Ascorbic Acid Drink after Submaximal Physical Activity can Maintain the Superoxide Dismutase Levels in East Java Student Regiment

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Abstract

Objective - This study aimed to analyze the effect of ascorbic acid after submaximal physical activity on reducing levels of malondialdehyde (MDA) and increasing levels of superoxide dismutase (SOD) to Student Regiment in East Java.

Methods - This study was a true experiment with a randomized control group posttest-only design used 28 male subjects aged 19-23 years with body mass index (BMI) 18.5-24.9 kg/m^2, and randomly divided into four groups. Namely G_1 (n=7, control without submaximal physical activity+placebo), G_2 (n=7, submaximal physical activity+placebo), G_3 (n=7, submaximal physical activity+AA 500 mg), and G_4 (n=7, submaximal physical activity+AA 1,000 mg). The submaximal physical activity was 2.400 meters running in 12 minutes or when the 60-70% of HRmax achieved. The drinks containing 500 mg and 1.000 mg ascorbic acid (AA) for G3 and G4 was given after exercise test. Measurement of serum MDA and SOD used the Thiobarbituric Acid Reactive Substances (TBARs) method. The data was statistical analyzed using SPSS software with a significant level (p<0.05).

Results - The results of MDA levels showed in G_1 (308.18±61.81 ng/mL), G_2 (338.42±125.78 ng/mL), G_3 (290.54±69.18 ng/mL), G_4 (279.83±39.10 ng/mL) and there was no significantly difference among groups (p=0.557). The mean levels of SOD was significant among groups ((p=0.000), and the SOD level in each group was G_1 (23.19±0.77 units/mL), G_2 (24.81±0.87 units/mL), G_3 (25.27±0.79 units/mL), G_4 (25.57±0.47 units/mL).

Conclusion - It can be concluded that 500 mg and 1.000 mg ascorbic acid drinks after submaximal physical activity can increase SOD levels of students regiment in East Java. Ascorbic acid drink may maintain the SOD level by lessening the use of antioxidant endogen and it may also increase the SOD level, however futher research is needed to be conducted to figure out the mechamism.

Keywords: *Submaximal physical activity, malondialdehyde, superoxide dismutase, student regiment*.

Introduction

The student regiment (MENWA) is a component of the state defense reserve that comes from students. Student Regiment Units are located on campuses in Indonesia. The Student Regiment must undergo basic military education and training to become a member. The MENWA as basic military education and training is a series of activities that involve many physical factors such as running, push up, sit up, and long marches which are useful for improving physical abilities. Physical activity is a very important factor in the prevention and treatment of various diseases such as coronary heart disease, type 2 diabetes mellitus, and symptoms related

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to metabolic syndrome, therefore physical activity is often used as an approach to improve and to maintain physical and health.[1,2] Moreover, physical activity carried out regularly, measured, and continuously can improve cognitive function,[3] and prevent premature aging.[4] Physical activity can induce a variety of physiological changes depending on the intensity and dose of physical activity undertaken.[5] During physical activity, there is an increase in oxygen demand 100x from the normal requirement.[6] The increase in oxygen demand makes the metabolic system in the mitochondria increase, this can lead to an increase in reactive oxygen species (ROS) and result in oxidative stress which will have an impact on injury and the death of cells in the body.[7] Physical activity that is carried out with high intensity and is competitive in nature has the potential to trigger an imbalance between the production of free radicals and antioxidants in the body, which can increase the occurrence of oxidative stress.[8,9]

In conditions of oxidative stress, it can cause cell, tissue, or organ damage that can lead to degenerative diseases.[10] Oxidative stress is an imbalance condition between free radical production and antioxidants.[9] Oxidative stress conditions characterized by increased production of ROS that have implications for various diseases such as hypertension, atherosclerosis, diabetes, stroke, chronic kidney disease (CKD), heart failure, and other chronic diseases.[11,12] Therefore, a balance is needed from the production of antioxidants in the body. The role of antioxidants is to reduce or stop chain reactions by eliminating free radicals or inhibiting other oxidation reactions.[13] Antioxidants can be in the form of endogenous antioxidants, which are found in the body, and exogenous antioxidants, which come from outside the body, such as from food and supplements. The human body is also equipped with endogenous enzymatic antioxidants including superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase.[14] Ascorbic acid (AA) is a water-soluble vitamin. Besides, AA is also found in several food sources. A person cannot synthesize exogenous antioxidants which are very important in inhibiting the occurrence of ROS, so consumption of AA is very important to meet the body's needs. Consumption of AA supplementation is highly recommended to prevent oxidative stress after physical activity as protection against oxidative stress.[15] However, the effect of AA in inhibiting the occurrence of oxidative stress in male adolescents is still unclear, especially in adolescent boys who are exposed to high-intensity physical activity.

On this basis, this study aimed to prove the effect of giving ascorbic acid after submaximal physical activity on reducing levels of malondialdehyde (MDA) and increasing levels of SOD of Student Regiment in East Java. This study hypothesized that submaximal physical activity increased levels of MDA and decreased levels of SOD, while the administration of ascorbic acid after submaximal physical activity reduced MDA levels and increased SOD levels to Students Regiment in East Java.

Materials and Methods

Experiment design

This study was a true experiment with a randomized control group posttest-only design using 28 male subjects (19-23 years old), body mass index (BMI) 18.5-24.9 kg/m^2. The subjects were randomly divided into four groups, G_1 (n=7, control without submaximal physical activity+placebo), G_2 (n=7, submaximal physical activity+placebo), G_3 (n=7, submaximal physical activity+AA 500 mg), and G_4 (n=7, submaximal physical activity+AA 1000 mg). All research procedures had been approved by the Ethical Research Committee of the Faculty of Medicine, Universitas Airlangga (34/EC/KEPK/FKUA/2020).

Ascorbic acid (AA) and exercise protocol

The submaximal physical activity was a distance of 2.400 meters running at the Brawijaya Regional Military Command Stadium in Surabaya at 06.00 a.m. The AA drinks 500 mg and 1000 mg was given after doing submaximal physical activity for G_3 and G_4. Monitoring heart rate was applied during submaximal physical activity used Polar Heart Rate Monitoring (Polar H10 Heart Rate Sensor, USA, Inc).

Anthropometric measurements

Measurement of height used a stadiometer (SECA, Chino, CA while weight measurement used an electronic scale (Tech 05®, China). BMI was measured

by calculating body weight (kg) divided by body height in meters squared (m^2).[16,17]

Physiological condition

Blood pressure was measured using the OMRON digital tension meter (OMRON Model HEM-7130 L, Omron Co., Ltd. JAPAN). Resting heart rate was measured using a Pulse Oximeter (PO 30 Pulse Oximeter, Beurer North America LP, 900 N Federal Highway, Suite 300, Hallandale Beach, FL 33009).

Blood samples and blood analysis

Blood sampling was fulfilled on 3 ml Cubital veins. At the time of drawing blood, the subject was in a sleeping position. Blood sampling was taken 60 minutes after giving a drink containing 500 mg and 1,000 mg of ascorbic acid. Blood was centrifuged for 15 minutes at 3000 rpm. Measurement of MDA levels and SOD levels used the Thiobarbituric Acid Reactive Substances (TBARs) method.[12]

Statistical Analysis

Data analysis techniques used statistical software packet for social science (SPSS) version 17 (Chicago, IL, USA). The normality test used the Shapiro-Wilk test, while the homogeneity test used the Levene test. Data that were normally distributed and had homogeneous variants were tested using One-way ANOVA and continued with the post hoc Least Significant Difference (LSD) test. All data were presented as mean±SD. All statistical analyzes used a significant level ($p<0.05$).

Results

The results of the descriptive analysis of the characteristics of research subjects in each group can be seen in Table 1.

Table 1. The results of the analysis of the characteristics of the research subjects

Variable	Group				ANOVA p-value
	G1 (n=7)	G2 (n=7)	G3 (n=7)	G4 (n=7)	
Age (years)	19.29±0.49	19.86±1.46	19.86±1.46	20.14±1.07	0.598
Body Weight (kg)	65.00±5.42	63.14±6.20	59.00±5.97	58.86±5.98	0.158
Body Height (m)	1.69±0.03	1.70±0.06	1.68±0.03	1.66±0.07	0.540
Body Mass Index (kg/m2)	22.72±1.44	21.96±1.19	21.05±2.02	21.34±1.21	0.191
Systolic Blood Pressure (mmHg)	112.86±7.56	117.14±9.51	110.00±8.16	112.86±9.51	0.508
Diastolic Blood Pressure (mmHg)	80.00±5.77	81.43±3.78	82.86±4.88	81.43±6.90	0.811
Resting Heart Rate (bpm)	66.86±10.51	70.86±6.82	72.57±9.36	69.14±9.44	0.686

G_1 (control without submaximal physical activity + placebo), G_2 (submaximal physical activity + placebo), G_3 (submaximal physical activity + AA 500 mg), G_4 (submaximal physical activity + AA 1.000 mg). One way-ANOVA. Data are presented as mean±SD.

Based on Table 5.1, the results of the One way-ANOVA test showed that the mean data on the characteristics of research subjects in each group did not have a significant difference ($p>0.05$). The results of the analysis of MDA levels were presented in Figure 1.

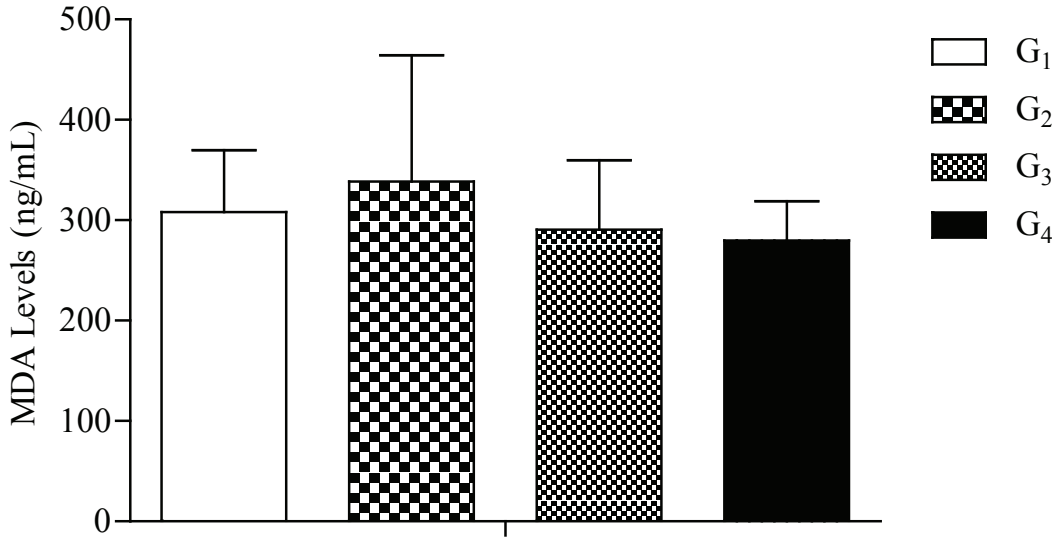

Fig. 1 The Average of MDA Levels in Each Group. G_1 (control without submaximal physical activity + placebo), G_2 (submaximal physical activity + placebo), G_3 (submaximal physical activity + AA 500 mg), G_4 (submaximal physical activity + AA 1.000 mg). The data were shown as the mean (SD).

Based on Figure 1, it could be seen that MDA levels in G_2 were higher than G_1, G_3, and G_4. One-way-ANOVA test results showed that there was no significant difference in the mean MDA levels in each group (p>0.05). The results of the analysis of MDA levels were presented in Figure 2.

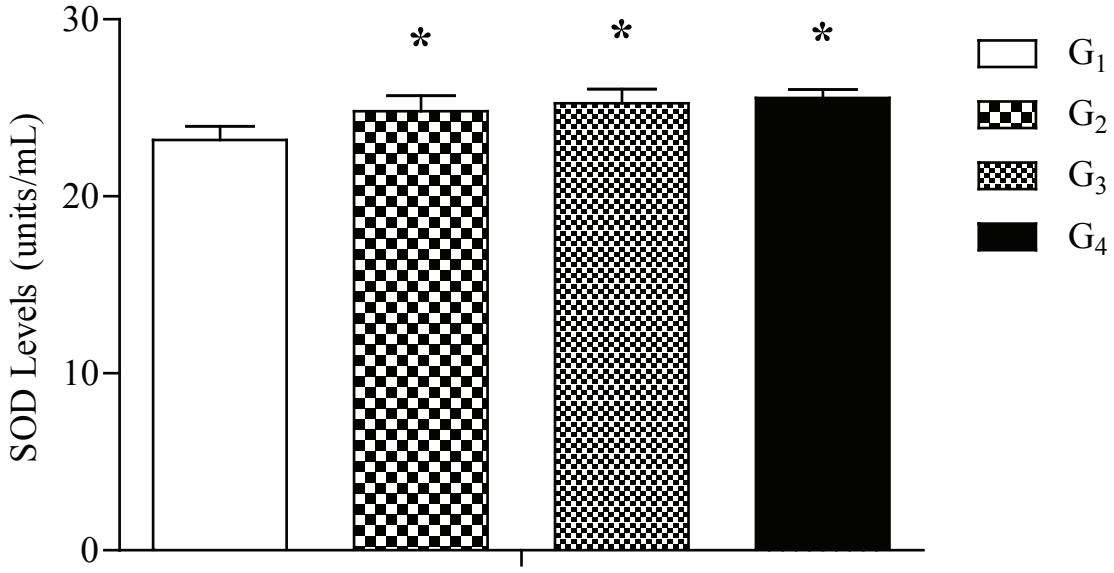

Fig. 2 The Average of SOD in Each Group. G_1 (control without submaximal physical activity + placebo), G_2 (submaximal physical activity + placebo), G_3 (submaximal physical activity + AA 500 mg), G_4 (submaximal physical activity + AA 1.000 mg). The data are shown as the mean (SD). Statistically significant differences are denoted as p<0.05 vs. control group (G_1).

Based on Figure 2 proved that the mean levels of SOD in each group experienced a significant difference (p<0.05). The results of the LSD post hoc test exhibited that there was a significant difference in the mean levels of SOD between G_2 and G_1 (Sig. 0.000), G_3 and G_1 (Sig. 0.000), G_4 and G_1 (Sig. 0.000), while G_2 and G_3 did not show a significant difference. Significant (Sig. 0.257), as well as G_2 with G_4 (Sig. 0.068) and G_3 with G_4 (Sig. 0.460).

Discussion

The results showed that the average levels of malondialdehyde (MDA) in G_2 (338.42±125.78 ng/mL) were higher than that of G_1 (308.18±61.81 ng/mL), G_3 (290.54±69.18 ng/mL), and G_4 (279.83±39.10 ng/mL). One-way-ANOVA test results revealed that there was no significant difference in the mean MDA levels in each group (p>0.05). These results are in line with the results of research conducted by Souissi *et al*.[18] concluded that there was an increase in MDA levels in athletes after carrying out physical activity in the form of running. However, these results differ from the results of a study conducted by Azizbeigi *et al*.[19] concluded that there was a significant decrease in malondialdehyde levels after the resistance training intervention for 8 weeks. This difference was probably due to the previous study providing chronic intervention (8 weeks), whereas in our study it only provided one intervention (acute) of submaximal physical activity so that the body's physiological adaptation process had not yet occurred. Furthermore, physical activity completed with high intensity and was competitive in nature had the potential to trigger an imbalance between the production of free radicals and antioxidants in the body, which could increase the occurrence of oxidative stress.[8,9] Oxidative stress can be identified by looking at changes in levels of malondialdehyde (MDA).[20] The reaction between ROS and polyunsaturated fatty acids (on the cell wall) will result in the formation of aldehydes, such as MDA, through the lipid peroxidation process. Some studies have shown that MDA is a stable and accurate measurement component of lipid peroxidation and has helped explain the role of oxidative stress in many diseases.[21]

On the other hand, increased production of ROS after submaximal physical activity can oxidize various molecules which will cause damage to lipids, proteins, and deoxyribonucleic acid (DNA).[22] If an atom/molecule contains one or more unpaired electrons and can stand alone, it is called a free radical.[23] The absorption of oxygen is higher during submaximal physical activity than at rest, due to the increased energy demand in many body tissues which will have an impact on increased free radical production during respiration in the mitochondria.[24] Submaximal physical activity can also increase the occurrence of lipid peroxidation and cause significant muscle damage.[25] This can have an impact on increasing levels of MDA in the blood, as a marker of lipid peroxidation caused by oxidative stress.[26] Lipid peroxidation causes damage to cell membranes.[27] Therefore, this marker can also be an indirect marker of oxidative stress.[28] Besides, the metabolic changes that occur during submaximal physical activity are caused by the release of catecholamines, this may play an important role in the increase in ROS in the body.[29] Increased oxygen consumption, release of catecholamines, excess lactic acid, the activity of enzymes such as xanthine oxidase, and free radical formation by mitochondria are sources that can increase the production of ROS after submaximal physical activity.[30]

The results proved that the average MDA level in G_4 (279.83±39.10 ng/mL) was lower than that of G_1 (308.18±61.81 ng/mL), G_2 (338.42±125.78 ng/mL), and G_3 (290.54±69.18 ng/mL). One-way-ANOVA test results revealed that there was no significant difference in the mean MDA levels in each group (p>0.05). Our results proved that there was a decrease in MDA levels in G_4 (submaximal physical activity + AA 1.000 mg) although not significant compared to G_1 (control without submaximal physical activity + placebo), G_2 (submaximal physical activity + placebo), and G_3 (submaximal physical activity + AA 500 mg). These results were almost the same as previous studies conducted by Aryanugraha *et al*.[31] concluded that the combination of Vit. C 500 mg and Vit. E 200 IU for 5 days in diving athletes after physical activity intervention showed no significant difference in malondialdehyde levels, but there was a tendency to decrease MDA levels. Likewise, the research results of Popovic *et al*.[32] reported that Vit. C significantly decreased serum MDA levels in the regular exercise group and the acute

exercise group. The decrease in MDA levels in this study was probably due to the influence of exogenous antioxidant levels obtained from drinks containing 1000 mg AA. Endogenous antioxidants can increase the destructive effect of oxidants in cells so that there is no increase in oxidative stress which is marked by a decrease in MDA production.[33] In addition, during the early 1980s, Lester Packer's laboratory investigated the role of antioxidant nutrients in the protection of cells and organelles from free radical-mediated oxidative damage.[34] The results of the study reported that ascorbic acid supplementation (1 gram) can inhibit the increase in free radical production and prevent lipid peroxidation and the formation of MDA.[35] Vit. C works as an electron donor, donates electrons in intracellular and extracellular biochemical reactions, and can remove reactive oxygen species in neutrophil cells, monocytes, lens proteins, and the retina.[31] Therefore, consume Vit. C can be used to reduce oxidative stress after giving physical activity.[36]

Based on the results of the study showed that the average levels of superoxide dismutase (SOD) in G_4 (25.57±0.47 units/mL) were higher than G_1 (23.19±0.77 units/mL), G_2 (24.81±0.87 units/mL), and G_3 (25.27±0.79 units/mL). One way-ANOVA test results found that the mean levels of SOD in each group experienced a significant difference (p<0.05). The results of the LSD post hoc test showed that there was a significant difference in the mean levels of SOD between G_2 with G_1 (Sig. 0.000), G_3 with G_1 (Sig. 0.000), G_4 with G_1 (Sig. 0.000), while G_2 with G_3 did not show a significant difference significant (Sig. 0.257), as well as G_2 with G_4 (Sig. 0.068) and G_3 with G_4 (Sig. 0.460). This result is in line with the results of research conducted by Yimcharoen *et al*.[37] reported that giving ascorbic acid supplementation at a dose of 1000 mg to healthy women after 30 minutes of ergocycle physical activity increased SOD levels compared to the control group. Ascorbic acid is an antioxidant that can neutralize oxidative stress through the donation/ electron transfer process.[38] Ascorbic acid with the right dose can reduce and inhibit ROS. Furthermore, studies with human plasma have shown that ascorbic acid is effective in preventing lipid peroxidation caused by the accumulation of ROS.[39] Ascorbic acid acts by donating electrons to prevent other compounds from being oxidized and scavenging superoxide anions, hydroxyl radicals, and lipid hydroperoxides.[32] Ascorbic acid supplementation as an exogenous antioxidant can reduce free radicals, so it can inhibit lipid peroxidation and prevent cell damage.[37] Ascorbic acid supplementation can significantly reduce serum MDA levels and suppress lipid peroxidation, so this confirms that ascorbic acid has an antioxidant capacity to prevent oxidative stress after physical activity.[32]

Physical activity has been shown to increase skeletal muscle contraction which in turn can increase the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), thereby promoting increased oxidative stress in skeletal muscle cells.[40] Ascorbic acid is an organic compound that has a role as a catalyst in metabolic processes which is usually in the form of coenzymes.[41] Ascorbic acid can be obtained from food.[42] Ascorbic acid is responsible for scavenging free radicals. Ascorbic acid is involved in several biochemical metabolic pathways that are important during physical activity. Based on the results of our study, it was shown that the consumption of ascorbic acid 1000 mg immediately after submaximal physical activity could increase the endogenous antioxidant power as evidenced by an increase in SOD levels and a decrease in MDA levels. During physical activity, glucose uptake by muscle work increases 7 to 20 times the basal level.[37] Increased glucose metabolism is associated with increased production of ROS. ROS is produced when glucose levels are high or low. High glucose levels stimulate the production of ROS through activation of Nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase.[43] Ascorbic acid is a water-soluble vitamin found in the body. Ascorbic acid used orally will be absorbed from the digestive tract into the systemic circulation. The bioavailability of ascorbic acid in the digestive tract is in the form of ascorbic acid and dehydroascorbate acid.[44] Dehydroascorbate transport occurs using facilitated diffusion, dehydroascorbate is reduced to ascorbic acid as soon as it enters the cell membrane.[45] This dehydroascorbate is transported by facilitated diffusion using four of the 14 types of glucose transporter, namely Glucose transporter 1 (GLUT 1), Glucose transporter 2 (GLUT 2), Glucose transporter 3 (GLUT 3), and Glucose transporter 4 (GLUT 4).[46]

Glucose transporter (GLUT) is competitively inhibited by glucose. When there is excess glucose in plasma, the intestine will reduce the dehydroascorbate transport facilitated by GLUT.[47] Distribution and transportation vary between different Glucose Transporters (GLUTs). Thus, GLUT 1 is expressed in various cells throughout the body.[48] GLUT2 is mainly expressed in the liver, spleen, and basolateral membrane of intestinal and renal epithelial cells.[46] GLUT3 is found mainly in the brain and neurons and GLUT4 in skeletal and cardiac muscle cells, as well as in adipose tissue.[48] After absorption through the intestinal epithelium, ascorbic acid is released into the bloodstream.[49] Here, ascorbic acid is easily oxidized and the dehydroascorbate which is produced rapidly enters via the GLUT1 transporter in erythrocytes.[50] The mechanism of dehydroascorbate being reduced to ascorbic acid is a continuous process in the cellular cytoplasm, which is responsible for extinguishing free radicals, such as superoxide anions.[51]

Conclusion

It can be concluded that 500 mg and 1.000 mg ascorbic acid drinks after submaximal physical activity cannot reduce MDA levels, conversely, it can increase SOD levels of students regiment in East Java. The ascorbic acid drink may maintain the SOD level by lessening the use of antioxidant endogen and it may also increase the SOD level, however, further research is needed to be conducted to figure out the mechanisms.

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