



# Selenomethionine Supplementation Contributes to Reducing Oxidative Stress and Inflammation Markers following Exercise-Induced Muscle Damage

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## Abstract

**Background.** Exercise-induced muscle damage (EIMD) is a temporary response to intense or prolonged exercise that can cause muscle pain, inflammation, and impaired muscle function. Antioxidant supplementation is a proposed strategy to reduce EIMD symptoms by targeting reactive oxygen and nitrogen species (RONS) involved in the process.

**Objective.** The study aimed to investigate the effect of Selenomethionine supplementation on malondialdehyde (MDA) and C-reactive protein (CRP) levels resulting from Exercise-Induced Muscle Damage (EIMD).

**Materials and methods.** This study used a randomized pretest-posttest control group design, involving a total of 32 male recreational students from the Universitas Negeri Surabaya, (age  $19.25 \pm 0.76$  years, body mass  $63.16 \pm 3.38$  kg, height  $167.28 \pm 4.54$  cm, body fat  $19.60\% \pm 4.57\%$ ). The participants were randomly assigned to the selenomethionine group (SEM, 100  $\mu$ g/day) or placebo group (PLA, corn starch 100 mg/day) for a period of 28 days (4 weeks). On days 1 (baseline) and 29, participants underwent a single bout of EIMD. Blood samples were collected 24 hours post-EIMD to measure MDA and CRP concentrations in plasma. The statistical analysis was conducted using paired sample t-test.

**Results.** The placebo group experienced a significant increase in plasma MDA and CRP concentrations after EIMD compared with baseline values ( $p < 0.05$ ). However, the SeMet group showed lower plasma MDA and CRP levels than the placebo group.

**Conclusions.** Daily Selenomethionine supplementation for 28 days has been found to reduce oxidative stress by lowering MDA levels in the blood and to decrease inflammation by reducing CRP levels post-exercise-induced muscle damage. This indicates a lower risk of EIMD due to reduced oxidative stress and inflammation.

**Keywords:** exercise, oxidative stress, inflammation, immune function.

## Introduction

There have been numerous reports indicating that exercise-induced muscle damage (EIMD) commonly occurs following strenuous unaccustomed exercise, especially when the activity involves a high frequency of eccentric contractions (Irawan et al., 2021; Irawan et al., 2024). This condition is frequently observed in exercises like running, plyometrics, strength training, and other high-

intensity activities that involve eccentric muscle actions, such as movements that involve lengthening, braking, or deceleration. The harm caused by EIMD can result in muscle tenderness, soreness, and reduced functionality. This condition can result in impaired immune function by marking an increase in IL-6 concentrations (Fernández-Lázaro et al., 2020; Irawan et al., 2021). This confirms that intense exercise, particularly those involving high-intensity eccentric muscle contractions, can increase the risk of muscle damage (Fernández-Lázaro et al., 2020; Kong et al., 2018). There are several indicators of direct and indirect muscle damage, including ultrastructural disturbances, delayed-onset muscle soreness, continued decline in muscle

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function, and changes in many serum markers such as creatine kinase (CK) and lactate dehydrogenase (LDH), anti and pro-inflammatory cytokines (IL-6, IL-2, IL-10, and TNF- $\alpha$ ), and superoxide dismutase (SOD) (Nanavati et al., 2022; Souissi et al., 2020; Suzuki et al., 2020).

One study revealed that both strenuous exercise and intensive exercise can result in a decrease in the ability of mitochondrial membranes and an increase in lipid peroxidation (Bazzucchi et al., 2019; Del Giudice & Gangestad, 2018). The study by Souissi (Souissi et al., 2020), which aimed to examine the modality of running exercise against oxidative stress, concluded that intermittent or 15/15 intermittent exercise caused less radical damage compared to intermittent 30/30 exercise. This proves that high-intensity exercise can result in oxidative stress characterized by increased activity of oxidative stress markers including malondialdehyde (MDA), advanced oxidation protein products (AOPP), SOD, and glutathione peroxidase (GPX) (Ammar et al., 2020; Matta et al., 2021; Souissi et al., 2020). Another study concluded the association of muscle damage with oxidative stress (O'Connor et al., 2022; Suzuki, 2018).

The mechanism of immune function disorders and muscle damage due to exercise has not been widely explained, although there are clinical symptoms due to exercise, including swelling, muscle pain, and increased circulating cytokines as markers of inflammation (Arazi et al., 2021; Cicchella et al., 2021; Tidball & Villalta, 2010). Inflammatory response serves as a marker of damage to both muscle fibers and other body parts, along with mechanical disturbances that occur during high-intensity exercise (Ruhe et al., 2020; Wadley et al., 2019). Disruption due to exercise-induced muscle damage could lead to a decrease in the athlete's performance, with muscle ability decreasing between 20-50%, requiring 2-7 days for full recovery (Kyriakidou et al., 2021; Lee et al., 2021). This damage is associated with an inflammatory response that involves many mediators, one of which is an increase in circulating IL-6 cytokines and inflammation (Ellingsgaard et al., 2019; Irawan et al., 2022).

Some studies suggest that the increased risk of muscle damage and inflammation during exercise is caused by free radicals (He et al., 2018; Martinez-Ferran et al., 2020). Therefore, the use of antioxidants may help reduce the risk of muscle damage and inflammation. Several studies have focused on efforts to reduce the risk of muscle damage using drugs (Brendler et al., 2021), antioxidant supplements (Righi et al., 2020; Wang et al., 2019), and other potential agents (He et al., 2018; Lima et al., 2019; Massaro et al., 2019). These studies yield varying results, indicating the need for further research to reduce the risk of impaired immune function and inflammation.

Many studies have been conducted regarding the effectiveness of antioxidant supplements against oxidative stress and inflammation due to EIMD. However, research on the effects of Selenomethionine (SeMet) on EIMD is very limited. Therefore, the present study was designed to primarily investigate the effect of Selenomethionine supplementation on MDA and CRP resulting from Exercise-Induced Muscle Damage (EIMD) in students from the Sports Science Department at Universitas Negeri Surabaya.

## Materials and Methods

### Study Participants

Thirty-two male students from the Sports Science Department at Universitas Negeri Surabaya were included in the study. They were healthy, had a normal BMI, and were non-smokers. Participants were randomly assigned to either the SeMet Group (SEM,  $n = 16$ ) or the Placebo Group (PLA,  $n = 16$ ). All participants had the same body weight, and there were no significant differences in anthropometric parameters between groups. Before recruiting participants, the research team carefully explained the purpose and procedures of the experiment to participants, and all participants filled out an informed consent form before participating.

### Study Organization

This study was an experiment with a pretest-posttest control group design. The design was developed to investigate the effectiveness of 100 $\mu$ g Selenomethionine (SeMet) capsule supplementation for 28 days (4 weeks) against changes in MDA and inflammation (CRP).

The research was conducted for one month with an initial screening on the first day to determine the participants' condition. All participants were not allowed to do sports or heavy physical activity for 30 days until the end of the study; only experimental exercise was allowed. Data on plasma concentrations levels of MDA and CRP 24 hours post-exercise before the 28-day supplementation period served as baseline data (D2), while plasma levels of MDA and CRP 24 hours post-exercise after 28 days of SeMet supplementation became posttest data (D30). On the first day (D0), participants filled out a physical fitness capacity form that included their weekly running frequency and volume, history of injuries and illnesses, and medications used in the 2 weeks before the start of the study.

The 10 sets of 10 Countermovement Jumps (CMJs), with a recovery of 1 min between sets protocol was chosen to obtain the EIMD effect. This type of Depth Jump Protocol (DP) has been proven to induce high-intensity muscle fatigue and eccentric muscle damage, which can be useful for assessing the effectiveness of supplements or interventions on muscle recovery (Dos Reis et al., 2023). All participants performed CMJs on Day 1 (D1) and Day 29 (D29) as a damaging exercise (EIMD). The MDA and CRP were obtained 24 hours post-exercise (D2 and D30). Participants were not injured and were asked to refrain from all physical activity and avoid taking anti-inflammatory drugs, treatments, and additional dietary supplements during the 30-day period of this study. A summary of the study design is presented in Figure 1.

The supplementation consisted of capsules containing 100  $\mu$ g of SeMet (SEM), while the placebo consisted of similar capsules containing 100 mg of corn starch (PLA). The Selenomethionine capsules were manufactured by Thorne (Poland), a commercially available product that ensures quality and safety for the product. The subjects consumed one SeMet (SEM) or Placebo (PLA) capsule daily with breakfast for 28 days (4 weeks). All participants were not allowed to engage in sports or heavy physical activity for 30 days until the end of the study. Additionally, participants were prohibited from participating in any recovery

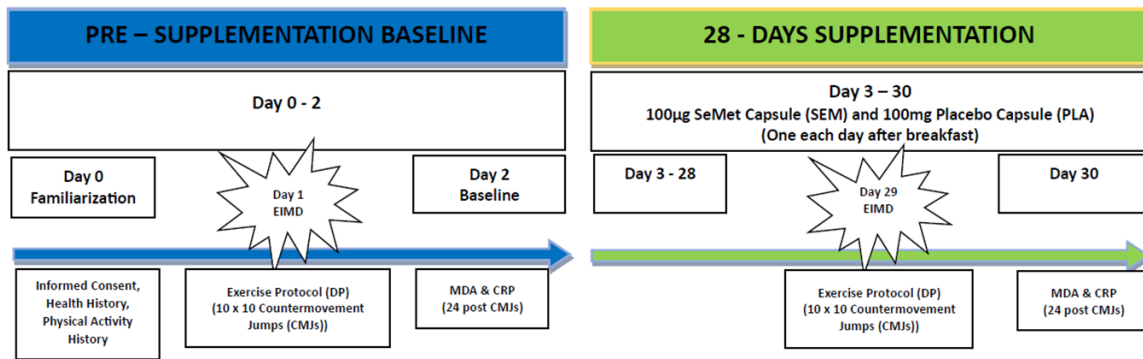


Fig 1. Study design

programs such as massage or cryotherapy. Before engaging in exercise, participants were required to be pain-free and injury-free, as confirmed by their completion of the Physical Activity Readiness Questionnaire (PAR-Q) and deemed fit for physical activity. Blood samples were taken, with up to 5 cc collected from the cubital vein and treated with EDTA. The centrifugation process was performed at 3000 rpm for 15 minutes, after which the plasma was stored in a freezer at a temperature of -20 °C until analyzed. Plasma MDA and CRP levels were measured using 100 µl of plasma 24 hours post-damaging exercise before (D2) and after the 30-day supplementation period (D30). Plasma concentrations of Malondialdehyde (MDA) were determined by a high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit using 96-well plates from Elabscience MDA ELISA kit, and plasma concentrations of CRP were analyzed using the DBC hs-CRP ELISA kit. The assessment of MDA and CRP plasma concentrations was conducted at the Sports Science Laboratory of Universitas Negeri Surabaya and Airlangga University Hospital Research Laboratory Installation, to obtain MDA and CRP plasma concentration data.

**Statistical Analysis**

The data was processed both manually and digitally to convert it into usable information. The distribution of the data was checked using the Kolmogorov-Smirnov method, and it was found that all samples had a normal distribution. With this confirmed, descriptive statistics were calculated for each measured variable. Independent t-tests were used to identify any differences between groups, and paired t-tests were used to examine changes within each group from pre-test to post-test. IBM SPSS Statistic 22.0 was used to analyze all of the data.

**Results**

A total of 32 male Sports Sciences Department students of Universitas Negeri Surabaya were willing to participate in this study. The participants were randomly divided into two groups: the SeMet Group (SEM, n=16) and the placebo group (PLA, n=16). The mean age of the SEM was 19.19 (±0.65) years old, while the mean age of the PLA was 19.31 (±0.87) years old. Tanita’s Body Fat Monitor BC 730 was used to obtain data on participants’ body weight and fat percentage, while the Multistage Fitness Test was used to determine individual aerobic fitness (VO<sub>2</sub>max). The characteristics obtained from this study can be seen in Table 1.

Table 1 above shows the anthropometric characteristics of the participants, including their IPAQ level. Based on Table 1, it can be seen that the physical activity level of all participants is in the moderate category.

The next step is to carry out prerequisite tests, which include normality tests and homogeneity tests. The normality test was conducted to determine whether a given dataset follows a normal distribution or not, and homogeneity tests were used to determine whether a sample or population is homogeneous. The result of the normality test helps determine the hypothesis test. A Kolmogorov-Smirnov test for normality for MDA and CRP, whether pretest and posttest, reported a p-value of >0.05, revealing that the distribution was normal. Likewise, with the homogeneity test using the Levene test, all variables MDA and CRP had significance values >0.05, which means that the data can be considered homogeneous.

Based on this, the next hypothesis test used is parametric statistics, specifically the paired t-test. The pretest and posttest t-tests in the SEM group and the PLA group aim to determine whether there is a decrease in plasma levels of MDA and CRP.

Table 1. Respondent characteristic

Indicators	SEM (n=16)	PLA (n=16)	Categories
Age (years)	19.19 ± 0.65	19.31 ± 0.87	
Height (cm)	165.50 ± 4.3	168.06 ± 4.71	
Weight (kg)	62.75 ± 3.75	63.56 ± 3.24	
BMI (kg/m <sup>2</sup> )	22.63 ± 0.66	22.50 ± 0.66	Normal
Fat Percentage (%)	20.88 ± 3.57	18.31 ± 3.55	General Fitness
IPAQ (METs-min/week)	1161.19 ± 217.22	1287.63 ± 127.87	Average
VO <sub>2</sub> max (mL/(kg·min))	35.67 ± 4.09	37.81 ± 3.71	Below average

### *Selenomethionine supplementation and changes on oxidative stress marker (MDA)*

When comparing plasma concentrations of oxidative stress markers (MDA) before and after SeMet supplementation (after 4 weeks of supplementation), we found different results in both SEM and PLA (Table 2).

**Table 2.** Changes in MDA plasma levels between pretest and posttest

MDA	SEM (µmol/ml)	PLA (µmol/ml)	p-values
Pre	15.42 ± 0.83	16.04 ± 1.06	0.000
Post	12.99 ± 0.075	15.16 ± 1.00	0.000

Table 2 above shows changes in plasma MDA levels before (pre) and after (post) 4 weeks for both SEM and PLA. There was a significant decrease in plasma MDA levels in the SEM group ( $P < 0.001$ ). On the other hand, in the PLA group, there was no significant change because the t-test value in the PLA group was 0.60 ( $P > 0.001$ ). Based on this, it can be said that supplementation of SeMet caused a decrease in MDA levels, whereas in the placebo group, there was no decrease in MDA levels. Notably, the control group showed little or no change in MDA levels.

### *Selenomethionine supplementation and changes on inflammatory marker (CRP)*

CRP is commonly used as a marker of inflammatory events following intense exercise. In a comparative analysis of plasma levels of inflammatory markers before and after SeMet supplementation (after a 4-week supplementation period), we observed a significant decrease in CRP levels in the SEM but not PLA (Table 3).

**Table 3.** The alterations in CRP plasma levels between pretest and posttest

CRP	SEM (mg/L)	PLA (mg/L)	p-values
Pre	6.79 ± 0.18	7.10 ± 0.19	0.000
Post	5.52 ± 0.23	6.86 ± 0.29	0.000

Table 3 above indicates the alterations in CRP plasma levels before (pre) and after (post) a 4-week supplementation period in both the SEM and PLA groups. The data reveal a significant decrease in CRP plasma levels in the SEM group ( $P < 0.001$ ). On the other hand, the t-test value of PLA was 0.69 ( $P > 0.001$ ), which means there were no significant changes in the placebo group. Hence, it can be deduced that SeMet supplementation results in a more substantial decrease in CRP plasma levels compared to the placebo.

## **Discussion**

Exercise-induced muscle damage (EIMD) can occur due to unaccustomed exercise, causing a temporary decrease in muscle strength, increased passive tension, increased pain and muscle inflammation, and elevated levels of intramuscular proteins in the bloodstream (Tanabe et al., 2021). EIMD is a common symptom that occurs

when engaging in prolonged or high-intensity exercise. It is a temporary and natural part of the muscle's repair and adaptation process (Owens et al., 2019). This response can lead to a temporary decrease in muscle function, as well as symptoms such as muscle soreness, swelling, and elevated levels of proteins in the blood, which are all indicative of muscle damage (Sánchez Díaz et al., 2022).

Research has shown that muscle damage is often accompanied by oxidative stress (OS), which is characterized by an increase in reactive oxygen species (ROS), the concentration of the biomarker produced depends on the degree of fatigue or muscle damage. One of study Elevated MDA and CRP has been reported during exercise with EIMD and has similarly been attributed to a shift to more glycolytic energy production (Kruk et al., 2021; O'Connor et al., 2022; Thirupathi et al., 2021). Therefore, inflammatory processes have always been associated with OS and both are directly involved in muscle damage and should be analyzed and controlled together (Amalraj et al., 2020).

This study aimed to examine the impact of a 28-day supplementation of Selenomethionine on MDA levels as an oxidative stress marker and CRP levels as an inflammation marker following Exercise-Induced Muscle Damage. The results of this study indicate an increase in oxidative stress marker (MDA) and inflammation marker (CRP) 24 hours post a single bout of CMJs as a damaging exercise protocol, as shown in Table 2 and Table 3. Muscle contraction during exercise triggers the production of ROS in active muscle fibers, primarily originating from skeletal muscle. This generation of ROS results in oxidative damage in different tissues, such as increased protein oxidation and lipid peroxidation, accelerated muscle fatigue, and exercise-induced adaptations in muscle fiber contraction. These effects are linked to the activation of relevant biochemical signaling pathways (Ammar et al., 2020; Powers et al., 2020).

The level of cellular oxidative stress can be assessed by measuring MDA a marker of lipid peroxidation (Guerrero et al., 2021). High levels of free radicals or ROS are known to directly initiate the chain peroxidation reactions of membrane lipids (Lin et al., 2021), resulting in increased oxidative stress and damage to organs and tissues. The results of this study showed that daily intake of SeMet for 28 days effectively reduces the oxidative stress marker 24 hours post a single bout of CMJs-induced EIMD through a significant reduction in MDA plasma concentrations, as it inhibits lipid peroxidation. A possible reason for this phenomenon may lie in decreased membrane permeability and reduced leakage of components due to the inhibition of lipid peroxidation (Brendler et al., 2021; Williamson & Davison, 2020). This could be due to SeMet's potential antioxidant properties (Lior et al., 2019).

Selenium is considered the most vital micronutrient for both humans and animals. Selenium is an essential nutrient that serves as a vital cofactor for numerous enzymes, enabling them to function properly. Its antioxidant properties have been extensively researched, and it has also been found to play a significant role in the immune system's functioning and overall defense mechanisms (Mal'tseva et al., 2022). Notably, selenium can be incorporated into proteins in the body, where it can bind non-specifically to the amino acid methionine, allowing it to participate in various cellular activities (Goldsztein et al., 2022).

One of the ways selenium's antioxidant properties works is by activating glutathione peroxidase, an enzyme that contains selenium. This enzyme helps to neutralize free radicals by converting harmful compounds like hydrogen peroxide and organic hydroperoxides into harmless ones, such as water and alcohols (Bjørklund et al., 2022). Selenium-dependent enzymes, such as GPX1–4 and GPX6 and thioredoxin reductases (TrxR 1-3), directly combat oxidative stress. Cytosolic GPX4 is essential for embryonic development and cell survival. GPX1 is the most abundant selenoprotein and a critical metabolic form of selenium for combating severe oxidative stress (Weaver & Skouta, 2022).

In this study, CRP plasma concentrations in blood were measured as an inflammatory marker. As shown in the results above, there is an increase in CRP plasma concentrations 24 hours post single bout pencak silat exercise. The results of statistical analysis showed that there is a difference in CRP plasma concentrations before and after 28 days of SeMet supplementation. Recent studies have also shown a slight increase in CRP levels as an inflammatory marker after CMJs as an EIMD (Cerqueira et al., 2020; Costello et al., 2018; Kawamura & Muraoka, 2018). The presence of this inflammatory marker (CRP) is suggested to be the result of muscle cell damage as a consequence of high-intensity exercise (Boukhris et al., 2020; Mal'tseva et al., 2022). The present study showed that daily SeMet intake demonstrated to be efficient in attenuating CRP plasma concentrations 24 hours post EIMD.

Research has consistently shown that eccentric exercise, particularly when new or unfamiliar, can cause muscle damage due to the intense force exerted on the muscles (Heiss et al., 2019). This damage leads to the infiltration of inflammatory cells, destruction of cell membranes, and breakdown of the extracellular matrix. The excessive inflammatory response that follows can ultimately lead to additional tissue damage (Taherkhani et al., 2020). Previous evidence has suggested that an acute bout of exercise increases NF- $\kappa$ B activity. NF- $\kappa$ B is a critical regulator of various physiological and pathological processes, including inflammation and oxidative stress (Suzuki et al., 2020).

Recent studies have found that selenium can affect immune cell function and transcription pathways, such as NF- $\kappa$ B (Prabhu et al., 2002). Selenium acts as an anti-inflammatory by inhibiting NF- $\kappa$ B activation and regulating cytokine production at the gene expression level. NF- $\kappa$ B activation triggers inflammatory markers like CRP and TNF- $\alpha$  (Maehira et al., 2003).

Given the scarcity of studies discussing exercise-induced muscle injury mechanisms, on the other hand, this scheme supports the need for further research to reassess the effects of long Selenomethionine supplementation on antioxidant defense systems, muscle performance, and hormonal responses to determine potential improvements in athletic performance.

## Conclusions

Our research indicates that daily Selenomethionine supplementation for 28 days reduces oxidative stress by lowering MDA levels in the blood and decreases inflammation by reducing CRP levels post-exercise-induced muscle damage. Thus, it can be said that Selenomethionine

supplementation significantly attenuates exercise-induced oxidative stress and inflammatory markers, respectively, suggesting that selenium may be a valuable adjunct to optimize exercise performance and reduce muscle damage.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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# Приєм доіавок селенометіоніну сприяє зменшенню оксидативного стресу та маркерів запалення після пошкодження м'язів внаслідок виконання фізичних вправ

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Авторський вклад: А – дизайн дослідження; В – збір даних; С – статаналіз; D – підготовка рукопису; Е – збір коштів

Реферат. Стаття: 8 с., 3 табл., 1 рис., 53 джерела.

**Історія питання.** Пошкодження м'язів внаслідок виконання фізичних вправ (ПМВФВ) — це тимчасова реакція на інтенсивні або тривалі фізичні навантаження, що може викликати біль у м'язах, запалення та порушення м'язової функції. Антиоксидантні доіавки є запропонованою стратегією щодо зменшення симптомів ПМВФВ шляхом впливу на реактивні форми кисню та азоту (РФКА), які беруть участь у цьому процесі.

**Мета дослідження.** Метою дослідження було вивчення впливу доіавок селенометіоніну на рівень малонового діалдегіду (МДА) та С-реактивного білка (СРБ), що виникають внаслідок пошкодження м'язів, спричиненого фізичними вправами.

**Матеріали та методи.** У цьому дослідженні використано метод рандомізованого перед- і післятестового контролю, в якому взяли участь 32 студенти чоловічої статі рекреаційного профілю навчання з Сурабайського державного університету (Індонезія), (вік  $19,25 \pm 0,76$  років, маса тіла  $63,16 \pm 3,38$  кг, зріст  $167,28 \pm 4,54$  см, вміст жиру в організмі  $19,60\% \pm 4,57\%$ ). Учасники були розподілені методом рандомізації до групи, яка приймала селенометіонін (СЕМ, 100 мкг/добу) або плацебо (ПЛА, кукурудзяний крохмаль 100 мг/добу) на період 28 днів (4 тижні). На 1-й (початковий) та 29-й дні дослідження, учасники зазнали однократного впливу пошкодження м'язів внаслідок виконання фізичних вправ. Зразки крові відбирали через 24 години після ПМВФВ з метою вимірювання концентрації МДА та СРБ у плазмі. Статистичний аналіз проведено за допомогою t-критерію парних вибірок.

**Результати.** У групі застосування плацебо спостерігалось значне підвищення концентрації МДА та СРБ у плазмі крові після пошкодження м'язів внаслідок виконання фізичних вправ, порівняно з вихідними значеннями ( $p < 0,05$ ). Однак в групі, яка приймала селенометіонін, рівень МДА та СРБ у плазмі крові був нижчим, ніж у групі, що отримувала плацебо.

**Висновки.** Встановлено, що щоденний прийом доіавок селенометіоніну протягом 28 днів зменшує оксидативний стрес шляхом зниження рівня МДА в крові та послаблює запалення шляхом зниження рівня С-реактивного білка після пошкодження м'язів внаслідок фізичних вправ. Це вказує на нижчий ризик розвитку ПМВФВ завдяки зменшенню показників оксидативного стресу та запалення.

**Ключові слова:** фізичні вправи, оксидативний стрес, запалення, функція імунної системи.

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