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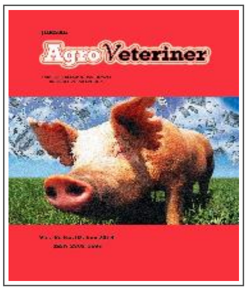

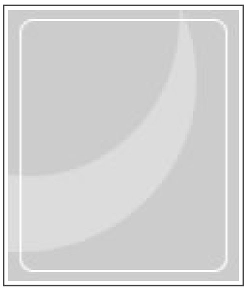

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EFFECT OF COMBINED 500 mg VITAMIN C AND 200 IU VITAMIN E ON PLASMA MALONDIALDEHYDE LEVEL AFTER PHYSICAL EXERCISE IN DIVING ATHLETES

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ABSTRAK

*Setiap hari kita terpapar oksidan. Oksidan yang berlebihan dapat menyebabkan berbagai penyakit dalam tubuh manusia. Oksidan mungkin muncul sebagai bagian dari aktivitas metabolik biasa, gaya hidup, diet, paparan eksogen, dan aktivitas fisik. Atlet, termasuk atlet diving, adalah kelompok yang memiliki faktor risiko stres oksidatif karena latihan fisik dengan intensitas tinggi. Vitamin C dan vitamin E merupakan bagian dari sistem pertahanan tubuh terhadap oksidan. Namun, kombinasi dosis 500 mg vitamin C dan 200 IU vitamin E dalam atlet diving masih belum jelas. Tiga belas atlet diving digunakan sebagai subjek penelitian dibagi menjadi dua kelompok. Kelompok A diberi kombinasi 500 mg vitamin C dan 200 IU vitamin E selama lima hari dan kelompok B diberi plasebo. Pada hari keenam, pra-latihan plasma MDA diambil pada menit ke-0, maka setiap atlet diberi larutan glukosa mg 100 air + 200 cc. Kami mulai menghitung menit sejak asupan glukosa. Kemudian, para atlet yang menjalani latihan fisik dengan menyelam. Pada menit ke-30 dan 60 menit setelah asupan glukosa kami mengambil post-prandial MDA plasma. Hasil analisis data dengan menggunakan paired T menunjukkan perbedaan yang signifikan dalam latihan plasma kadar MDA dengan plasma MDA tingkat 30 'PP di grup A dan B (p <0,05). Perbedaan tidak signifikan (p> 0,05) diperoleh dari analisis plasma MDA 30 'PP dengan plasma MDA dan MDA plasma 60' PP pra-latihan dengan plasma MDA 60 'PP. Uji t tidak berpasangan antara kelompok A dan kelompok B menunjukkan tidak ada perbedaan yang signifikan dalam plasma kadar MDA dalam waktu pengambilan (p> 0,05). Dari hasil tersebut, dapat disimpulkan bahwa kombinasi dosis 500 mg vitamin C dan 200 IU vitamin E tidak mempengaruhi kadar MDA plasma pada atlet, tapi ada kecenderungan sedikit meningkat berarti MDA. Hal ini bisa disebabkan oleh efek antioksidan eksogen dalam mengurangi radikal bebas. **(FMI 2012;48:156-162)***

Kata kunci: *antioksidan, vitamin C, vitamin E, MDA*

ABSTRACT

*Every day we are exposed to oxidants. Excessive oxidants may cause various diseases in human body. Oxidants may appear as part of a regular metabolic activity, lifestyle, diet, exogenous exposure, and physical activity. Athletes, including diving athletes, are group that have a risk factor of oxidative stress because of high-intensity physical exercise. Vitamin C and vitamin E are part of the body's defense system against oxidants. However, combination dose of 500 mg of vitamin C and 200 IU of vitamin E in the diving athletes is still not clear. Thirteen diving athletes used as research subjects were divided into two groups. Group A was given a combination of 500 mg of vitamin C and 200 IU of vitamin E for five days and group B was given placebo. On the sixth day, pre-exercise plasma MDA was taken in minute 0, then each athlete was given 100 mg glucose solution + 200 cc water. We started counting minutes since the intake of glucose. Then, the athletes were undergoing physical exercise by diving. In the 30th minute and 60 minutes after glucose intake we took post-prandial plasma MDA. Results of data analysis using paired T test showed significant difference in exercise plasma MDA levels with plasma MDA levels 30' PP in group A and B (p < 0,05). Not-significant differences (p > 0.05) was obtained from the analysis of plasma MDA 30' PP with plasma MDA and MDA plasma 60' PP pre-exercise with plasma MDA 60' PP. Unpaired t test between group A and group B showed no significant difference in plasma MDA levels in any retrieval time (p > 0,05). From these results, it can be concluded that the combination dose of 500 mg vitamin C and 200 IU of vitamin E does not affect plasma MDA levels in athletes, but there is a trend of slightly increase in mean MDA. This could be due to the effects of exogenous antioxidants in reducing free radicals. **(FMI 2012;48:156-162)***

Keywords: *antioxidants, vitamin C, vitamin E, MDA*

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INTRODUCTION

Every day we are exposed to oxidants. Oxidant is an oxidizing compound that accepts electrons (Takeuchi 2006). The human body to consume approximately 250 grams (175 liters) per day of oxygen and 3-5% of the amount is also converted into oxidants. Excess oxidant can cause various diseases in the human body (Danusanto 2003, Murray et al 2006).

Oxidants can appear as part of the regular metabolic activity, lifestyle, diet, exposure to exogenous, and physical activity (Miharja 2005). Athletes, including athletes dive, a group who have risk factors for oxidative stress occurs due to high-intensity physical exercise. During maximal exercise, whole body oxygen consumption increased 20 times, while the oxygen consumption of the muscle fibers in the estimate increased 100-fold. The use of this excess oxygen causes the release of electrons from the mitochondrial superoxide to form oxidants. Relative hypoxia while in a network of several organs that are not as active as the kidneys, liver and intestines to compensate the increase in the supply of blood to active muscles and skin can also cause oxidant (Cooper 1994). One example is the oxidation reaction peroxidation reaction (auto-oxidation) of lipids (Murray et al 2006). The reaction was initiated by a compound oxidants (free radicals) that are formed radical fat (L) which after going through some process is formed Malondialdehyde (MDA), 9-hydroxy-nonenal, ethane (C2H6) and pentane (C5H12) (de Zwart et al 1999). MDA is used as a reference that determines how many oxidants are formed in the body (Hendromartono 2000). If the oxidant production exceeds the antioxidant, oxidative stress will occur (Taha 2011). Free radicals are normal and continue over time (Tjay & Rahardja 2002). Our body is able to ward off free radicals by making endogenous antioxidants to counteract free radicals in the body. However, the endogenous antioxidant, is still too often perceived.

Vitamin C or L-ascorbic acid is a non-enzymatic antioxidant that is soluble in water. While vitamin E is fat soluble. These compounds are part of the body's defense system against reactive oxygen species in the plasma and cells as a reductant (Zakaria, 1996, Foyer 1993). In previous studies, Bryant et al (2003) tested 1 gram of vitamin C and 400 IU of vitamin E either separately or in combination to lactic acid and levels of malonaldehyde (MDA) cyclist. The result is a separate vitamin E is more effective than vitamin C and vitamin C and E as well. And 1 gram of vitamin C could increase the plasma levels of MDA. However, testing for dose combination of 500 mg of vitamin C and 200 IU of vitamin E in submarine athletes is still unclear.

From the above, it is known that high-intensity exercise can increase free radicals. Antioxidants, vitamins C and E combination, is necessary to prevent unintended consequences. With this research is expected to know the influence of a combination of vitamins C and E on plasma MDA levels were formed in athletes dive diving activities. This study aims to determine the effect of the combination of 500 mg of vitamin C and 200 IU of vitamin E dose on plasma MDA levels in athletes dive diving activities.

MATERIALS AND METHODS

This type of research is experimental to determine the effect of vitamin C 500 mg and vitamin E 200 IU on fasting plasma levels of MDA, 30 minutes postprandial (PP) and 60 minutes of PP. This study used a cross-sectional study design. This study observed risk factors (independent variables) with effect (dependent variable) by measuring levels serially the MDA plasma moment.

The study population was diving athlete Surabaya. Samples were taken from population studies that met the inclusion criteria, namely Surabaya diving athletes over 18 years old and healthy. While the exclusion criteria are non-athletes and athletes submarine Surabaya less than 18 years old and unhealthy. In experimental studies of the samples were taken by 15 people (Gay & Diehl 1996). To reduce the possibility of sample drop out in the middle of the necessary corrections to the study of the sample, so the sample size is 16. The sampling technique in this research is done by purposive sampling. The sample was selected through a conditional selection process conducted by the researcher.

Data were taken experimentally. Group A as the group treated and group B as a control group. Documenting age, sex, and weight carried during the briefing athletes. The first day until the fifth day of a group taking 500 mg of vitamin C supplements and E 200 IU after dinner every day and were asked not to eat foods other than normal. Whereas group B taking placebo. On the sixth day group A and B were collected. After data retrieval weight and age of the athlete, done taking fasting plasma MDA minute 0, then at each athlete is given a solution of glucose intake of 100 mg + 200 cc of water. Calculation of minutes was started since the intake of glucose. Then, the athletes carried out physical exercise that is diving simultaneously with the recorded time with a stopwatch. In the 30th minute and 60 after the intake of glucose taken post-prandial plasma MDA.

Blood sampling performed intravenously on dorsum. Blood was drawn as many as 2 cc. Blood that has been taken is inserted into the tube without anticoagulant allowed to stand for 30 minutes at room temperature and then in a centrifuge at 2000 rpm for 5 minutes to separate serum from the blood. Serum obtained is used to determine plasma levels of MDA. Measurement of plasma MDA levels is done through tests thiobarbituric acid-reactive substance (TBARS). Basic examination is simple spectrophotometric reaction, in which one molecule of MDA will be split into two molecules of 2-thiobarbituric acid. This reaction is run at pH 2-3. TBA will give pink-chromogen which can be checked spectrophotometrically. TBA test in addition to measuring the levels of MDA formed by the process of lipid peroxidation aldehydes also measure non-volatile products resulting from the heat generated at the time of measurement of actual levels of serum MDA (Reilly et al, 1991, König & Berg 2002).

The collected data will be inserted into the table and then analyzed using SPSS software. The data were then analyzed using univariate analysis. Univariate analysis was performed descriptive statistics for each variable of the research include plasma MDA levels, antioxidants are vitamin C 500 mg and E 200 IU, age, sex and weight. Each data will be determined measures of central tendency and standard deviation (SD), if the data has a normal distribution, then the measures of central tendency are the mean and the size of the deployment is elementary. If the data has a normal distribution, then the measure of central tendency is the median and the size of the deployment is the minimum-maximum values (Dahlan 2011).

To look for differences between the mean levels of insulin in the blood antioxidant content of vitamin C 500 mg and E 200 IU used unpaired t test. Unpaired t test is performed if the data has a normal distribution. To determine whether or not to use the normal distribution Kolmogorow-Smirnov normality test, the test value of p> 0.05 states whereas if the data were normally distributed data is not normal distribution of data transformation, if it still shows a normal distribution can using Mann-Whitney. Neither the unpaired t test nor Mann Whitney if the value of p <0.05 compared to the data distribution has a significant mean difference between the two groups (Dahlan 2011). Figure 1 shows the distribution of the sex of the study sample.

RESULTS

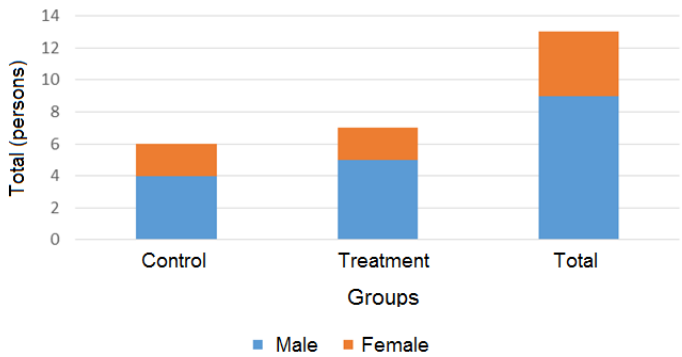

Figure 1. Distribution of Gender

Table 1. Variables of Age, Weight, and MDA

Variables	n_k	Control	n_p	Treatment
Age	6	15.833± 0.753 years	7	16.143± 1.464 years
Bodyweight	6	60.833± 5.456 kg	7	59.143± 7.221 kg
MDA Pre-exercise	6	16.855 ± 3.211 nmol/dL	7	16.529 ± 2.103 nmol/dL
MDA 30’ PP	6	8.774 ± 3.644 nmol/dL	5	12.035 ± 1.972 nmol/dL
MDA 60’ PP	6	14.574 ± 7.660 nmol/dL	7	16.733 ± 6.066 nmol/dL

Of the total of 13 samples, nine were male. Male gender also dominates the sample in the control group and the treatment group. Table 1 shows the mean ± SD age, weight, and MDA in the control and treatment groups, nk is the number of samples of the control group. np is the number of samples treated groups. The variables measured in this study consisted of age, weight, pre-exercise MDA, MDA 30 'PP, and MDA 60'PP. The measurement results are presented in Table 1.

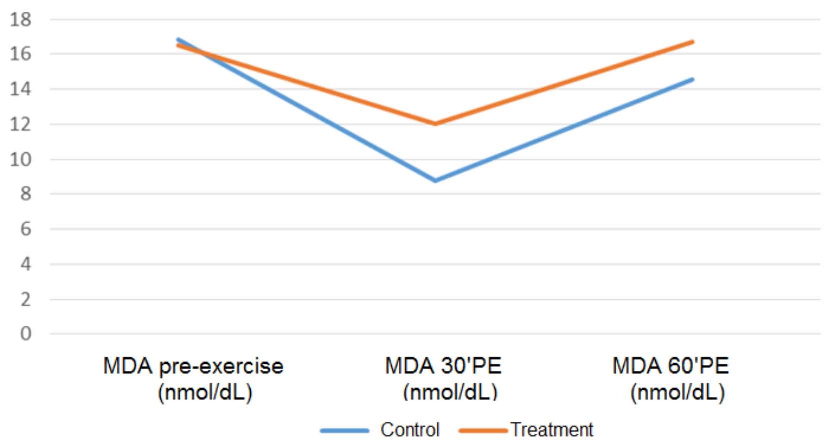

Figure 2. Graph Mean Plasma MDA

Plasma MDA levels were measured from blood drawn in three stages: before exercise, thirty minutes after glucose administration. and sixty minutes after administration of glucose. Figure 2 shows the mean MDA series in the control group and the treatment group. Data were tested for normality using the Shapiro-Wilk because of the small sample size. Value Shapiro Wilk test for normal distribution of the variables age, weight, pre-exercise MDA, MDA 30'PP, and MDA 60'PP more than 0.05 (p> 0.05), the conclusion is the distribution of normal data for all variables.

Table 2. Normality Test Variables

Variables	Groups	Shapiro-Wilk P
Age	Control	0.212
	Treatment	0.269
Bodyweight	Control	0.69
	Treatment	0.904
MDA Pre-exercise	Control	0.226
	Treatment	0.310
MDA 30’ PP	Control	0.491
	Treatment	0.987
MDA 60’ PP	Control	0.323
	Treatment	0.404

In the paired T test MDA MDA pre-exercise to 30'PP obtained significance value of 0.005 T test (p <0.05), it can be concluded there are differences between the mean scores were significantly between MDA MDA pre-exercise and MDA 30'PP. While the paired T test MDA MDA pre-exercise to 30'PP and MDA MDA pre-exercise to 60'PP obtained significant value T test row by 0.072 and 0.563 (p <0.05), it can be concluded there is no difference mean score of MDA were significantly between these variables.

Table 3. Paired T Test Control Group

Variables	N	Mean
MDA Pre-exercise	6	16.855 ± 3.211 [a]
MDA 30’ PP	6	12.035 ± 3.644 [b]
MDA 60’ PP	6	14.574 ± 7.660 [ab]

Specification:
a, b, ab: significant difference when meeting with different alphabets
n: number of samples

Table 4. Paired T Test Treatment Group

Variables	N	Mean
MDA Pre-exercise	7	16.529 ± 2.103[a]
MDA 30’ PP	5	8.774 ± 1.972 [b]
MDA 60’ PP	7	16.733 ± 6.066[ab]

Specification:
a, b, ab: significant difference when meeting with different alphabets
n: number of samples

In the paired T test MDA MDA pre-exercise to 30'PP obtained significance value of 0.044 T test (p <0.05), it can be concluded there are differences between the mean scores were significantly between MDA MDA pre-exercise and MDA 30'PP. While the paired T test MDA MDA pre-exercise to 30'PP and MDA MDA pre-exercise to 60'PP obtained significant value T test row by 0.149 and 0.920 (p <0.05), it can be concluded there is no difference mean score of MDA were significantly between these variables.

Table 5. unpaired t test in the control group to the treatment group

Variables	n_k	Mean Control	n_p	Mean Treatment	p
MDA Pre-exercise	6	16.855 ± 3.211	7	16.529 ± 2.103	0.830
MDA 30' PP	6	12.035 ± 3.644	5	8.774 ± 1.971	0.108
MDA 60' PP	6	14.574 ± 7.660	7	16.733 ± 6.066	0.582

Specification:
nk: number of samples of the control group
np: number of samples of the treatment group

Unpaired t test was conducted to determine whether there were significant differences between the two groups. This test compares the control group and the group treated with the same variable. Significance value unpaired t test on all three variables is smaller than 0.05 ($p > 0.05$) it can be concluded there is no significant difference between the mean MDA control and treatment groups in three variables.

DISCUSSION

This research was carried out by observing the effect of the combination of vitamin C 500 mg and vitamin E 200 IU against malondialdehyde levels (MDA) plasma due to physical exercise in athletes dive. Physical exercise can result in free radicals which can be determined with increased lipid peroxidation parameters, namely MDA. Decreased levels of plasma MDA is used for the protective effects of antioxidant vitamin C and vitamin E against free radicals caused by physical exercise.

In the control group, the subjects were given a placebo, showed pre-exercise plasma MDA levels by an average of 16.855 ± 3.211 nmol/dL. Furthermore, subjects consuming glucose and dive as far as 100 m. Results of plasma MDA levels on average thirty minutes post-prandial of 8.774 ± 3.644 nmol/dL. With a paired t test found a significant difference between plasma MDA and MDA pre-exercise thirty minutes post-prandial. These results indicate that the decrease of MDA in blood plasma in an interval of 30 minutes post-prandial. The formation of free radicals generated primarily by the contraction of skeletal muscle (Powers & Jackson 2008). MDA is one result of free radicals. Furthermore, the MDA will be in circulation and measured levels. MDA decreased indicating the possible use of MDA for the body to then be processed into energy. MDA metabolic pathways involving oxidation by Aldehide dehydrogenase in mitochondria and decarboxylation produce carbon dioxide and acetate (Siu & Draper 1982). Acetate containing an acetyl group which can bind with coenzyme A to acetyl-CoA. This enzyme is useful to metabolize carbohydrates that provide energy for physical activity. In addition, glucose consumption conducted pre-exercise athletes also affect the plasma MDA. Increased levels of glucose can cause lipid peroxidation (Jain et al 1996).

Measurement of plasma MDA average after sixty minutes post-prandial is 14.574 ± 7.660 nmol/dL. Paired t test found a significant difference between the levels of plasma MDA thirty minutes post-prandial plasma MDA levels sixty minutes post-prandial. These results indicate that an increase in plasma MDA levels were not significant. MDA is the previous muscle has been in circulation. Resulting in accumulation and increased levels of plasma MDA, although not significantly. Increased plasma MDA can be influenced by endogenous antioxidant activity of subjects. The endogenous antioxidants can improve the destructive effects of oxidants in the cell so that no significant increase (Sen et al 2000).

In the treatment group, the subjects were given a combination of vitamin C 500 mg and vitamin E 200 IU, showed pre-exercise plasma MDA levels by an average of 16.529 ± 2.103 nmol/dL. Furthermore, subjects perform the same procedure as the control group. Results of plasma MDA levels thirty minutes by an average of 12.035 ± 1.972 nmol/dL. With a paired t test found a significant difference between plasma MDA and MDA pre-exercise thirty minutes post-prandial. These results indicate that the decrease of MDA in blood plasma in an interval of 30 minutes post-prandial. This result is not much different from the control group. Vitamin C works as an electron donor, donating electrons to the intracellular and extracellular biochemical reactions, and is able to eliminate the reactive oxygen species in cells neutrophils, monocytes, lens proteins, and the retina. Vitamin E serves to break the chains of fatty peroxide to donate hydrogen ions in the reaction, so it can lower blood fat levels peroxide.

Measurement of plasma MDA average after sixty minutes post-prandial is 16.733 ± 6.066 nmol/dL. Paired t test found a significant difference between the levels of plasma MDA thirty minutes post-prandial plasma MDA levels sixty minutes post-prandial. These results indicate that an increase in plasma MDA levels were not significant. MDA is released from the muscles are in circulation muted by endogenous antioxidants and exogenous antioxidants. This makes the increase in plasma MDA levels increased but not significantly. Unpaired t test was conducted to test whether there are differences in average levels of plasma MDA series between the control group and the treatment group. The result was no significant difference of the three variables, namely the average plasma MDA pre-exercise, plasma MDA average of thirty minutes post-prandial, and MDA Plasma average sixty minutes post-prandial between groups. This analysis shows that there are differences in average plasma MDA levels between groups that did not receive supplements of vitamins and supplements the group receiving 500 mg of vitamin C and vitamin E 200 IU. Vitamins C and E are antioxidants that are expected to dampen oxidative stress shown on plasma MDA levels. These findings refute the previous hypotheses. Similar results were also obtained in the study the effects of antioxidant supplements of vitamin C and vitamin E for oxidative stress and muscle injury (Bloomer et al 2007).

Factors that may affect the results of this study include the dose and type of antioxidant factors, lifestyle factors research subjects, and the timing of antioxidant factors. Dosage and type of antioxidants used in this study is a combination of 500 mg of vitamin C and vitamin E doses of 200 IU. From the results of this study found that there was no significant difference between MDA treatment and control groups. Whereas in previous studies, Bryant et al (2003) tested 1 gram of vitamin C and 400 IU of vitamin E either separately or in combination to lactic acid and MDA levels cyclist. The result is a separate vitamin E is more effective than vitamin C and vitamin C and E as well. And 1 gram of vitamin C could increase the plasma levels of MDA. Lifestyle subjects affecting plasma MDA levels, especially smoking and alcohol consumption. Daily active smokers had a mean plasma MDA concentration higher than non-smokers. The positive correlation was also found in the plasma MDA and weekly alcohol consumption (Nielsen et al 1997). Gender also affects the plasma MDA levels, but not for age or age and gender as well. In this study, administration of antioxidants conducted over five days. In previous studies, Ariyando (2011), given the antioxidant curcumin 45 mg capsule preparations for thirty days, which resulted in a significant increase SOD levels, lower levels of MDA, and increase the antioxidant status in the second trimester pregnant women.

CONCLUSION

The combination of 500 mg of vitamin C and 200 IU of vitamin E dose did not affect the plasma MDA levels in athletes, but there is a tendency of smaller increase in mean MDA. This may be due to the effects of exogenous antioxidants that can help reduce free radicals.

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