Study of Pre and Post Supplementation Green Tea Extracts to MDA Levels

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Abstract
High intensity exercise (HI) induces oxidative stress. Running for 2400 meters is one of the simple exercise form which can be counted as HI. During HI, increase of oxygen consumption happened in muscle cell resulted in an increase of oxidant level. The aim of the study was to measure the effects of Indonesian green tea supplementation, before and after HI to the level of lipid peroxidation (TBARS-Malonyldialdehyde (MDA)). The subjects of study were 42 male students from Faculty of Medicine, Universitas Padjadjaran (FMUP) and Faculty of Physical Exercise, Indonesia University of Education (FPOK-UPI). The subjects were divided into trained and untrained subject. Subjects were divided into groups using Astrand Harvard step test. The purpose of this test was measuring the level of VO2 max. Blood samples for MDA level were collected at 3 hours before and after high intensity aerobic exercise. Oxidative stress was induced by 2400 meters run. The result showed that green tea supplement-ation was effective to decrease MDA plasma level especially after exercise. Green tea could serve as natural antioxidant resources, which potentially can be used as potential sport supplemental drink.

Key words: Aerobic exercise, Malonyldialdehyde (MDA), pre and post exercise

Studi Pra dan Pasca Pemberian Suplemen Ekstrak Teh Hijau terhadap Kadar MDA

Abstrak
High intensity exercise (HI) dapat menginduksi stres oksidatif. Berlari sejauh 2400 meter merupakan salah satu bentuk latihan sederhana yang dapat digolongkan sebagai HI. Selama HI, konsumsi oksigen pada sel otot meningkat yang dapat menyebabkan peningkatan kadar oksidan. Penelitian ini bertujuan untuk mengukur efek dari suplemen teh hijau Indonesia sebelum dan setelah HI terhadap kadar lipid peroksida (TBARS-*Malonyldialdehyde* (MDA)). Subjek penelitian ini adalah 42 mahasiswa laki-laki dari Fakultas Kedokteran Universitas Padjadjaran dan Fakultas Pendidikan Olahraga dan Kesehatan, Universitas Pendidikan Indonesia. Subjek penelitian dibagi ke dalam kelompok terlatih dan tidak terlatih dengan menggunakan cara pengujian *Astrand Harvard.* Tes ini bertujuan untuk mengukur kadar *VO2 max*. Sampel darah untuk kadar MDA diambil 3 jam sebelum dan setelah *high intensity aerobic exercise*. Stres oksidatif diinduksi dengan berlari sejauh 2400 meter. Hasil penelitian menunjukkan bahwa suplemen teh hijau efektif dalam menurunkan kadar MDA dalam plasma khususnya setelah berlari. Teh hijau dapat menjadi sumber antioksidan alami yang berpotensi sebagai minuman suplemen olahraga.

Kata kunci: *Aerobic exercise*, *Malonyldialdehyde* (MDA), sebelum dan setelah berlari

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Introduction

Exercise improve cell capacity to neutralize the oxidant by increasing production of endogenous antioxidant. Powers *et al.,* have been reported that treadmill training increase antioxidant activity such as: peroxydase and superoxide gluthation dysmutase. Intensity of exercise is important factor to optimalize the purpose of exercise. High intensity aerobic exercise increases muscle strength and power via stimulates oxidative phosphorylation and generated more energy (ATP).[1] Exercise also upregulates function of mitochondria metabolic enzyme which is involved in oxidative phosphorylation and glucose metabolism in skeletal muscle.[2] Single exercise activity, high intensity aerobic exercise, generated energy through oxidative phosporylation in mitochondria. Unfortunenately, oxidative phosphorylation doesnot only ATP, it is also generates hazardous additional product, called the free radicals. An excess of free radicals will decrease exercise performance.[3,4]

Free radicals is additional side products which are generated during high intensity exercise.[5] The accumulation of free radicals could be alter exercise performance of athletes.[6,7] Free radicals oxydize lipids of cell membrane lipids and inactivates enzyme the cell membrane which decreased cell`s function.[3] Free radicals have unstable nature, it can bound fastly with the lipids in the membrane which would lead to disruption of fatty acid composition in cell membrane and also leakage of enzymes and chemotactic factors.[4] In short, free radicals will damage muscle cells and then reduce muscle performance. Human has special defense mechanisms to prevent cellular damage due to the emergence of free radicals. Naturally, cell's defense system produces endogenous antioxidant enzymes such as Super Oxide Dysmutase (SOD), catalase and gluthation peroxydase. Function of this endogenous antioxidants is protection for membrane cell from oxidation by free radicals. When production of free radicals were higher than endogenous antioxidant formation, excess of free radical will oxidize and damage cell membrane.[4,8,9]

Exogenous antioxidant may be required to resolve the free radicals excess. Exogenous antioxidants can be derived from variety of sources, one of widely known one is green tea. Green tea is a popular beverages drink, but it's effectiveness as exercise supplement is still need to be elaborated further. Henning *et al.* and Mao-Jung *et al.*, reported about ingestion and bioavaibility of green tea, which gave us information to predict best time to give supplementation.[10,11] Recently, people's know more about green tea benefit as antioxidant. Green tea has many important substances such as flavonoids, tea flavins and teanine.[12,13] Among all substances, flavonoid has an important function such as neutralizing free radicals activity through several pathway. It acts as electron donor (giving electron), as metal chelating agent and as pro-oxidant enzyme inhibition.[4,12,14] Green tea active compound is called polyphenol which has strong antioxidant effects. Therefore, green tea can be a good candidate of natural antioxidant for protecting cell from oxidative stress and also for improving exercise performance.[15,16]

Taken together, exogenous antioxidant may be required to optimize muscle performance during high endurance exercise. Natural antioxidant such as green tea is a good candidate to support demand of antioxidant to neutralize free radicals. The purpose of present study is to find the best timing to give green tea supplement to improve exercise performance.

Methods

Informed consent

Preparation of research: Prior to the study, the subjects were given an explanation of the purpose, objectives and procedures of the study and were asked for their willingness to join as

volunteer research subjects. All procedure was agreed by all participants and following ethical comitte of Faculty of Medicine, Universitas Padjadjaran, Sumedang, Indonesia.

Subject experiment

Amount of 42 male, 18–23 years old, at good health condition, agreed to all research procedure were subjects in this study. After screening by questionnaire and Astrand Harvard step test. Subjects which were students from Faculty of Medicine, Universitas Padjadjaran (FMUP) and Faculty of Physical Exercise, Indonesia University of Education (FPOK-UPI). The subjects were divided into trained and untrained subject. Subjects were divided into groups using were divided into 6 groups with 6 subjects each. For training criteria, subject must perform a routine exercise for 30–60 min per day and 3 to 5 times per week, in the past one year. Only subjects inside those criteria and had good VO2 max level were included into trained group experiment. During the quarantine and study period, all subjects were prohibited from taking any medicine, food or drink that may contain antioxidants.They were quarantined in the location from 48 hours before test. Based on Bompaand Tudor, run 2400 m was used as high intensity aerobic exercise. All subjects performed a maximum efforts on this 2400 m run.

Body Mass Index (BMI)

Criteria for BMI determination is adopted from World Health Organization criteria for BMI (2000). Category for BMI is divided into: underweight: <18.5, normal: 18.5–24.9, overweight/pre-obes: 25.0–29.9, Obes I: 30–34.9, obes II: 35–39.9, and obes III: ≥40.

Group

Subjects were divided into 6 groups; Group I: untrained received placebo; Group II: trained received placebo before HI; Group III: untrained received green tea after HI; Group IV: trained who received placebo; Group V: trained group received green tea before HI; Group VI: trained group received green tea after HI.

VO2 max test

We utilized Astrand Harvard step test to measureVO2 max levels of each subjects. We used specialized size chair for men (60x40x40 cm, length x wide x height). Heart rate were monitored every minutes as follows: 1 minutes before test, at min 1, 2, 3, 4 and 5 using polar watch. Results were converted using astrand table. This test was performed one week before quarantine time.

Exercise protocol

Initial blood sample was taken for measuring MDA level before exercise. Before running, subjects did warming up to reduce the occurrence of sports injuries. Blood sampling was done at 3 hours before and after running 2400 m. Groups II and V was given a cup green tea just before running, the nall subject run 2400 m with outrest. After finished run, subjects from group III and VI were given 2 minutes rest, then they drank a cup of green tea. Blood sample was examined to compare MDA levels prior and post of 2400 run/HI. Blood sample was stored into 1.5 ml eppendorf tube which contained EDTA powder to prevent blood coagulation, then sample was stored in -80ºC until use.

Green tea sample

We used green tea as antioxidant agent. Green tea extracts was kindly provided by RK Beverages Company. Every subject in supplemented group drank a cup of green tea in 400 ml water (water temperature for preparation was 70ºC and flavonoid dose is 150 mg of EGCG).

MDA levels analysis

MDA blood was measured by the following procedure. One ml of blood sample was put

into tube with anticoagulant. Test tube inverted 8–10 times to mix blood sample with anticoagulant completely, then put for 30 min in the ice. Samples were centrifuged at 4000 rpm for 10 min. Plasma was carefully taken and used for MDA levels test. Test tube filled with 700 µL of blood plasma were analyzed and mixed with 200 µL of sodium dodecylsulfates (SDS) solution, 50 µL of a solution of 5% Butylatedhydroxytoluene (BHT), 50µL of a solution of EDTA, 1,500 µL acetic glacial and 1500 µL thiobarbituric acid (TBA). The mixture was then heated in a water bath at 100ºC for 60 minutes, then it is removed from heat and cooled in ice bath and centrifuged at 2335 rpm for 10 min. MDA levels was measured based on absorbance of mixture (spectrophotometer at 532 nm). MDA experiment was performed in duplicate and the average value was converted to blood MDA concentration in µmolliter^{-1}.

Statistical analysis

Analysis of the research data is processed with SPSS V.13.0 for windows with significance level $p \leq 0.05$. Differences of means between the groups analyzed with unpaired t-test and followed by oneway ANOVA.

Results

Subjects experiment have similar characteristic and BMI was in normal range

The result performed a general health examination to all subjects. Subject with obese and underweight criteria were excluded from this experiment (Table 1). All subjects received the same diet for 48 hours before experiment and they are quarantined to regulate them to have same schedule activity. This situation was intended to reduce confounding factors might alter free radical content, antioxidant and waste product of MDA in plasma.

Subject with good VO2 max only was involved in the trained group

We performed two screening tests for VO2 max confirmation. Firstly, simple questionnaire was used to separate subjects into trained and untrained groups. Secondly, Astrand Harvard step test. There were 16 people who has VO2 max level in good category those subjects were subjected for trained group. Other 16 subject who has VO2 max levels average and poor were grouped into untrained group. A strand step test was used to divide subjects into trained and untrained group (open bar: good; black bar: average; horizontal line: poor; diagonal line: very poor). A strand-Harvard step test was performed to define and confirm level of VO2 max of every subject. Only subjects with a good levels of VO2 max were included into trained group experiment (Figure 1).

Running performance was improved by green tea supplementation

Running time was faster in untrained group which received green tea supplementation both

Table 1 Physical characteristics of the subjects

	Age	Weight (kg)	Height (cm)	BMI Untrained
Untrained	20±0.3774	62.09±2.352	1.68±0.105	21.81±0.594
Trained	20.33±0.2516	60.271±0.7046	1.71±0.095	20.61±0.2193

There were no significant differences between groups subject. All subjects were in good health condition.

before and after exercise compared to untrained group without supplementation. Subject who received green tea extract showed an improved running time around ~20% compared to group without supplementation. It is assumed improvement in running time maybe only suggestive effects of supplementation (Figure 2). However, to clarify the mechanism behind this improvement, we showed the measurement MDA level before and after running exercise.

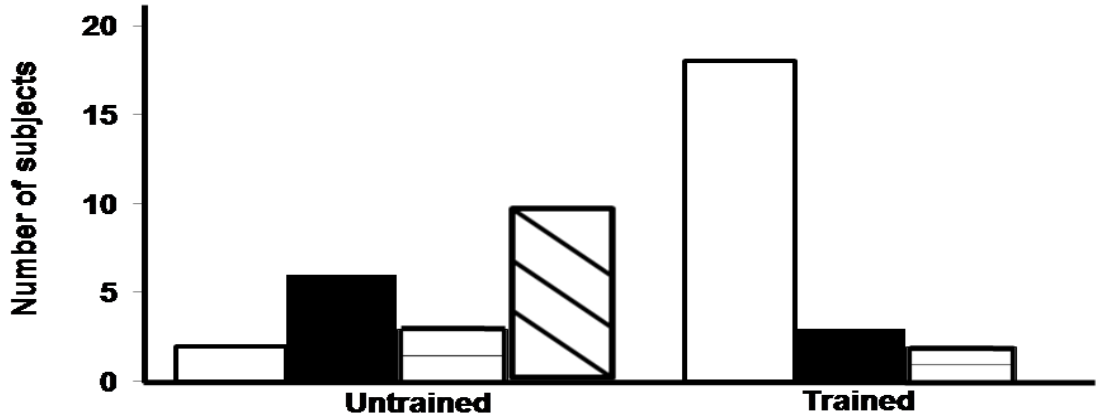
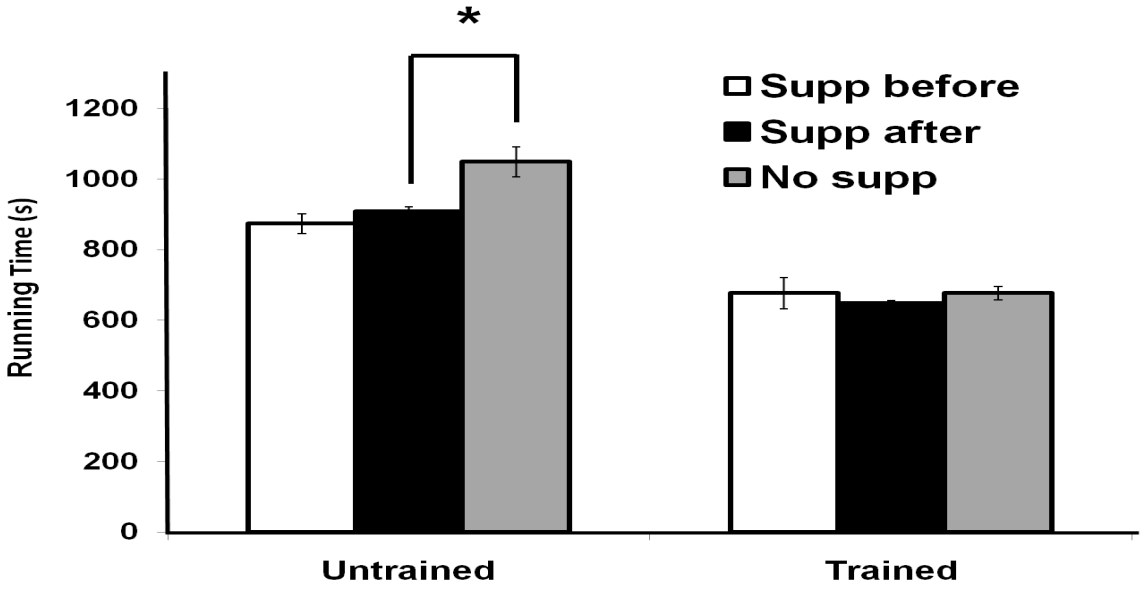


Figure 1 Distribution of subjects of VO2 max levels

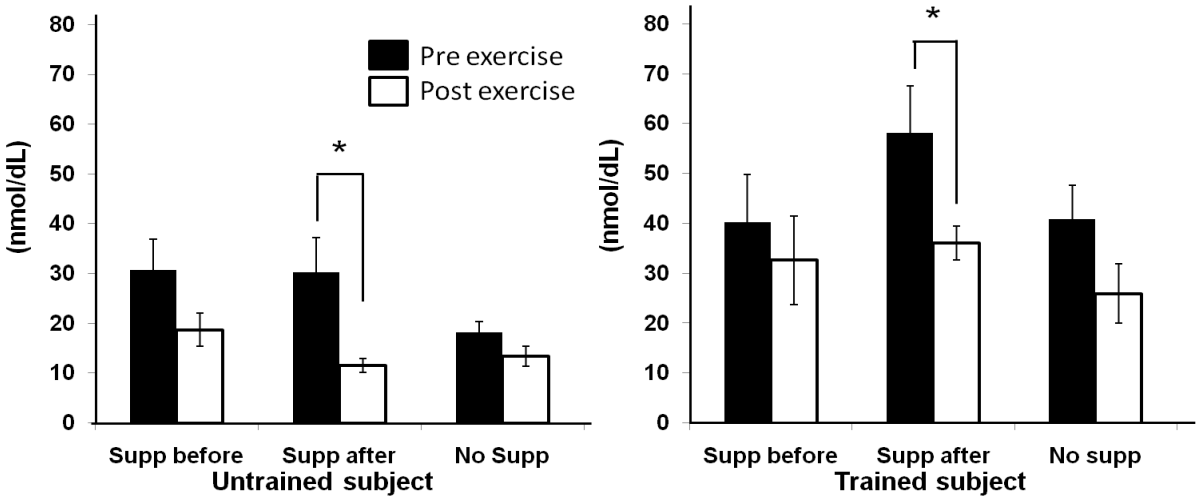


Time laps	Untrained	Trained
Supp before	872.85±27.6	676.28±13.1
Supp after	906.85±44.23	648.42±7.48
No supp	1048.42±42.4	675.85±19.3

Figure 2 Running time. The running performance was significantly improved in untrained group experiment after supplemented by green tea extract. *p<0.05, was analyzed by unpaired t-test, and considered as significant

Green tea ingestion reduces MDA level

We observed lower MDA levels after green tea supplementation in untrained group. The MDA levels at untrained group which received green tea supplementation after exercise were decreased around 40%, compared to other groups. In the trained group, we found no significant difference in MDA levels before or after exercise compared to control group. In addition, we also found that baseline level of MDA in trained group was higher compared to untrained group (Figure 3).



Untrained	Pre exercise	Post exercise
Supp before	30.68± 6.2	18.7±3.3
Supp after	30.27±6.8	11.51±1.38
No supp	18.25±2.2	13.4±2.04
Trained	**Pre exercise**	**Post exercise**
Supp before	40.16±9.6	32.65±8.8
Supp after	58.14± 9.3	36.14±3.4
No supp	40.91± 6.7	25.9±5.9

Figure 3 Green tea significantly reduced MDA levels. Supplementation green tea after exercise reduced more of MDA level compared to group who received green tea supplemen tation before exercise. *p<0.05,was tested by one way ANOVA and considered as significant (n=6 people/group)

Discussion

This study compared the effect of pre or post supplementation of green tea extract to neutralize and counter negative effects of free radicals in trained and untrained groups after single high intensity exercise. All six group performed a single high intensity aerobic exercise which stimulate many free radical formation in cell. Distance 2400 meter is well known as high intensity aerobic exercise.[17] Oxidant mostly is generated as side product of electron transfers chain in Krebs Cycle in mitochondria. High production of antioxidant will decrease exercise performance, as simple parameter is our study, result of running time

showed that groups without supplementation had a longer running time (Figure 2). It is assumed this exercise performance alteration was caused by increasing MDA levels and also suggestive effect of green tea supplementation.

Interestingly, this study also observed that training increased baseline levels of MDA in the serum which is also consistent with previous report[18], therefore this is reasonable if MDA baseline level in trained group was higher compare to untrained group (Figure 3). Leewenburgh and Heinecke have been reported that adaptation to free radical in trained group may be better compared to untrained group.[19] MDA is the waste product of damage in membrane lipids which is caused by series of oxidation-reduction process. Direct measurement of reactive free radical level is very difficult because of free radical only has very short half-life (10^{-6}–10^{-12} s). Thus, indirect method to measure free radical via measuring level of oxidation damage products such as (MDA) is the best solution to quantify free radical activity.[20,21]

Moreover, MDA has been widely used as an indicator of oxidative stress.[4] High free radical content make a significant decrease in exercise performance. Moreover in high intensity exercise, free radical formation is higher compared to endogenous antioxidant formation. Under imbalance condition, exogenous antioxidant may have a role to help cell system to neutralize excess of exercise generated oxidant.

Green tea may improve athlete performance via increasing cell capacity to neutralize free radicals. Green tea can stimulate endurance capacity and lipid metabolism to generate more energy.[22] Green tea may stimulate endogenous antioxidant level such as: Glutathione Peroxydase (GPx) and Super Oxide Dysmutase (SOD). Furthermore, it may be compulsory to measure levels of GPx and SOD at before and after exercise. We also considered whether mitochondria have some roles in modification or adaptation in such situation.

Taken together, reduction of athlete performance caused by high intensity aerobic exercise can be minimalized by giving green tea supplement. A benefit could be obtained by giving green tea supplement in reducing level damage effect to tissues. Dose and timing of supplementation is important to get an optimal effect for improving exercise performance. Further experiment, it is also interesting to look into others potential natural antioxidant such as: red fruit or wine (revastrol) which were used to neutralize free radicals during exercise MDA levels.

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Conclusion

Green tea supplementation was effective to decrease MDA plasma level especially after exercise.

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