JPJO 9 (2) (2024) 161-168

Jurnal Pendidikan Jasmani dan Olahraga

Available online at:
https://ejournal.upi.edu/index.php/penjas/article/view/63202
DOI: https://doi.org/10.17509/jpjo.v9i2.63202

Correlation between Antioxidant Intake with Pro-Oxidants (MDA) and Endogenous Antioxidants (SOD) in Football Athletes

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Article Info

Article History :
Received October 2023
Revised Mei 2024
Accepted August 2024
Available online September 2024

Keywords :
Football, MDA, Micronutrients, SOD

Abstract

Soccer is one of the high intensity activities requiring an intensive training since the regulatory process may increase the oxidative stress in the form of Reactive Oxygen Species (ROS). Free radicals occurring due to the imbalance of endogenous antioxidants can be measured by examining the malondialdehyde (MDA) and Superoxide Dismutase (SOD) levels as endogenous antioxidants. This outcome will affect the formation of Hemoglobin (Hb), automatically affecting the athlete ability to bind oxygen and their VO2Max. The average SOD level of soccer athletes at the Student Sports Education and Training Center (PPLP) was 45.945 U/mg. This study is a descriptive study with a cross-sectional design aimed to determine the correlation between antioxidant intake with pro-oxidants (MDA) and endogenous antioxidants (SOD) at the same research period. In this study, the total sampling technique was chosen. A total of 33 soccer athletes of PPLP Medan, from both the main and reserve teams, participated in this study. Data were analyzed using univariate, bivariate, and multivariate analysis by employing linear regression statistical tests. The results showed that there was a correlation between antioxidant intake with MDA and SOD levels of soccer athletes with $p>0.005$. The results of multivariate analysis showed that the most influential variable on MDA levels was vitamin C intake, affecting the decrease in MDA levels by 5.545 times. Meanwhile, for SOD levels, the most influential variable was zinc intake, affecting the increase by 6.354 times. In conclusion, there is a correlation between antioxidant intake with MDA and SOD levels, where MDA levels are strongly influenced by vitamin C intake while SOD levels are influenced by zinc intake.

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INTRODUCTION

Football is the most popular sport in the world and also in Indonesia. Based on the ranking realized by the world football body (FIFA), Indonesia position in the Southeast Asia region is still below Vietnam (96) and Thailand (111) (Kristianto et al., 2020). Football is a sport that requires strength and endurance (Dieny & Putriana. 2016). Professional soccer athletes often overtrain, especially before a match or competition. This will result in a high rate of nutrient metabolism and a lack of oxygen uptake during heavy physical activities which will stimulate the release of superoxide radicals, including Reactive Oxygen Species (ROS) (Yavari et al. 2015). The long-term consequences of this process will result in an imbalance between pro-oxidants and antioxidants (Puspaningtyas et al. 2018). The results of the study conducted by Siahaan in 2024 found that soccer athletes in PPLP North Sumatra had average superoxide dismutase (SOD) levels of 45,945 U/mg (Siahaan et al. 2024).

The emergence of ROS can be characterized by an increase in Malondialdehyde (MDA) levels in blood serum (Harun et al., 2017). MDA is one of the biomarkers for examining free radicals in the body which can describe the degree of oxidative stress in soccer athletes (Puspaningtyas et al. 2018). Antioxidant status in the body can be in the form of Glutathione Peroxidase (GPx), Catalase, and Superoxide Dismutase (SOD) (Park & Kwak, 2016). Endogenous antioxidants are the first barrier to fight excessive free radicals in the body, which will indicate an oxidation process in cell membranes (Zaetun et al., 2019).

An increase in MDA levels and a decrease in SOD levels can cause disruption in the formation of Hemoglobin (Hb) needed by a soccer athlete in its capacity to uptake oxygen, known as Vo2max. Low Hb levels indicate anemia in soccer athletes and is often referred to as sports anemia (Dieny & Putriana. 2016). The results of a study conducted by Senturk in 2017 stated that increased oxidative stress can result in sports anemia, making soccer athletes vulnerable to injury (Şentürk et al., 2017).

Anemia in athletes does not only occur due to the lack of Hb-forming substances (Fe, Folic Acid, and B12) but can also be caused by disruptions of the balance of antioxidants and free radicals due to an overtraining. Sports anemia in soccer athletes can be prevented by consuming antioxidant sources, such as beta-carotene, vitamin E, vitamin C, and zinc. These sources can be obtained from foods, such as fruits, vegetables, and nuts, which are rich in antioxidants and have a high bioactive substances (Parwata 2016). The aim of this study was to analyze the correlation between antioxidants with pro-oxidants (MDA) and endogenous antioxidants (SOD) in non-professional youth soccer athletes. As its novelty, this study discussed how antioxidant intake affected free radicals (MDA), which could affect the activity of the SOD enzyme in the body, indirectly affecting the performance of a soccer athlete.

METHODS

This research used descriptive observational with a cross-sectional design by analyzing the correlation between intake of antioxidant nutrients (vitamin A, vitamin C, and Zinc) with MDA and SOD levels in football athletes which was carried out on 12-28 February 2023.

Participants

Determination of the sample in this study was carried out based on the inclusion criteria, namely football athletes who lived and joined trainings at PPLP Medan, aged 15-18 years, were not sick, consumed food provided by PPLP Medan, and were willing to fill informed consent. Exclusion criteria included soccer players who were sparring partners, aged over 19 years, and were still snacking outside of the food provided by the PPLP Medan. Based on the criteria determined by the researchers, 33 people were found and selected using the total sampling technique. All of them were PPLP football athletes, including the core and reserve players.

Procedure

The In collecting data, the researchers were assisted by 4 enumerators. They were students from the Department of Nutrition, receiving a briefing for 2 days to equalize perceptions. The data collection on nutritional intake of vitamin A, vitamin C, and zinc was carried out using the 24-hour food recall method for 3 non-consecutive days. The nutritional intake data were processed using the Nutrisurvey program. The data collection was carried out on Saturday, Tuesday, and Thursday.

Data Analysis

The examination of MDA and SOD levels was carried out by taking a blood sample from the upper left arm using a 2.5 cc syringe by a medical laboratory technology analyst. Then, the blood was put into a tube containing ethylenediaminetetraacetic acid (EDTA) solution and examined at the Molecular Laboratory of the Faculty of Medicine, Brawijaya University, Malang, Indonesia. MDA and SOD examinations were carried out using the enzyme-linked immunosorbent assay (ELISA) method with a spectrophotometer. Data analysis of this study consisted of univariate analysis, bivariate analysis, and multivariate analysis. Univariate analysis describing each variable was presented in a frequency table. Bivariate analysis used the Pearson correlation test because the data were normally distributed based on the Kolmogorov Smirnov test. Meanwhile, multivariate analysis was carried out to find out the independent variable that had the highest influence on the dependent variable using a linear regression statistical test (Notoadmojo, 2018). This research has received approval from the Health Research Ethics Committee of the Medan Health Polytechnic of the Ministry of Health number: 01.1500/KEPK/POLTEKKES KEMENKES MEDAN 2023.

RESULT

Description of Sample Characteristics

The sample characteristic data in this study are used to support the obtained research results. In this study, the sample characteristics consisted of age, current education, length of time spent at PPLP Medan, and nutritional status. These characteristics can be seen in Table 1.

Table 1. Distribution of Sample Characteristics

Sample Characteristics	Variable	(n)	(%)
Age	15 Year	6	18,2
	16 Year	5	15,2
	17 Year	14	42,4
	18 Year	8	24,2
Current Education	High School/ Vocational School	33	100
Length of Time at PPLP	<1 Year	8	24,2
	>1 Year	25	75,8
Nutritional Status (IMT/U)	Normal	30	90,9
	Overweight	3	9,1

Table 1 shows that the age of the samples was dominated by 17-year-old, totaling 14 people (42.4%) with the youngest being 15-year-old and the oldest being 18-year-old. Based on the nutritional status of the sample, 30 people (90.9%) had normal nutritional status and 3 people (9.1%) had overweight nutritional status.

Antioxidant Intake of Soccer Athletes

Table 2 shows that the results of interviews using the food recall method for three non-consecutive days revealed that the average micronutrient intake in football athletes, specifically for antioxidant intake, was above the 2019 nutritional adequacy rate (AKG) for vitamin A (700 re), vitamin C (90 mg), and zinc (17 mg). It was similar to the average MDA and SOD levels, which gained 17.51 n/mol for MDA and 45.94 u/ml for SOD.

Table 2. Distribution of means, SD, Vitamin A, Vitamin C,

Indicators	Min.	Max.	Mean	SD	% AKG	
					15	16-18
Vitamin A	623	756	688	31,2	114 %	98 %
Vitamin C	68,3	91,2	82,4	21,5	109 %	91 %
Zinc	3,5	36,6	9,5	7,0	86 %	86 %
MDA	12,682	43,68	17,5	5,5		
SOD	39	51,22	45,9	3,4		

Based on test results using the Pearson correlation method, vitamin C was the antioxidant substance having the strongest relationship with MDA (Free Radicals) with a very strong relationship (-0.807). This means that the lower the intake of vitamin C, the higher the MDA levels of soccer athletes. Meanwhile, zinc intake gained a very strong relationship with endogenous SOD levels (0.798), meaning that the higher the zinc intake, the higher the endogenous SOD levels.

The results of multiple linear regression in Table 4 show that the antioxidant intake of vitamin C had the highest influence on MDA levels of football athletes. In this study, the vitamin C intake coefficient value of 0.322 would produce an MDA constant of 5.545 mg/dl, showing that vitamin C intake affected the decrease in MDA levels by 5.545 times.

The results of multiple linear regression in Table 5 show that zinc antioxidant intake had the highest influence on SOD levels of football athletes. In this study, the zinc intake coefficient value was 0.256, producing an SOD constant of 6.354 mg/dl, indicating that zinc intake influenced the decrease in SOD levels by 6.354 times.

Table 3. Correlation Analysis of Antioxidant Intake with MDA and SOD in Football Athletes

Nutritional intake	n	MDA		SOD	
		R	P	R	p
Vitamin A	33	- 0,441	0,032	0,235	0,045
Vitamin C	33	- 0,807	0,001	0,625	0,037
Zinc	33	- 0,447	0,025	0,798	0,002

Table 4. Results of Multivariate Analysis of Micronutrient Intake with MDA of Football Athletes

Variable	B	SD	Beta	p	95% CI
Constant	5,545	1,34		0,000	0,085-0,670
Vitamin A	0,125	0,001	0,187	0,001	
Vitamin C	0,322	0,001	0,470	0,002	
Zinc	0,053	0,025	0,250	0,002	

Table 5. Results of Multivariate Analysis of Micronutrient Intake with SOD in Football Athletes

Variable	B	SD	Beta	p	95% CI
Constant	6,354	7,0		0,056	0,182-0,794
Vitamin A	0,028	0,001	0,560	0,043	
Vitamin C	0,157	0,003	0,675	0,001	
Zinc	0,256	0,002	0,668	0,342	

DISCUSSION

Sample Characteristics

In this study, the age range was 15-18 years. PPLP selects its athletes to become outstanding athletes representing North Sumatra to achieve their peak performance so that when selecting athletes, especially for football, selected athletes are those who have achieved good results at the sub-district, district, and club levels. Peak performance occurs from the age of 17 until 24. It is expected that the Ministry of Youth and Sports, in this case PPLP, will be able to accommodate athletes of this age, who are at the high school education level (Jumalong, 2014).

Nutritional status was measured using body weight and height to obtain a body mass index which was then compared with age (BMI/U). The obtained results showed that there were 30 athletes with normal nutritional status (90.9%) and only 3 people (9.1%) with overweight nutritional status. These results are in accordance with the research conducted by Alfitasari in 2019, stating that the majority of football athletes at the clubs that she studied had normal nutritional status, amounted to 14 people (87.5%) (Alfitasari et al., 2019). Nutritional status determines the fitness of a football athlete to perform optimally because overweight will affect the movement and explosive ball dribbling ability will be disrupted.

Correlation between Antioxidant Intake with MDA

The results of this study showed a significant relationship between antioxidant intake (vitamin A, vitamin C, and Zinc) and MDA. Vitamin C had the strongest relationship with MDA (r = -0.807). This shows that the more vitamin C increases, the more the MDA will decrease. Malondialdehyde (MDA) is one of the results of lipid peroxidation caused by free radicals during the maximum physical exercise or high intensity endurance training (Sinaga, 2017). Vitamin C is the vitamin most commonly used as an antioxidant. Antioxidant content, such as vitamin C, phenolics, flavonoids, anthocyanins, iron, zinc, L-ergotien selenium, terpenoids, sterols, ß-glucans, saponins, and carotenoids can help prevent oxidative damage during exercise and prevent increases in MDA levels in soccer athletes with a high intensity training (Adawiah et al., 2015). Vitamin C has another name, namely ascorbic acid, which is a water-soluble vitamin and is available in several food sources. Vitamin C at the right dose functions as an effective antioxidant in inhibiting free radicals (Wibawa et al., 2020). Vitamin C is needed for regeneration and neutralizing free radicals by donating H+ ions. Not only does it neutralize hydroxyl, alkoxyl, and peroxyl radicals with H+ donations, ascorbate can also neutralize radical forms of other antioxidants, such as GSH. Beta carotene is an effective scavenger of Alkoxyl and Peroxyl radicals (Sivaranjani et al., 2013).

Vitamin C is chemically capable of reacting with most free radicals and oxidants in the body. The recommended daily intake for adult women is 75 mg and for adult men is 90 mg (Almatsier, 2018). The recommended daily intake for adult women is 75 mg equivalent to 100 gr papaya (1 medium piece) and for adult men is 90 mg equivalent to 50 gr guava (1 small piece) (Almatsier 2018). Vitamin C supplements are recommended to be given after a heavy physical activity as a protection and antioxidant against oxidative stress (Bunpo et al., 2019). The decrease in Vitamin C concentration during training, especially in a heavy training, is in line with the statement of Puspaningtyas (2018), stating that for athletes with a heavy activity both during training and when entering competitions, vitamin C functions as the part of the first defense mechanism against ROS by preventing the formation of radicals, hydroxyl, and also acts as a hydrogen donor so that MDA decreases (Puspaningtyas et al. 2018) (Vargas-Mendoza et al. 2019).

Research conducted by Lin in 2018 stated that zinc can significantly reduce MDA serum levels and suppress lipid peroxidation, so this confirms that zinc has antioxidant capacity in preventing oxidative stress caused by the excessive activity (overtraining) (Lin et al. 2018). Zinc seeks to protect itself from the danger of free radicals by making the endogenous antioxidant system effective, including superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase, and ceruloplasmin. Apart from that, zinc is also the main constituent of Superoxide Dismutase (SOD), which plays a role in protecting cells from inflammations and the toxic effects of Reactive Oxygen Species (ROS). Zinc is also a mineral that helps maintain the balance of nutrient metabolism, including fat metabolism products, so that lipid peroxidation does not occur. Zinc from food sources, such as meat, nuts, tofu, tempeh, milk, and eggs, can reduce levels of oxidative stress, inflammation, and MDA (Santulli et al. 2023).

Vitamin A has a role in cellular immunity involving white blood cells, both mononuclear and polynuclear, as well as NK (natural killer) cells. These cells act as cells that capture antigens, process them, and present them to T cells. It is known as presenting cells or APC (antigen presenting cells) (Hans, K. B., & Jana, 2018). Vitamin A intake has an effect on a person immune system, specifically in the cellular and humoral immunity, because vitamin A as an antioxidant can increase immunological responses for cellular responses and humoral responses, in addition to being needed to maintain the integrity of the function and structure of the epithelium, thereby increasing the activity of T cells and B cells to suppress the formation of free radicals (Keleidari et al. 2014). Vitamin A, including its derivative B-carotene, acts indirectly in providing cell membrane protections and maintaining cell membrane integrity by preventing the formation of lipid peroxidation which will automatically suppress the formation of MDA (Park and Kwak 2016).

Correlation between Antioxidant Intake with SOD

The results of this study showed a significant relationship between antioxidant intake (vitamin A, vitamin C, and Zinc) and SOD. Zinc had the strongest relationship with SOD (r = 0.789). Zinc is an antioxidant that comes from micro minerals so it is also called an essential mineral (Almatsier, 2018). Zinc main function is as a co-enzyme which helps enzymes work optimally, apart from cell proliferations, especially mucosal cells. Zinc as a co-enzyme is needed in the activity of the SOD (Superoxide Dismutase) enzyme which has an important role in the body defense system, especially against the activity of reactive oxygen compounds (Altobelli et al., 2020). The presence of SOD as an enzyme will function optimally as the body first defense against the lipid peroxidation process if there is sufficient zinc in the body and it works optimally. Zinc works as an antioxidant, acts as an enzyme catalyst, and takes part in the metabolism of lipids, carbohydrates, and proteins, so that if zinc intake is sufficient to meet needs, SOD will work optimally. SOD, as an endogenous antioxidant that is able to reduce free radicals, will prevent damage to DNA and RNA, proteins and lipids which play a role in the process of forming new free radicals (Alexander et al. 2020).

Vitamin C works physiologically and biochemically as an electron donor and reducing agent which makes vitamin C effective as an antioxidant because it can quickly break the reaction chain of SOR (Reactive Oxygen Species) and SNR (Reactive Nitrogen Species) (T. Tarigan et al., 2018). Vitamin C easily donates electrons to free radicals, so cells including immune cells are protected from damage caused by free radicals (N. Tarigan et al., 2022). Apart from having a direct role as an antioxidant, vitamin C is able to stimulate and increase the work of SOD as an endogenous antioxidant by also being able to capture superoxide free radicals and minimize the damage caused by oxidative stress. The presence of vitamin C can lighten the work of SOD so that SOD activity in inhibiting free radicals becomes higher (Fery & Amaliah, 2021).

Vitamin A as an antioxidant will help the performance of endogenous antioxidants, in this case SOD, which can function to donate electrons to ROS so that free radicals in the form of ROS will reduce the negative impact of free radicals (Silva et al. 2021). Vitamin A has an epithelium on the cell membrane so that, with the presence of vitamin A, the ability of free radicals to damage cells is smaller or lower. Vitamin A, which stimulates the work of SOD, can also maintain cell integrity and defense from damage caused by free radicals in the body (Cantorna et al., 2019). Basically, vitamin A as an antioxidant functions to protect oxidized compounds due to excessive activity by football athletes. The metabolic process of nutrients consumed by football athletes also causes an increase in the blood lipid profile of a football athlete which can also cause the formation of ROS. The presence of vitamin A, which also influences the work of SOD apart from being protective, is also able to suppress the formation of ROS as free radicals (Menal-Puey, Martínez-Biarge, and Marques-Lopes 2019) (dos Reis et al. 2018). The habit of consuming vegetables and fruits as a source of vitamins and minerals for athletes must be the priority in their lives because, in addition to increase the stamina, fruits and vegetables are also able to prevent injuries due to overtraining by reducing the occurrence of oxidative stress in athletes. For this reason, a strict and continuous supervision from each sport coach is needed so that the maximum athlete performance is achieved when competing.

CONCLUSION

There is a relationship between antioxidant intake and SOD and Malondialdehyde (MDA) levels in soccer athletes, $p<0.05$. Vitamin C gained the strongest relationship with MDA ($r = -0.807$). This shows that the more vitamin C increases, the more the MDA will decrease. Moreover, zinc had the strongest relationship with SOD ($r = 0.789$). Meanwhile, vitamin A as an antioxidant could also increase immunological responses for cellular responses and humoral responses, so that it could protect oxidized compounds due to an excessive activity in football athletes. This research is a basic solution for athletes, coaches, and PPLP coaches to provide exogenous anti-oxidant sources from fruits and vegetables. The fulfillment of fruit consumptions for 200-250 gr/day and vegetables for150-200 gr/day can be applied and also become a habit for every athlete of various sports.

ACKNOWLEDGEMENT

The researchers would like to thank PPLP Medan for willing to allow football athletes to participate in this research.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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