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RESEARCH ARTICLE

Attenuating Plasma Cytokines Response After High-Intensity Exercise Through Selenium Supplementation

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Abstract

High-intensity exercise are associated with muscle injury and an acute inflammatory response characterized by an increase in cytokines. Interleukin 6 (IL-6) is one of the proinflammatory cytokines that is most highly elevated by high-intensity exercise, which in turn reduces muscle strength, limits range of motion, and causes general discomfort, affecting performance and training programs. The purpose of this study was to investigate the effect of Selenium supplementation on IL-6 and CRP post high-intensity exercise. This research used an experimental pre-post control group design with 36 male students from the sports science department of Universitas Negeri Surabaya. Participants were randomly assigned to either a selenium supplementation group (n=18) or a placebo group (n=18) and instructed to consume one capsule daily for 21 days in a double-blind manner. Following a 21-day supplementation period, on the 22nd day, participants performed a 100 Drop Jump (DJ) exercise protocol to induce cytokines response. IL-6 and CRP plasma concentration were assessed immediately after, 24 hr and 48 hr after exercise. This study's results demonstrated that during the 24 to 48-hour period following high-intensity exercise, both groups experienced a reduction IL-6 and CRP plasma concentration compared to immediately after. The study's findings indicated that the group supplemented with selenium exhibited a significantly more pronounced decrease in IL-6 with P=0.000 (<0.05) and CRP plasma concentration with P=0.006 (<0.05) compared to the placebo group. In conclusions, Selenium supplementation may lower inflammatory response following high-intensity exercise, as it effectively decreases IL-6 and CRP plasma concentration the bloodstream.

Keywords
Exercise, Muscle Damage, Inflammation, Sports Injured, Supplement

INTRODUCTION

Regular physical activity of light to moderate intensity has been consistently linked to a wide range of health benefits, including a reduced risk of chronic conditions such as cardiovascular disease, type 2 diabetes, certain cancers, as well as neurodegenerative disorders like dementia and Alzheimer's disease (Fuller et al., 2020). However, high-intensity exercise triggers a substantial release of pro-inflammatory cytokines and free radicals from activated leukocytes, which play a role in muscle damage and tissue injury (Suzuki et al., 2020).

It has been stated in many studies that all forms of high-intensity exercise are associated with muscle injury and an acute inflammatory response (Middelbeek et al., 2021). Increased levels of a number of pro- and anti-inflammatory cytokines, naturally occurring cytokine inhibitors, and chemokines are observed. These studies explain that the presence of muscle injury and inflammatory response is reflected by the elevated activity of both

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inflammatory and anti-inflammatory cytokines in the bloodstream (Fleckenstein et al., 2021; Taherkhani et al., 2020) such as tumor necrosisfactor (TNF)-alpha, interleukin (IL)-1 beta, IL-1 receptor antagonist (IL-1ra), TNF-receptors (TNF-R), IL-10, IL-8, Interleukin 6 (IL-6) (Lin et al., 2021), and C-Reactive Protein (CRP) (Boukhris et al., 2020), which are typically released from damaged muscle cells.

Larger amounts of IL-6 are produced in response to exercise than any other cytokine, IL-6 is produced locally in the skeletal muscle in response to exercise. IL-6 production peaks shortly after the training session ends due to reduced muscle glycogen availability, followed by a rapid decline to baseline concentrations. However, if the exercise is accompanied by muscle damage, IL-6 will increase again to signal the acute-phase response and tissue repair during the recovery period (Nash et al., 2023) and experienced a peak increase 24 hours after completing the exercise session (Hennigar et al., 2017).

The level of inflammatory response from high-intensity exercise can vary based on factors such as the type, intensity, and duration of the exercise, as well as the individual's sex and age (Daniela et al., 2022). This process is the body's natural response to muscle damage and inflammation, as the body tries to repair and adapt to new levels of exertion (Suzuki, 2021). While the inflammatory response is a common part of the healing process, uncontrolled inflammation can prolong skeletal muscle recovery after intense exercise or exercise-related injury, muscle soreness, temporary decrease in muscle force, edema, which can influence the magnitude of performance decrement and the time course recovery following high-intensity exercise (Ringuet et al., 2021; Tidball & Villalta, 2010).

This shows that exercise, particularly high-intensity training, is known to induce inflammation through the release of pro-inflammatory cytokines and free radicals, which can contribute to muscle damage and delayed recovery as mention above. While various nutritional supplements are commonly used by athletes and fitness enthusiasts to enhance performance and recovery, their effectiveness in modulating exercise-induced inflammation remains unclear. Some supplements may reduce inflammation and oxidative stress, promoting quicker recovery, while others might exacerbate the inflammatory response or have minimal impact.

Selenium is a vital trace element that is crucial for human health. It acts as a potent antioxidant and has been shown to possess anti-inflammatory properties. Selenoproteins, in which selenium is a key component, are essential for various functions including redox catalytic activity, structural support, and transport processes (Zheng et al., 2022).

Selenium (Se) is associated with antioxidant defense, support for thyroid hormone production, regulation of testosterone metabolism, preservation of DNA structure, modulation of vitamin E (alpha-tocopherol), promotion of anti-cancer mechanisms, and improvement of muscle performance (Kuršvietienė et al., 2020).

Previous research has indicated that elevated levels of selenium and selenoenzymes (such as GPx and Se protein) in the bloodstream have been observed during the initial stages of severe illnesses marked by inflammation and oxidative stress (Hariharan & Dharmaraj, 2020). Consequently, additional studies are required to validate the potential advantages of selenium, particularly in mitigating the likelihood of exercise-induced inflammatory response.

The original value of this study lies in its exploration of selenium supplementation as a potential strategy to modulate exercise-induced inflammation resulting from high-intensity exercise. Selenium, a key antioxidant, may play a role in reducing oxidative stress and the release of pro-inflammatory cytokines following exercise.

The study seeks to offer fresh perspectives on the efficacy of selenium in lessening muscle damage, facilitating recovery, and improving exercise performance. By delving into selenium's impact on inflammation and recovery, the study fills a gap in existing literature and provides evidence-based recommendations for athletes and individuals seeking to enhance recovery and mitigate inflammation-related performance effects. Understanding the impact of supplementation on post-exercise inflammation is vital for enhancing recovery and performance in athletes.

The study aimed to explore the physiological effects of 21 days of selenium supplementation on IL-6 and CRP as indicators of inflammation triggered by high-intensity exercise. Through investigating various supplementation approaches, the study sought to shed light on the potential of selenium supplementation in reducing muscle damage, expediting recovery, and optimizing performance. Ultimately, this research contributes to a deeper understanding of how supplementation can be utilized to manage inflammation in athletes and active individuals.

MATERIALS AND METHODS

Subjects

This study was a randomized, double-blind, placebo-controlled experiment. Thirty-six healthy, non-smoking recreational male students from the Sports Science Department at Universitas Negeri Surabaya were randomly and double-blindly assigned to either a selenium supplementation group (n=18) or a placebo group (n=18). Participants were selected based on specific criteria, excluding those currently undergoing drug treatment, using chemical drugs, or taking any supplements. Both group was low risk of bias.

All participants were required to have had no intense exercise in the past month and no prior pain or discomfort. They were also instructed to refrain from taking any medication or undergoing any therapy during the study period. Furthermore, participants were directed to avoid any strenuous physical activities not only during the 4-week study period, but also for 48 hours after the experiment, to ensure a consistent and controlled environment.

Before the study began, the research team ensured that all participants had identical physical measurements across the groups. The team then explained the study's goals and procedures to the participants, who provided their informed consent. Prior to joining the study, participants were asked to confirm they had no pain or injuries by completing the Physical Activity Readiness Questionnaire (PAR-Q). To complete the questionnaire, participants were accompanied by a fitness expert from the Faculty of Sports and Health Sciences while completing the questionnaire, which consists of seven questions that cover medical history, symptoms, and physical limitations.

This study adhered to ethical guidelines and was granted permission by the Health Research Ethics Committee of the Faculty of Public Health at Airlangga University, and was deemed acceptable with a specific identification number: 104/EA/KEPK/2023.

Experimental Procedures

The study lasted for a month and began with an initial visit to gather baseline information. Participants in both the Selenium group and the Placebo group recorded their initial data, including body weight, height, fat percentage, physical activity level, and VO2Max, to ensure that the groups were similar at the start. To measure VO2Max, we used the Multistage Fitness Test (MFT). Before starting the study, all participants provided informed consent after being fully informed about the study's objectives and procedures.

Within this study, the Selenium group (n=18) was administered capsules, each containing 200µg of selenium capsules (commercially available products), and the Placebo group (n=18) received placebo capsules containing 100mg of corn starch. Both groups consumed one capsule per day after breakfast for a 21-day period.

Following a 21-day supplementation period, on the 22nd day, Participants performed a Drop Jump (DJ) exercise protocol that has been proven to induce muscle damage. Before the protocol, participants were instructed on the proper technique to ensure correct form and maximum effort. The exercise protocol consisted of five sets of 20 maximal drop jumps from a height of 60 cm with a 10-second interval between jumps and a 2-minute rest between sets. This protocol has been shown to cause significant muscle damage in previous studies (Kirby et al., 2012).The timeline of this present study showed in figure 1.

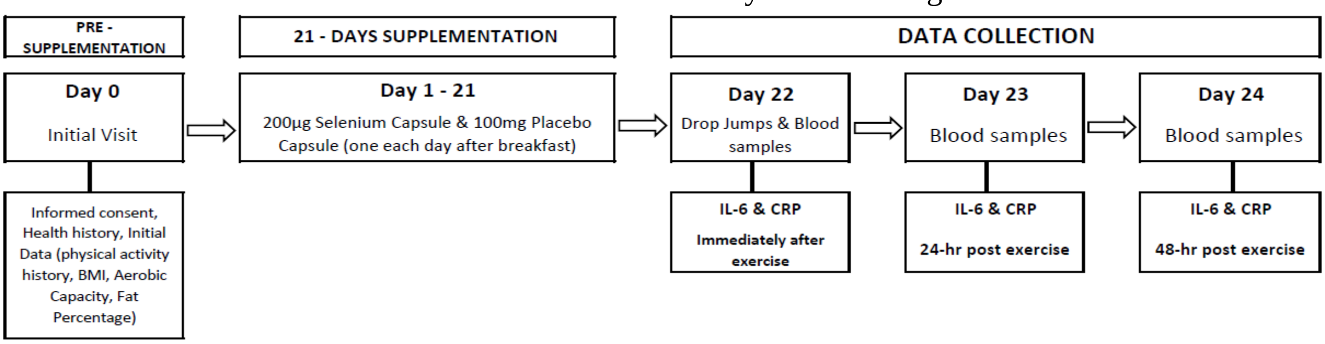

Figure 1. Timeline of the study

Data Collection Tools

Assessment of IL-6

IL-6 in the blood was obtained through the cubital vein, collecting up to 10 cc and placing it into an SST (Serum Separator Tube). Subsequently, the tube containing frozen blood was centrifuged for 15 minutes to separate the plasma from the blood. The plasma formed in the tube was then transferred into several polypropylene tubes, which were closed and labeled. Plasma IL-6 levels were measured using the ELISA method (Ruhee et al., 2020).

ELISA stands for Enzyme-Linked Immunosorbent Assay, a common technique used to detect and quantify specific proteins, antibodies, or other substances in a sample. In this case, the ELISA IL-6 method was likely used to measure the plasma levels of IL-6 in the blood. A commercially available ELISA kit (brand Finetest EH0205) was used for this purpose.

Assessment of C-Reactive Protein (C-RP)

Plasma Level of C-RP was measured using High-Sensitivity C-Reactive Protein (Hs-CRP) method (Boukhris et al., 2020). High-sensitivity CRP (Hs-CRP) is a specific type of CRP that is sensitive enough to detect even small increases in CRP levels. A commercially available Hs-CRP Assay (brand Abbexa) was used for this purpose. .

Statistical Analysis

Data collected was processed both manually and digitally to convert it into information. A one-way repeated measures (immediately, 24, and 48 hours after exercise) ANOVA was used to analyze the data statistically focusing on subject characteristics. The normality test utilized was the Kolmogorov–Smirnov test. The Mann–Whitney U-test was applied for non-normally distributed data, while the independent t-test was used for normally distributed data. The significance level can be written as $p < 0.005$.

RESULTS

Subjects

A total of 36 recreational male students of the Sports Science Department, Universitas Negeri Surabaya students, with an average age of 19.56 years (±0.89) and a mean BMI of 20.93 (±1.14), participated in this study. All participants willingly agreed to become respondents, providing informed consent. The participants were then randomly divided into two groups, Selenium group and Placebo group.

The study examined the characteristics of the participants in the Selenium group (n=18) and the participants in the Placebo group (n=18). The average age of the Selenium group was 19.69 years, while the average age of the Placebo group was 19.44 years. This study also analyzed other characteristics, such as height, weight, BMI, body fat percentage, physical activity level, and aerobic capacity, to ensure that the two groups were normally distributed and homogeneous. The results indicated that the differences between the groups have p-values greater than 0.05 ($p > 0.05$), which means that the data is normally distributed. The Subject characteristics of both groups (Selenium group and Placebo group) are shown in Table 1.

Table 1. Subject characteristic

	Subject Characteristic		
	Selenium (M ± SD)	Placebo (M ± SD)	*P-values*
Age (years)	19.11 ± 0.68	19.21 ± 0.76	0.673
Height (cm)	166.72 ± 4.10	166.42 ± 3.22	0.273
Weight (kg)	62.44 ± 3.54	62.67 ± 3.75	0.404
BMI (kg/m3)	22.45 ± 0.70	21.18 ± 1.14	0.586
Body Fat (%)	12.44 ± 1.22	12.26 ± 0.88	0.185
Physical Activity Level (MET)	1332.26 ± 242.18	1292.48 ± 228.42	0.654
Maximum Oxygen Intake (mL/(kg·min))	36.75 ± 4.54	33.82 ± 5.52	0.112

Interleukin 6 (IL-6)

High-intensity exercise increased plasma IL-6 concentrations. Repeated-measures tests showed that plasma IL-6 concentrations increased significantly (P < 0.05) at baseline (immediately after exercise) in both groups. The increase in plasma IL-6 concentrations immediately after exercise was followed by a significant decrease at 24 and 48 hours (P < 0.05) in both groups as the recovery period progressed. However, although all post-exercise assessments showed decreased plasma IL-6 concentrations in both Selenium (P < 0.024) and PLA (P < 0.001) compared to baseline, it was observed that Selenium decreased plasma IL-6 concentrations more than PLA at 24 and 48 hours post-exercise (P < 0.020). Changes in plasma IL-6 concentrations at baseline, 24 hours, and 48 hours can be seen in Figure 2.

C-Reactive Protein (CRP)

High-intensity exercise causes a significant increase in plasma CRP concentration immediately after exercise, followed by a decrease in plasma CRP concentration over the next 24 and 48 hours during the recovery periods. In both groups, the initial increase in CRP was statistically significant (P < 0.05). However, the rate of decline in plasma CRP concentration was faster in the Selenium group compared to the Placebo group. In fact, the difference between the two groups was statistically significant at 24 and 48 hours post-exercise (P < 0.05). This is shown in Figure 3, which plots the changes in plasma CRP concentration levels over time.

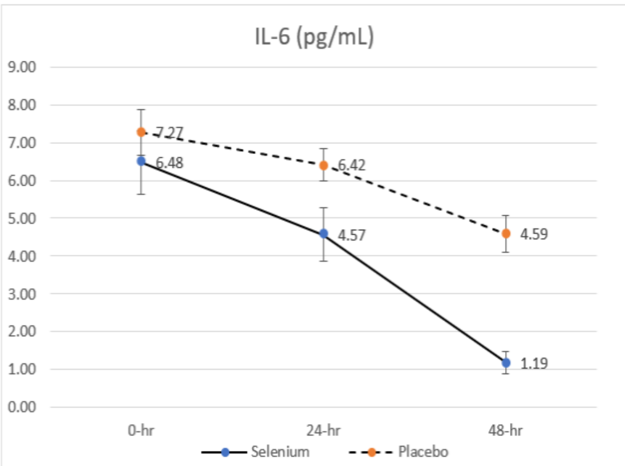

Figure 2. Plasma IL-6 concentration for Selenium group and Placebo group subjects across time

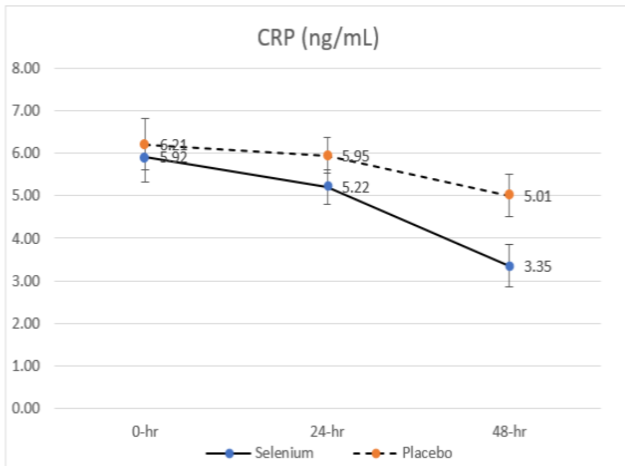

Figure 3. Plasma CRP concentration for Selenium group and Placebo group subjects across time

Table 2. Time after exercise, in hours

	Groups	0-hr (M ± SD)	24-hr (M ± SD)	48-hr (M ± SD)	P Value
Interleukin 6 (IL-6)	Selenium	6.48 ± 0.84	4.57 ± 0.70	1.19 ± 0.29	0.000
	Placebo	7.27 ± 0.60	6.42 ± 0.42	4.59 ± 0.49	
C-Reactive Protein (CRP)	Selenium	5.92 ± 0.61	5.22 ± 0.59	3.35 ± 0.49	0.006
	Placebo	6.21 ± 0.30	5.95 ± 0.40	5.01 ± 0.49	

DISCUSSION

During high-intensity exercise, Interleukin 6 (IL-6) is produced and released from contracting muscles into the circulation (Villar-Fincheira et al., 2021), reaching peak circulating concentrations immediately after completion of high-intensity exercise, then rapidly decreasing with recovery to baseline concentration (Islam et al., 2024). However, if the exercise is accompanied by muscle damage, immune cells infiltrate the muscle and release IL-6 to signal the acute-phase response and tissue repair (Philippou et al., 2021).

High-intensity exercise with inadequate recovery may result in an increase in cytokines, especially IL-6 and CRP, as an inflammatory response in circulation, leading to detrimental physiological effects such as increased muscle damage and decreased nutrient absorption (Takami et al., 2024). It may also interfere with the body's normal ability to adapt to exercise, making it more difficult to recover and train effectively (Peake, 2019). The primary objective of this study was to investigate the physiological effects of 3 weeks of selenium supplementation on the inflammatory response, specifically Interleukin 6 (IL-6) and C-Reactive Protein (CRP) as markers of inflammation after high-intensity exercise.

IL-6 is an important inflammatory marker that plays a critical role in the body's response to exercise-induced muscle damage and inflammation. The mechanical damage to a muscle fiber appears to trigger an inflammatory response, which is not limited to the damaged muscle but also extends to the entire body (Stožer et al., 2020).

The results of this study indicated that there were increased levels of IL-6 and CRP in both groups (Selenium group and Placebo group) immediately after exercise as a baseline, followed by decreases at 24 hours and 48 hours after exercise as part of the recovery process (figure 2 and figure 3). The main finding of this study was that the selenium-supplemented group had a significantly greater reduction in plasma IL-6 and CRP compared to the Placebo group ($p<0.05$). This means that daily supplementation of selenium for 3 weeks (21-days) can reduce the risk of inflammatory response following high-intensity exercise.

High-intensity exercise may trigger an increase in IL-6 levels, possibly due to the generation of free radicals during exercise (Pal et al., 2014). These free radicals, also known as reactive oxygen species (ROS), play a role in cell signaling and affect various cellular processes. Excessive ROS levels due to high-intensity exercise can stimulate increased plasma IL-6 levels, resulting in damage to biomolecules such as lipids, proteins, and nucleic acids. This can potentially cause cell damage through increased skeletal muscle IL-6 expression (Jakubczyk et al., 2020). The body's antioxidant system helps counteract the oxidative stress caused by the overproduction of ROS during intense exercise (Di Meo et al., 2019).

Increased oxidative stress, which triggers the production of Reactive Oxygen Species (ROS) during exercise. ROS are general mediators of signal transduction pathways and have the ability to induce cytokine production from various cell types (Aguiar et al., 2020; Dos Reis et al., 2023). In this case, researchers have focused their research on administering antioxidant supplements before high-intensity exercise in an effort to enhance antioxidant and anti-inflammatory properties, reducing factors that contribute to the exercise-induced inflammatory response. Among the supplements studied are vitamin C (Righi et al., 2020), vitamin E (Martínez-Ferrán et al., 2022), BCAA (Greer et al., 2007), curcumin (Rahmat et al., 2021), and Vitamin D (Żebrowska et al., 2020).

Therefore, they identified the release of reactive oxygen species (ROS) due to oxidative stress as a significant stimulus for exercise-induced cytokine production. Their study aimed to determine whether implementing specific measures could reduce the severity of muscle injury and inflammation that occurs as a result of the exercise-induced inflammatory response.

Selenium (Se) is an essential trace element that plays key roles in antioxidant and immune systems (Mal'tseva et al., 2022). It is a crucial component of selenoproteins, involved in redox catalysis, structure, and transportation. Selenium functions include antioxidant defense, thyroid hormone synthesis, testosterone metabolism, DNA integrity maintenance, vitamin E modulation, cancer prevention, and muscle performance enhancement (Bryan et al., 2023).

Multiple studies have shown that Selenium supplementation boosts the activity of plasma Glutathione Peroxidase (GPx), a potent antioxidant enzyme that relies on Selenium. This suggests that the relationship between GPx and Selenium is crucial for antioxidant defense against Reactive Oxygen Species (ROS) (Bjørklund et al., 2022). The interplay between ROS generation during exercise and Selenium's antioxidant protection may play a significant role in enhancing exercise performance.

Furthermore, the present study showed that 3 weeks selenium supplementation reduced the increase in IL-6 and CRP plasma concentrations in the blood. This suggests that selenium supplementation can help alleviate muscle damage caused by high-intensity exercise. The decrease in IL-6 and CRP plasma concentrations was attributed to selenium's ability to restrain the NF-kappa B pathway (Wang et al., 2022). NF-kappa B is a protein that regulates inflammation, and when it's active, it increases the production of inflammatory markers like IL-6 and CRP (Maehira et al., 2003).

Selenium supplementation re duced the activity of NF-kappa B, which in turn decreased the production of these inflammatory markers. Selenium also reduced nitric oxide (NO) production by regulating the expression of nitric oxide synthase (NOS) and inducible NOS (iNOS) genes (Jomova et al., 2023). NO is a molecule that can contribute to oxidative stress and inflammation, so reducing its production may have contributed to the anti-inflammatory effects of selenium supplementation.

Conclussion

In conclusion, Selenium supplementation may help reduce the rise in IL-6 and CRP plasma concentration levels induced by intense exercise by enhancing the activity of the Se-dependent enzyme GPx. This suggests that Selenium supplementation could boost antioxidant capacity in active individuals.

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Conflict of Interest

All authors have no conflicts of interest regarding this article. This research was funded by Directorate of Research, Technology And Community Service, Ministry of Education, Culture, Research And Technology of Indonesia 2022.

Ethics Statement

This study was conducted in accordance with ethical guidelines and was approved by the Health Research Ethics Committee at the Faculty of Public Health, Airlangga University, with the reference number: 104/EA/KEPK/2023.

Participants were fully informed and gave their consent, which included details about the research, potential risks, benefits, confidentiality, and their rights. The research strictly adhered to the principles of the Declaration of Helsinki, prioritizing the rights and well-being of participants in all aspects of the study, from design to implementation.

Author Contribution

Study design, RJI and TM; Data Collection, RJI, PAN and NR; Statistical analysis, TrM and SRN; Data iterpretation, TM, TrM, NR,; Literature search, RJI, SRN, NR, and PAN. All authors have read and approved the published version of the manuscript

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