

Response of Trained Long-Distance Runners to Lowering Superoxide Dismutase Levels via High Intensity Interval Training (HIIT)

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Keywords

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Abstract

Background of study: Superoxide Dismutase is essential for shielding cells from the harm caused by free radicals. Oxidative stress and an increase in free radicals can result from high-intensity exercise.

Aims and scope of paper: The purpose of this study is to ascertain how high-intensity interval training (HIIT) reaction long-distance runners' superoxide dismutase levels.

Methods: A pre-experimental research design with a single-group pretest-posttest design was the methodology employed in this study. The HIIT training approach, on the other hand, was performed ten times. The individual was taught to perform interval running by separating two minutes of sprinting and two minutes of walking. A Borg scale was used to track the subject's intensity during exercise, keeping it between 7 and 10. Up to 10 µl of the subject's blood was drawn from a vein in the cubital region both before and after the workout. Following collection, the blood samples were analyzed in the University of Brawijaya's Faculty of Medicine's Clinical Physiology Laboratory. In a paired sample T-test, data are calculated to determine outcomes.

Result: If the p-value is greater than 0.05 (0.393), it is known that Ho is accepted and H1 is rejected, meaning that the program offered to the subject does not have a significant effect on athletes.

Conclusion: The HIIT program in this study proved that there was no significant decrease in SOD levels in long-distance athletes.

1. Introduction

According to Widiyatmoko and Hadi (2018), running is a sport that is frequently done as a requirement to always stay fit and healthy. Physical and spiritual fitness can be achieved by appropriate exercise, but excessive or irregular activity is also harmful to one's health (Sandi, 2016). Because one of the causes of oxidative stress is high-intensity exercise, which might produce free radicals (Gisti Rahmawati, Farida Nur Rachmawati, 2014). An imbalance between pro-oxidants (free radicals) and antioxidants is known as oxidative stress. Stated differently, the body contains more pro-oxidants than antioxidants (Candrawati, 2013). The antioxidant enzyme superoxide dismutase (SOD) serves as the body's first line of defense against free radicals (Astuti et al., 2008). As exercise intensity and duration increase, antioxidants that protect cells are depleted, leading to oxidative stress (Werdhasari, 2014). Intensity and duration have a significant impact on the link between oxidative stress and physical exercise (Yavari, Javadi, Mirmiran, & Bahadoran, 2015).

Free radicals are rather unstable molecules (Sayuti, 2015). Reactive molecules, sometimes referred to as reactive oxygen species (ROS), look for their electron pairs to remain stable (Masaki, 2010). Cells are subject to oxidative stress when the production of ROS surpasses the available antioxidant capability. On the other hand, ROS generation promotes cell growth, signalling, and survival when it is balanced with antioxidant capability. (2015, Suryadinata). ROS are produced by processes that are catalysed by the cytochrome p450 enzyme system or oxidase family enzymes (Izyumov et al., 2010). Under typical circumstances, reactive oxygen species (ROS) serve as a "redox messenger" that regulates intercellular pathways. Oxidative stress is caused by natural ROS generation that is out of balance with the tissues' antioxidant capability. This state leads to irreversible damage to cellular components and cell death by intrinsic apoptosis via mitochondria. Overproduction of ROS can result from damage to the respiratory chain in mitochondria, which can worsen oxidative processes in other sections of the cell as well as in the mitochondria (Schöttker et al., 2015). Oxidative phosphorylation brought on by intense physical activity is the body's primary source of ROS (Arsana, 2016). Since the ROS chain reaction can only be halted by forcing two ROS to react with one another to pair two unpaired electrons, more antioxidants, such as vitamin E, vitamin C, flavonoids, and uric acid, are required. Rahman and Hidayat (2019).

VO2Max High-intensity interval training is one sport that helps develop cardiovascular endurance (HIIT), gaining proficiency in both technical and strategic abilities (Wiswadewa et al., 2017). High intensity interval training (HIIT) is a program that alternates high intensity intervals with high intensity training above 85–90% VO2max (Fleg, 2016). Frequent exercise will speed up the body's metabolism, boost oxygen consumption, and improve heart function (Bimrew Sendekie Belay, 2014).

By slowing down the oxidation process, antioxidants can be thought of as substances that can balance or neutralize free radicals before they harm cells. Uric acid, albumin, glutathione, bilirubin, thiols, vitamins, and phenols are examples of non-enzymatic endogenous antioxidants. Enzymatic endogenous antioxidants include catalase, glutathione peroxidase, and superoxide dismutase (SOD). Endogenous antioxidants will normally balance the production of ROS. In order to prevent and defend against age-related disorders, endogenous antioxidants work to maintain optimal cellular equilibrium and systemic homeostasis in general (Erejuwa et al., 2012; Rahman, 2007; Zalukhu et al., 2016). Exercise is one strategy to boost antioxidant levels. Exercise at submaximal intensity has been shown to preserve cardiorespiratory fitness and enhance health status (Hottenrott et al., 2012). Sports training physiologically stresses the body, which might result in an adaptation response. It is advised that training at a high enough intensity can cause particular adaptations that enable the body to operate more effectively, provided that the body can adjust to excessive demands on it (the overload principle) (Katch et al., 2013). Antioxidants like catalase (CAT), Superoxide Dismutase (SOD), and Hydroxyl Sulfur Glutathione (GSH) are also known to be produced in greater quantities when sports training is modified (de Araujo et al., 2016; Mayorga-Vega et al., 2013). Because cells will continue to produce reactive oxygen species (ROS) and free radicals during respiration and inflammatory situations, antioxidant defence is essential for a cell. By increasing the synthesis of endogenous antioxidants, sports training adaptation can help avoid oxidative stress, which can harm cells and their constituent parts (Putri, 2019).

Park and Kwak (2016) compiled the findings of earlier research on the impact of exercise on endogenous antioxidant capacity, among other things, and explained how participation in sports led to an increase in oxidative stress and a decrease in antioxidant capacity. These findings diverge from Ismaeel et al.'s (2019) research, It discovered that antioxidant status might be considerably raised by the resistance training paradigm. Similarly, de Souza et al. (2019) found that rats' antioxidant capacity might be increased by high-intensity interval training (HIIT) activities. According to Vatani and Golzar's (2012) research, while exercise can boost the antioxidant system and lower some cardiovascular risk factors in overweight individuals, resistance training combined with whey ingestion is more beneficial.

Vieira Junior et al. (2013) reported additional research on the improved antioxidant capacity brought on by exercise, which led to a 36.83% increase in antioxidant capacity (SOD). Similarly, exercise training increased antioxidant capacity, according to studies by Azizbeigi et al. (2014). However, the outcomes differed significantly based on the exercise model. Resistance training (RT) grew by 9.5%, endurance training (ET) climbed by 21.8%, and the concurrent training (CT) model increased by 14.5% respectively. This exposure revealed that studies on how exercise increases antioxidant capacity produced a wide range of findings. This is heavily impacted by numerous elements of Sports training techniques, such as variations in exercise dosages based on frequency, intensity, and duration, as well as exercise regimen and assessment techniques.

2. Method

By applying one or more treatments to research subjects and observing the causal relationship between independent and dependent variables, experimental research methods are employed to support this study. (Budiwanto, 2017). The type of research used is an experiment with a pre-experimental research design of one group pretest and posttest design. The study's sampling strategy One method of selecting samples that precisely meet the researcher's criteria is to use purposive sampling techniques. The number of samples used in this study was 8 long-distance runners with the following inclusion criteria: male aged 18-23 years, members of the PASI Malang City team, athletes who train and are still actively participating in championship events and have signed a letter of willingness to become subjects.

The study's HIIT training strategy involved interval running, where participants were told to walk for two minutes and sprint for two minutes for ten repetitions. Blood sampling was conducted by the Physiology Laboratory team of Brawijaya University before and after exercise through the vein in the cubiti section as much as 10 µl. After blood sampling, the sample was immediately given to the Clinical Physiology Laboratory of the Faculty of Medicine, Brawijaya University. Following a descriptive analysis of the laboratory test findings to assess the distribution of data, normality and homogeneity tests were performed as preconditions for hypothesis testing. If the results of the normality and homogeneity tests had been homogenous and normally distributed, the hypothesis test was then conducted using the paired sample T-test.

3. Results and Discussion

Eight athletes participated in this investigation, which was carried out at the State University of Malang's Cakrawala Field. A pre-experiment with a single-group pretest and posttest design was employed in the study.

This test employed an interval running paradigm for HIIT training, where participants were told to run for two minutes and walk for two minutes repeatedly for ten repetitions. Before engaging in HIIT exercise, the participants completed the informed consent form as the research subject for each sample, had their health examined, and filled out the biodata the researcher provided. They also had their height and weight taken to determine their BMI. Table 1 below shows the average age and BMI data

Table 1. Results of Data Analysis: Description of age, weight, height, and BMI

No	N	Age (year)	Weight (Kg)	Height (cm)	BMI
Mean	8	17,87	59	162,25	22,27
Min		18	43	140	17,22
Max		22	75	171	26,89

The results of the measurements that have been carried out on SOD levels before and after HIIT training. The results of the data analysis are presented in table form. Furthermore, the description of SOD levels can be seen in tables 2 below:

Table 2. Superoxide Dismutase (SOD) Analysis Result Data

No		Pretest (U/mL)	Posttest (U/mL)
1	Max	5,437	4,626
2	Min	1,545	1,449
3	Mean	3,52	2,98
4	SD	1,43	1,07

Table 2 above shows that the post-test value is smaller than the pre-test value. This indicates that SOD levels decreased during the post-test.

Table 3. Results of the Analysis of Normality Tests for Pretest and Posttest Data on SOD levels in long-distance runners.

	Statistic	Sig.	Interpretation
PRETEST	0,273	0,081	Normal
POSTTEST	0,160	0,200*	Normal

According to the normalcy test results, the pretest's significance value was 0.233, while the posttests was 0.894. It is possible to deduce that the significance value of the research data is normally distributed because the study's significance value is more than > 0.05 . To determine whether the data has a homogeneous (same) or non-homogeneous (different) variant, a homogeneity test will next be conducted.

Table 4. Results of Homogeneity Test Analysis

PRETEST DAN POSTTEST	Sig.	Interpretation
	0,243	Homogen

Both the pretest and posttest findings for the homogeneity test of SOD levels are 0.243. Since the study's significance value is known to be higher than > 0.05 , it may be said that the research data's significance value has a homogeneous variant. This indicates that the SOD level data from the pre-test and post-test groups exhibit similar or homogeneous variations. Additionally, the Paired Sample T-test will be used for Hypothesis Testing.

Table 5. Results of Paired T-Test Analysis

	Sig. (2-tailed)
PRETEST - POSTTEST	0,393

The paired T-test analysis yielded a sig value (2-tailed) of 0.393 > 0.05 , as shown in Table 5 above. Therefore, sig. (2-tailed) is greater than 0.05 based on the paired T-test results. According to these findings, the experimental $p > 0.05$, indicating that H_0 is accepted and H_1 is rejected. As a result, there is no effect of using HIIT to administer the SOD level test on raising SOD levels.

The purpose of the research is to determine how high-intensity interval training (HIIT) affects long-distance runners' levels of superoxide dismutase. This study supports the findings of Tarnajaya's (2018) research with the aim of testing the effects of high intensity exercise on superoxide dismutase enzyme levels in Wistar rats (*Rattus norvegicus*) where there was no significant effect on SOD levels in the control group (Tarnajaya, Pangkahila, & Siswanto, 2018). Ten repetitions of the HIIT program, which consists of two minutes of running and two minutes of walking, were used in the study. employing a pre-experimental research design that consists of a pretest and posttest for a single group and an experimental methodology. The average levels of SOD in this investigation were 0.393 > 0.05 , according to the data. This has demonstrated that HIIT training had no effect on the rise in SOD levels among PASI Malang City long-distance runners.

3.1. Subject Characteristics

Purposive sampling techniques were used to determine the number of individuals required for this study, which included eight male long-distance runners from PASI Malang City. It is known that the subjects' average age, according to normal BMI criteria, is 22.27. The selection of male subjects in this study is because, according to (Nuarti, Huldani, & Asnawati, 2019) there is more fat in women than muscle in women's bodies than in men, so women have a smaller VO₂ max than men. Because the concentration of hemoglobin in men is greater than vice versa (Nuarti et al., 2019). Men are particularly good at generating energy because hemoglobin is in tune with the binding of oxygen, which the body needs for the energy-burning process (Rohman Hakim, 2013). The objective of basic ability training, also known as physical training, is to raise the physiological Maximum Ability Limit from a physiological standpoint. This automatically and successfully enhances the athlete's mentality (Sinaga, 2018).

3.2. Effect of HIIT on SOD levels

The purpose of this study is to determine how High Intensity Interval Training (HIIT) affects Superoxide Dismutase levels. If no effect is seen, the value for raising SOD levels before and after exercise is not significant. The sig. value for the difference between the levels of Superoxide Dismutase before and after exercise is 0.081 and 0.200, respectively. Thus, it is possible to conclude that the data is regularly distributed. However, the homogeneity test yielded a result of 0.243, indicating that the data is homogeneous. After the data is declared normal and homogeneous, the hypothesis test is carried out using the Paired sample T-test. The results of the p-value calculation are known to be greater than 0.05 (0.393 > 0.05), so H₀ is accepted and H₁ is rejected. The HIIT training response does not significantly affect the decrease in Superoxide Dismutase levels in trained long-distance runners. Because the athletes' physical condition was used to heavier training, the HIIT training provided in this study was unable to surpass the maximum physical intensity for athletes who had trained, had physical condition, and had the stamina to compete (Tanskanen, Atalay, & Uusitalo, 2010). Antioxidants are needed by the body to neutralize free radicals and prevent damage caused by free radicals to normal cells. Antioxidants stabilize free radicals by supplementing the lack of electrons in free radicals and inhibiting the chain reaction of free radical formation that can cause oxidative stress. In general, antioxidants are divided into two, namely enzymatic antioxidants (endogenous antioxidants) and non-enzymatic antioxidants (exogenous antioxidants). Enzymatic antioxidants include glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). While non-enzymatic antioxidants include fat-soluble antioxidants (such as tocopherols, carotenoids, quinones, flavonoids etc.) and water-soluble antioxidants (such as ascorbic acid). By activating a chemical process to eliminate free radicals, endogenous antioxidants—enzymatic antioxidants—reduce oxidative damage (Berzosa C et al., 2011). By stopping the chain reaction and turning it into a more stable product, enzymatic antioxidants prevent the production of free radicals. This class of antioxidants is also known as chain breaking antioxidants. An enzyme called superoxide dismutase catalyzes the conversion of superoxide anion into O₂ and hydrogen peroxide (H₂O₂). Superoxide radicals can trigger radical chain events by removing electrons from cell membranes or other cell components, despite their low toxicity. It is possible for superoxide radicals to contribute to the production of hydroxyl radicals (Mittler R 2017). The findings of earlier studies have demonstrated that exercise causes the body's natural antioxidant levels to rise. According to Poblete Aro et al. (2015), sustained exercise at a moderate intensity can boost antioxidant capacity. According to a study by Vieira Junior et al. (2013), aerobic exercise five times a week for eight weeks increased the activity of antioxidant enzymes (catalase, glutathione peroxidase, and superoxide dismutase) and decreased MDA levels. According to the findings of the study by Ajabi and Mohammad (2013), cardiovascular activity three times a week for 12 weeks increased the antioxidants SOD and CAT and decreased the levels of MDA in blood plasma. According to research by de Sousa et al. (2017), which used the meta-analysis method and systematic review to examine the effect of exercise on antioxidants, antioxidant indicators tended to increase, and pro-oxidant indicators tended to decrease after training, irrespective of the population studied, exercise type, intensity, and volume. According to Dunggio's (2021) research, a 14-day walking exercise program increased SOD, however the difference was not statistically significant. The body might not be able to adjust to activity after 14 days of training.

4. Conclusion

HIIT response to decreased SOD levels in PASI long-distance runners in Malang city who were given a training intervention of 2 minutes of running and 2 minutes of walking for 10 repetitions. The HIIT program in this study proved that there was no significant decrease in SOD levels in long-distance athletes.

Author Contributions

All authors have equal contributions to the paper. All the authors have read and approved the final manuscript.

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Declaration of Conflicting Interests

The author declared no potential conflicts of interest for the research, authorship, and/ or publication of this article.

Data Availability

The datasets generated during and/ or analysed during the current study are available from the corresponding author on reasonable request.

Ethical Clearance

This study was reviewed and approved by The Research Ethics Committee of Malang State University with Ethical Exemption number .6.6.5/UN32.14.2.8/LT/2024

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