noted that other enzymes such as MDA are also indicators of cell membrane damage and oxidative stress. Overall, oxidative stress interrupts the performance of cell membranes, which can be evaluated by the measurement of plasma membrane (19, 20).

Selenium is a well-recognized antioxidant which acts as a cofactor of antioxidant enzymes. This essential element helps protect the body against free radicals causing damage to the cells. Substantial evidence suggests that free radical production leads to increased oxygen uptake over time. The indirect though significant impact of selenium supplements is to protect the cells against oxidative stress and free radical production during physical exercise (21).

Selenium with its antioxidant features plays an important role in the maintenance of healthy skin and hair (22, 23). Nature-made selenium contains 200 mg of this element with a natural origin, high absorption capacity, and prolonged retention (24, 25). Selenium exerts its antioxidant effects through glutathione peroxidase. Multiple studies have revealed that regular exercise and physical activity can lead to increased levels of glutathione peroxidase (26).

Moreover, selenium is capable of boosting the immune system and increasing the body resistance against various diseases (27). Several studies have evaluated the possible role of selenium in the prevention of diseases and some cancers, and have shown that it can reduce oxidative stress and improve the amount of antioxidants (28).

Selenium contributes to the maintenance of important antioxidants in the body (e.g., vitamin E and C) and reduces the damage caused by free radicals (27). This compound also plays a protective role against oxidative stress and is considered vital to the immune system. It activates glutathione peroxidase and is involved in antioxidant metabolism, which inhibits oxidative damage (11, 12, 19).

Several studies indicate that selenium deficiency during intense exercise can inhibit antioxidant enzymes in the liver and muscles (17, 28). Considering the scarcity of conducted studies on the antioxidant effects of selenium during sports activities, the present study was aimed to evaluate the changes in MDA and serum enzymes (CK and LDH) due to selenium intake among

physically-active female participants.

Materials and Methods

This applied research with a pretest-posttest, quasi-experimental design was conducted on the physically-active female participants, who were divided into two experimental groups.

Study sample

The study population (age: 21-24 years) consisted of young physically-active females majoring in physical education, with at least two years of experience in physical training at Shahid Madani University, Azerbaijan, Iran. The students had no prior history of medical conditions (e.g. cold or lung infection) over the past month. In total, the sample size was calculated to be 25, based on Morgan's table of sampling. However, 24 samples were enrolled in the present study. Due to sample dropout (n=4), a total of 20 participants were included.

The objectives of the study were explained to the participants. Before completing the consent forms, the subjects filled out the health questionnaire and 24-h dietary recall. First, anthropometric indices (e.g. height, weight, and body mass index or BMI) were measured in order to homogenize the samples and prevent the possible effects of these indices.

Research method

In this study, before the experiment and supplement intake, the participants completed the Bruce protocol stress test. Moreover, blood samples were taken from the participants prior to the experiment to measure the target indices and to evaluate the effects of exercise and supplement intake plus exercise. The subjects were asked to use selenium tablets (200µg/day) every afternoon for a period of 14 days. On the day 15, the Bruce protocol stress test was carried out and blood collection was performed again immediately after the test.

The control group (exercise group) only completed the Bruce protocol stress test, and blood tests were performed before and after the test. The intervention was conducted at 8:00 a.m. after 12 h of fasting. All the tests were performed under homogeneous conditions, i.e. similar time intervals (8:00-12:00 in the morning), temperature, ventilation, humidity, and ambient light.

In addition, the participants were prohibited from performing intense physical activities within 48 h before the test and were encouraged to adhere to similar diets (15). The subjects were young physically-active females with at least 2 h of physical activity per day.

Measurement of blood indices

The indices evaluated in this study included CK, LDH, and MDA levels. To collect the blood samples, 4 cc of blood was drawn from the vein of the left hand in a sitting position. Afterwards, the blood samples were allowed to clot for 10 min at 37°C and were immediately centrifuged at 3000 rpm for 3 min. In the next stage, the serum was separated from the clot and kept in 5.1 ml microtubes at -70°C until the onset of the interventions.

The total serum CK level was determined by applying a photometric method, based on Jaffe's reaction

(with a sensitivity of 1 IU/L and coefficient of variation of 1.6%), using a special kit (CK Kit, Pars Azmoon Co., Tehran, Iran) by an automated analyzer. On the other hand, LDH level was determined using an enzymatic colorimetric (DGKC) method with a sensitivity of 5 U/L and coefficient of variation of 2.1% (LDH kit, Pars Azmoon Co., Tehran, Iran); the measured values were expressed in liter.

Serum MDA level was determined based on reaction with thiobarbituric acid (TBA), extraction with normal butanol, absorption spectrophotometry, and absorbance comparison with a standard curve. The sensitivity of the applied method was estimated at 0.08 μm , and the coefficient of variation was 0.9%. The concentration of MDA was determined after the separation of the organic phase (lipid solution) and measurement of light absorption at a wavelength of 532 nm.

Seca stadiometer (Germany) was used to measure the height of the participants. In addition, Seca weighing scale (with an accuracy of 0.1 kg; Germany) was employed to measure the weight of the samples. In this study, a treadmill (Technogym, Italy) was used to perform the Bruce protocol stress test. In addition, Bejim timer was used to measure the time (15).

Kolmogorov-Smirnov test was applied to evaluate the normal distribution of the data. Paired-t test was performed for intra-group comparisons, while independent t-test was applied for inter-group comparisons. For statistical analysis, SPSS (version 18) software was used. P-value less than 0.05 was considered statistically significant.

Results

The demographic characteristics of the participants (e.g. height, weight, age, and BMI) are presented in Table 1. Based on the results of independent t-test, the mean CK and MDA concentrations did not significantly change in the two groups after the intake of supplements ($P=0.09$); however, the changes were less significant with supplement intake.

In terms of LDH level, the mean concentration significantly increased in the post-test compared to pre-test in the control group. On the other hand, selenium supplements caused a decline in this parameter; therefore, a significant difference was observed between the two groups ($P=0.03$) (Table 2).

The results of the present study revealed a significant difference between the two groups regarding the time of physical activity and performance improvement ($P<0.05$) (Table 3). Based on the results, exhaustive physical activity could significantly increase CK, LDH, and MDA levels. In addition, the results were indicative of the significant impact of selenium on LDH. Also, a decline was reported in the CK and MDA levels.

Table 1. Mean \pm SD of the participants' demographic characteristics

Characteristics (two groups)	Mean \pm SD
Height (m; n=20)	1.61 \pm 0.012
Weight (kg; n=20)	60.2 \pm 7.13
Age (year; n=20)	23.6 \pm 1.5
Body mass index (BMI) (kg/m ² ; n=20)	23.09 \pm 2.83

Table 2. The results of independent t-test regarding the changes in CK, MDA, and LDH levels in the two groups

Variables	Control group	Exercise + supplement group	P-value (supplement + exercise group)	P-value (control group)
Creatine kinase (CK) (L)	34.35 \pm 6.24	6.30 \pm 1.40	0.059	0.12
Lactate dehydrogenase (LDH) (L)	31.50 \pm 7.12	21.29 \pm 1.39	*0.00	0.34
Malondialdehyde (MDA) (ml)	2.10 \pm 1.14	1.05 \pm 1.92	0.491	0.611

The values represent mean \pm SD.

Table 3. The results of independent t-test for determining the difference in exercise duration in two different loadings of selenium supplements

Variables	Control group	Supplement group	P-value (control Group)	P-value (supplement + exercise group)
Performance time	14.5 \pm 1.5	10.5 \pm 1	0.95	*0.02

*P-value less than 0.05 indicates a significant difference.

Discussion

According to the results of the present study, exhaustive physical activity caused no major change in CK, LDH, or MDA levels in the control group. Meanwhile, intake of selenium supplements significantly decreased the LDH level. Moreover, CK and MDA levels were reduced, although the changes were statistically insignificant. Considering the increased levels of CK, LDH, and MDA after exhaustive exercise and the insignificant effects of supplements on the studied indices, it can be concluded that the observed changes were caused by physical activity, resulting in significant oxidative stress in the cells.

In the present study, increased level of MDA was reported during exhaustive aerobic activity in both

groups; however, a difference was observed between these groups. Since physical activity leads to lipid peroxidation and oxidative stress through different mechanisms (e.g., catecholamine activity, spontaneous catecholamine oxidation, metabolism of prostanooids, oxidation of nicotinamide adenine dinucleotide phosphate, and macrophage activity) (9, 12), it is not easy to decide which factor plays the most significant role.

Considering the heightened need for ATP during sports activities, oxidative phosphorylation and oxygen exchange rate in the electron-transport chain increase significantly. Increased oxidative stress following oxygen leak from the electron-transport chain of mitochondrion highlights the role of this organelle (1, 5,

7). Therefore, it seems that oxidative stress production and lipid peroxidation are associated with oxygen consumption (13, 15).

In addition, during physical activity the amount of catecholamines increases in response to the heightened metabolic needs of the tissues. Consequently, spontaneous oxidation of catecholamines leads to more oxidative stress and cellular damage (17). On the other hand, the ischemia/reperfusion process is also involved in oxidative stress (3, 8, 21).

The diversion of blood to the skin and active muscles leads to transient tissue hypoxia during exercise and an imbalance between the consumed and required oxygen in active tissues during high-intensity physical activities. ROS production increases as a consequence of re-oxygenating the tissues after reduced or discontinued physical exercise (15, 28). Therefore, damage to the cellular infrastructure, caused by improved ROS production, is accompanied by increased oxidative stress and decreased cellular performance. Muscle injury causes membrane damage, which in turn allows the muscle proteins such as CK and LDH to enter the intercellular fluid and finally pass through the bloodstream (12). According to the literature, the amount of CK in the plasma immediately increases after exercise and reaches its peak after 24 h (2, 19).

According to the findings of the present study, intake of selenium supplements led to decreased CK level after aerobic exercise, compared to the pre-exercise period; however, the difference was not statistically significant. Therefore, it can be concluded that selenium intake did not result in a significant difference with the control group in terms of CK level.

The less significant increase in muscle injury indices in the supplement + exercise group was in line with the results reported in several studies (14, 16, 17, 19). However, inconsistent with the current findings, some studies have revealed an increase in CK level after physical exercise in the supplement groups compared to the control groups (5, 15). Variables such as supplement dose, environmental conditions, physical status of the participants, type of exercise protocol, time and method of sampling, and type of the analyzing device may be involved in performance improvement and increased duration of physical activity.

Given the importance of CK and LDH enzymes as oxidative stress markers, the significant difference in their levels during rest and after exercise in the control group might be indicative of significant oxidative stress in the cells. Similar to CK, the level of LDH enzyme significantly increased after exercise in the control group, which might be attributed to the impact of aerobic exercise.

The aforementioned finding was in congruence with the results reported by Little et al. (2010) and Goldfarb et al. (2007), indicating that long-term exercise could lead to increased LDH and CK levels. On the other hand, the simultaneous use of selenium supplements and long-term physical activity significantly decreased the level of this enzyme after exercise.

Based on the reported findings, it can be stated that

selenium supplements cause significant changes in LDH level. Since the increased level of LDH during rest was indicative of the disrupted performance of cell membranes, aerobic exercise probably had no significant impacts on the function of cell membranes in the control group.

Another important finding of the current study was the time of reaching physical exhaustion. The improved performance period in this study could be suggestive of the elevated lactate threshold, as Savory et al. believed that reaching exhaustion in intense physical activities is attributed to excessive lactate accumulation (15).

It seems that the elevated lactate threshold might have led to the gradual and slow accumulation of these indices, thereby leading to delayed fatigue in the participants. These results were in line with the findings reported by blomer et al. (2006), who showed that after six weeks of exhaustive aerobic exercise, the subjects could reach the lactate threshold at a higher workload than the pre-exercise period (14).

In the current study, a difference was observed between the control and supplement + exercise groups regarding the time of reaching exhaustion. According to the results, selenium intake could increase the time of reaching physical exhaustion. On the other hand, according to several studies, depletion of muscle glycogen sources is the major inhibitory factor for aerobic exercises (18, 28).

Therefore, it seems that the major cause of fatigue and exhaustion due to intensive physical activity (even if accompanied by the intake of antioxidant supplements) is depletion of glycogen sources. Nevertheless, we cannot ignore the fact that a session of exhaustive exercise may lead to the increased production of lactic acid, which is mostly followed by lactate accumulation.

Conclusion

According to the results of the present study, selenium supplements at a physiological dosage might contribute to the overall physical performance of an individual through inhibiting free radical production in the body. As a result, it seems that selenium intake before intense, exhaustive physical activity can be beneficial for the cells and prevent oxidative stress in physically-active individuals. Nevertheless, the obtained results were not in accordance with some previous studies in terms of changes following exhaustive physical activity. Several factors may contribute to the discrepancy between the range of changes and the relationship between indices in the present study and previous research. These factors are as follows: 1) individual differences (e.g. race, age, gender, health status, and physical preparation); 2) factors contributing to physical pressure (e.g. type, frequency, intensity, and duration of applied pressure); 3) features of sports activities or muscle contractions (e.g. type, frequency, intensity, and duration); 4) environmental conditions; 5) nutrition (e.g. diet, consumed calories, use of medications, and dietary supplements); and 6) scientific conflicts in the present research and previous studies.

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