

ENZYMATIC ANTIOXIDANT PROTECTION IN PHYSICAL EXERCISE WITH ACTIVE AND PASSIVE INTERVAL

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ABSTRAK

Penelitian bertujuan untuk mengungkap respon latihan fisik dengan interval aktif dan pasif terhadap sistem proteksi antioksidan enzimatis. Sampel yang digunakan mahasiswa Jurusan Ilmu Keolahragaan, Universitas Negeri Malang, usia 19-21 tahun, Indeks Masa Tubuh (IMT) 19-23, tidak merokok, VO₂ maks 45-50 ml/kg BB. Pengambilan data dilakukan dengan cara orang coba mengayuh sepeda ergometer intensitas 80% dari Kapasitas Kerja Maksimal (KKM), selama 4 minutes, 4 kali ulangan, dengan interval istirahat aktif dan pasif. Interval istirahat aktif dilakukan dengan tetap mengayuh sepeda ergometer intensitas 40% dari KKM selama 4 minutes yang dilakukan 3 kali ulangan (kelompok I), sedangkan interval istirahat pasif dilakukan dengan cara orang coba tetap duduk diatas sadel sepeda ergometer selama 4 minutes yang dilakukan 3 kali ulangan (Kelompok II). Pemeriksaan SOD (superoxide dismutase), dari plasma darah, dilaksanakan sebelum latihan fisik dan 30 minutes setelah latihan fisik. Analisa data dilakukan dengan menggunakan uji beda anava signifikansi 5%. Berdasarkan hasil penelitian terdapat perbedaan yang bermakna kadar enzim SOD antara kelompok I dengan kelompok II, F 6.059 (p<0.05). Berdasarkan hasil penelitian dapat disimpulkan bahwa latihan fisik dengan interval aktif lebih banyak membutuhkan kadar enzim SOD untuk menetralkan ROS, dari pada latihan fisik interval pasif.

ABSTRACT

The research aims to uncover the response of physical exercise with intervals of active and passive protection against antioxidant enzymatic systems. The sample used student Sport Science Department, State University of Malang, aged 19-21 years, body mass index (BMI) 19-23, no smoking, VO₂ max 45-50 mL/kg. Data retrieval is done by people trying to pedal a bicycle ergometer intensity of 80% of Maximum Work Capacity (KKM), for 4 minutes, 4 replications, with active and passive rest interval. Active rest intervals performed by pedaling a bicycle ergometer fixed intensity of 40% of the KKM for 4 minutes at 3 times the replications (group I), whereas the passive rest interval is done by people trying to remain seated on the bicycle ergometer saddle for 4 minutes at 3 times the replications (Group II). Examination of SOD (superoxide dismutase), from blood plasma, carried out prior to physical exercise and 30 minutes after physical exercise. The data analysis performed using different test ANOVA 5% significance. Based on the results of the study there were significant differences between the SOD enzyme levels in group I with group II, F 6059 (p <0.05). Based on the research results can be concluded that physical exercise with intervals require more active enzyme levels of SOD to neutralize ROS, rather than passive physical exercise interval.

Keywords: physical exercise, internal, maximum working capacity, VO₂max, antioxidant, SOD

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INTRODUCTION

Physical exercise in recent decades has become fashionable and the "trend" for the people, both young, adults and parents (Gunjam 2009), as an effort to improve the health and physical fitness as well as prevent some chronic diseases (Araujode 2009). Be aware that physical exercise is not always always have a positive impact on health (Sjodin 1990), because physical exercise is also a risk (Rost 1993), both, physical exercise in maximum and submaximum intensity (Ronsen 2002) with intervals or continuously (Araujode 2009) which can cause problems in improving the health and physical fitness itself

(Boucard 1993). But this still seems to lack adequate attention.

During and after physical exercise changes the body's metabolism constantly (Kelkar 2008), which can cause stressors to the body (Mineto 2006). Stressors are not only physical stressors (Uchakin 2003), but also the psychological stressors, physiological and chemical (Hanzawa 2000). This can disrupt the body's homeostasis (Karcabey 2005), and potentially increase the reactive oxygen species (ROS) (Gunjan 2009), lipid peroxidation (Sverko 2005) and the imbalance between ROS generation with the ability of antioxidant systems

(Fearheller 2011). Although the increase in ROS is directly responded to the antioxidant enzymes, which is almost the same (Sureda 2005, Dekany 2008) during physical exercise possibility of ROS generation exceeds the antioxidant enzyme defense system capabilities (Aguilo 2005). According to Kelkar (2008) Physical exercise can increase oxygen demand of up to 10-20 times, and the active muscles can even be increased up to 100-200 times, whereas 4-5% of oxygen consumed will be free electrons in the respiratory chain and formed into one types of free radicals ie superoxide radicals (Wellman 2009). This is evidence that physical exercise is a stressor to the body chemicals that can result in increased tissue damage (Minetto 2006), increased enzyme creatine kinase (Sugiharto 2005), erythrocyte osmotic fragility (Sugiharto 2006) and leads to increased oxidative stress characterized by increased MDA (malodialdehydee) (Halliwell 1999, Sugiharto 2000, Fatouros 2004). Physical exercise is also a physical and psychological stressor, as evidenced by the intensity of physical exercise is exhausting increases endorphins and lowers cortisol secretion (Sugiharto 2008).

ROS generation occurs during and after physical training at rest interval lasts (Kelkar 2008). This increase is due to increased oxygen demand of tissue hypoxia, resulting in the fulfillment of re-oxygen (reperfusion) in the network (Sentiirk 2005). It also occurs due to the release lysolestin, due to chemical stress, during and after physical exercise (Levrout 2003). According Leelarugrayub, (2005) Physical exercise can be the secretion of adrenaline, decreased pH, lactate accumulation, acidity and increased metabolic stress and increased oxygen free radicals. During exercise also has the potential occurrence of ischemia, during ischemia adenylate kinase system is very active. With its active adenylate kinase, the ATP can be formed from a combination of two moles of ADP to ATP and AMP. AMP accumulation causes the build up hypoxanthin in skeletal muscle and plasma (Sjodin 1990). During a break hypoxanthin reshaped into AMP, but during physical exercise hypoxanthin converted to uric acid through the xanthin oxidase (Belviranli 2006). Kanthin oxidase is the enzyme superoxide free radical formation (Flaherty 1991), which can degrade the quality of health and physical fitness. When physical exercise enhances the formation of ROS, thus antioxidant enzymes SOD mainly used to neutralize ROS (Siirmen 2003), ROS are formed so that the higher the SOD is needed to neutralize (Sureda 2005).

Structuring a program of physical exercise, good and true, both from the aspect of energy metabolism, muscle performance system, cardiovascular system and the adaptation of anti-oxidants are very important to

improve the health, physical fitness and optimal performance. The program is very good interval training to improve performance and accelerate muscle recovery period, but based on the concept of free radicals can increase the possibility of hypoxia-reperfusion and resembles ischemia in the network, which can increase the formation of ROS. On that basis in this study to uncover the response of physical exercise with a rest interval of active and passive protection systems by using the enzymatic antioxidants SOD parameters measured from blood plasma, as performed by some previous researchers (Fadillioglu 2000, Fearheller Berzosa 2011 and 2011). According Sureda (2005) erythrocytes and blood plasma is a substance that is most vulnerable to ROS caused by physical exercise. The results of this study is expected to provide information on the healthful effects of physical exercise and increased achievement in optimum, as well as broaden the development of molecular-based sports science, which can be donated in building a healthy human resource and quality.

MATERIALS AND METHODS

This study aims to reveal the response of physical exercise with intervals of active and passive protection against anti-oxidant enzymatic system of erythrocytes in people instead of trying to trained athletes. This study is a research experiment in the Pre Test - Post Test Design Group, which consists of two treatment groups. A group of physical exercise with active rest interval and the second group of physical exercise with a passive rest interval. The sample in this study were students majoring in sports science are selected based on criteria of age 19-22 years, male, 19-23 BMI, not smoking, healthy, maximal oxygen capacity of 40-45 ml/kg/BW. Based on these criteria are then taken 16 people were further divided into two treatment groups at random. Physical exercise in this study is by pedaling a bicycle ergometer, the intensity of the KKM 80% for 4 minutes with 4 intervals of work, three intervals of rest. Rest intervals performed actively and passively. Active rest interval by performing light exercise pedaling a bicycle ergometer with intensity 40% of the KKM for 4 minutes, while passive rest interval, people try to remain seated on the bicycle ergometer for 4 minutes, and do not do physical activity.

Blood sampling was as much as 10-12 cc from cubital vein, performed before physical activity and 30 minutes after physical activity. Further testing is done free radical method to measure MDA with TBA (thiobarbituric acid) of Uchiyama & Mihara (1978) with units (nmol/g Hb), and testing of anti-oxidant enzymes SOD automatic measuring method of Wong's method

(1989) stated that the measurement results in units of U/mL. Testing anti-oxidants and free radicals in the laboratory of UB's Faculty of Medicine, Physiology Sciences Malang. Data analysis was performed using different test ANAVA with a significance level of 5%.

RESULTS

Based on the results of prior studies with SOD levels after exercise, the exercise group with the active interval showed a significant difference $t = 3370$ ($p < 0.05$), whereas in physical exercise with a passive rest interval there was no significant difference $t = 0.234$ ($p > 0.05$). Based on the results of different test ANAVA showed a significant difference between the levels of SOD in active physical exercise with intervals with intervals of passive physical exercise, 6059 ($p < 0.05$).

DISCUSSION

There is a significant difference in enzyme levels of SOD, 30 minutes after the interval of active physical training with physical exercise passive intervals ($p < 0.05$). Based on research results of SOD enzyme levels tended to decline in physical exercise with active rest intervals, compared with passive physical exercise intervals. The mean average before physical exercise in the exercise group with the active interval of 194.7165 U/mL, and after physical exercise 161.4566 U/mL, whereas physical exercise with a passive interval before exercise 161.3410 U/mL and after exercise 163.8384 U/mL, so it tends to increase, although not significant ($p > 0.05$).

Decrease in SOD enzyme levels showed that more ROS are formed at intervals of active physical exercise than physical exercise with a passive interval. The high ROS levels have consequences for the needs of the SOD enzyme. Due to the higher formation of ROS, the higher the levels of enzyme SOD needs to neutralize ROS. SOD is the first line of the body's defense system against ROS (Araujo 2009, Leelarugayub 2005) which is part of the protection system to prevent the reactivity of ROS (Belviranli 2006), and is very effective for protecting reactivity of cells from ROS (Dekany (2008). The enzyme SOD also an enzyme that serves as a catalyst in the dismutase process of hydrogen peroxide (Masuda 2006) and can convert superoxide anion into hydrogen peroxide and O_2 (Leelarugayub 2005). Because of that decreased levels of SOD enzyme in active physical exercise with intervals, can be understood as evidenced research results. There are significant differences between the MDA levels of active physical training with physical

exercise interval interval passive ($t = 7.799$) ($p < 0.05$). The average levels of MDA after exercise active interval 2.2974 nmol/g Hb, whereas in physical exercise passive interval 2.0886 nmol/g Hb. Based on the average physical exercise with the active interval is relatively higher compared with passive physical exercise with intervals, so that more required SOD enzyme.

Physical exercise has the potential for an increase in ROS (Halliwell 1999, Sjodin 1990). Even under conditions of resting ROS keep its form even very low levels of normal metabolic processes of aerobic organisms (Berzosa 2011). In this case Marciniak (2009) analogue, in resting conditions a person who weighs 70 kg, requires 3.5 ml O_2 /kg/minutes, which is equivalent to 350 l O_2 /hari (15 mol/day). An estimated 2-3% O_2 is converted to superoxide anion radical (0:30 to 0:45 mol), whereas during physical exercise O_2 consumption increased to 100%, so that the superoxide anion radical will be formed more than 100%. Even Sureda (2005) estimates that 2-5% of oxygen used is converted into radicals. Therefore, physical exercise has great potential to increase ROS, thereby required more levels of the enzyme SOD, which resulted in imbalance between the increased formation of ROS with antioxidants (Fearheller 2011, Belviranti 2006). The results showed a decrease in SOD in physical exercise with active rest intervals are suspected by the increase in ROS formation in the exercise.

Research using the same indicators (plasma SOD enzyme levels), which was reported by Sureda (2005), found a decrease in SOD after doing a test with a load on a bicycle ergometer rise. Likewise, Zbigniew (2010) who conducted the study using 40 amateur athletes, found a decrease in plasma SOD enzyme levels after conducting new long-distance running and there was indication of increase again after 24, although not the same as before doing long distance running. In another study conducted Aguilo (2005) by using the parameters of plasma MDA and SOD erythrocyte, also found an increased MDA and decreased antioxidant enzymes SOD, after doing a load test on a bicycle ergometer multilevel sub-maximum intensity. Results of research conducted by some experts also have the same indication with the results of research conducted by the researchers.

Based on the results of the study also showed a decrease in SOD levels of physical exercise with active rest intervals. Decrease in SOD in physical exercise is probably caused by the still strong influence of ROS are formed during exercise and 30 minutes after exercise, thus requiring more SOD to neutralize them. Due to the higher production of ROS are formed, the more SOD is

used to neutralize ROS, by changing anion superoxide into oxygen and hydrogen peroxide (H_2O_2) (Fairheller 2011), consequently the rest of SOD will decrease (Berzosa 2011). The same was reported Siirmen (2003). Found an increase in ROS formation, resulting in a growing number of SOD is required, resulting in decreased levels of SOD enzyme. As reported by Sjodin (1990) Physical exercise increases ROS production, it will require lots of SOD, resulting in decreased levels of SOD enzyme. Therefore the higher ROS formed higher SOD required to neutralize. Fadillioglu (2000) in his study found a decrease in ROS after exercise stops 24-48 hours, followed by a decrease in activity of antioxidant enzymes to neutralize ROS. The difference between the antioxidant that is formed and used to increase, thus increasing levels of enzyme SOD (Sureda 2005). According Dekany (2008) indeed has the properties of physical exercise "ambiguous", one side produces an increase in ROS, on the other hand also increases antioxidant protection system, depending on the intensity of exercise and the condition of the individual.

ROS production occurs in mitochondria caused by increased activity in the mitochondrial electron transfer system, as a result of increased metabolism (Siirmen 2003), and oxygen demand up to 20 times the resting conditions (Pinho 2006, Marciniak 2009). The process is greater in active physical exercise rest interval, compared to physical exercise with active rest intervals. As a result, ROS are formed more on physical exercise with active rest interval, the consequences are more levels of SOD enzyme is needed to neutralize these ROS. Besides physical exercise with active rest intervals is still relatively high activity of the muscles to support the physical exercise is primarily red muscle. In the red muscle is active rest interval much involved in supporting physical exercise. The inclusion of red muscle to bring the consequences of an increase in aerobic metabolism to meet energy needs (Gonchar 2005). This has led to an increase in mitochondrial electron transport system (Aguilo 2005). In the study Wellman (2009), the increase in mitochondria transport electron oxidation processes affecting the formation of ROS. Due to an increase in the oxidation process produces unstable oxygen molecules (Araujo 2009). Belviranli (2006) suggest an increase in ROS and also because of the increased autooxidation catecholamines during exercise that causes the production of O_2 -react with similar molecules such as protons (dismutase) and hydrogen peroxide (H_2O_2), as well as the inflammatory response when there is damage to muscles caused by overexertion. What's more active intervals of physical exercise involving many muscles, consequently the higher the distribution of blood flow, resulting in

increased muscle deoxygenation on active, the impact on increased formation of ROS (Laughlin 2008).

Formation of ROS through a mechanism that resembles the shape ischemic-reperfusion in cell superoxide anion (Levrant Sentiirk 2003, (2005) was greater in physical exercise with active rest intervals. As a result, anion superoxide formed relatively more on physical exercise with active rest intervals. The consequence is that there will be more SOD enzyme required to convert superoxide anion on physical exercise with active rest intervals. superoxide enzyme SOD transform into O_2 anion and hydrogen peroxide (H_2O_2) (Fairheller 2011). Thus the more superoxide anions are formed, the more SOD enzyme is also required to neutralize or turn it into hydrogen peroxide.

SOD enzyme levels gradually increased again after some time of physical exercise is stopped. Elevated levels of plasma SOD enzyme gradually as reported Zbigniew (2010), SOD enzyme levels gradually increased from after running, 12 hours and 24 hours after making the run. Elevated levels of enzyme SOD is suspected in addition to the acute response of the antioxidant enzyme system, also caused by a decrease in ROS formation after exercise is stopped. This does not occur at intervals of exercise with active rest, because in this form of exercise during the intervals of rest the muscles are still active, to do the activity. Therefore the needs for oxygen and energy requirement are still relatively high. Even during active rest aerobic metabolic processes are more dominant, active during the break because people try to keep doing the activity, although the intensity of 40% of the KKM. On the intensity of work, the red muscle remained active work. Under these conditions the formation of ROS was still relatively high. In contrast to physical exercise with a passive rest interval, during rest relative muscle activity, so it needs more oxygen and energy is relatively low. The consequence of ROS formation has decreased, so that the needs of the SOD enzyme levels used to neutralize ROS also decreased. This leads to increased levels of residual enzyme SOD is not used, so that the levels of SOD enzyme also increased. As depicted on the results of the study, the physical exercise with a passive rest interval ROS are formed relatively low, so there are many levels of SOD enzymes needed to neutralize ROS (Fairheller 2011). The consequence of this is not only a proper balance between ROS and SOD, but also the difference in levels of antioxidant enzymes that are used with the form (Berzosa 2011).

The effectiveness of antioxidant protection system in neutralizing ROS, in addition influenced by the intensity and duration of exercise (Gonchar 2005) is also influenced by the level of training (Aguilo 2005). As

noted previously that the samples used in the study were not trained athletes, the Sport Science students. The results showed that there is a tendency of MDA levels remained relatively unchanged, this may be one of them caused by training sample rate, so the effectiveness of the ability of the SOD enzyme protection system even better. Because physical exercise not only causes an increase in ROS, but also increase antioxidant enzyme that is almost simultaneous (Dekany 2008). Since the adaptation of exercise can increase antioxidant enzyme reserves, but also improve the response speed of the production of antioxidants, if mengalain oxidative stress during physical exercise (Fadillioğlu 2000). Research conducted by Dekany (2008) in trained men after physical exercise endurance not found a significant decrease in SOD, even on professional athletes increased antioxidant enzyme activity, which is caused by adaptation to exercise. People trained by Ji (1995) have the ability to respond and adapt to the antioxidant enzyme better than the untrained person. Therefore, ROS in people who are less trained than the untrained person.

CONCLUSION

Based on the results of the study there were differences in levels of SOD in active physical exercise with intervals with physical exercise with a passive interval. The more ROS are formed, the more levels of SOD enzymes used to neutralize them. Because of that physical exercise with intervals require more active SOD enzyme levels to neutralize SOR of the physical exercises with passive interval. Because of this difference in levels of SOD enzymes used are available with relatively lower, which indicated a decrease in SOD enzyme levels, whereas in passive physical exercise with intervals of SOD enzyme levels that are available to those used to neutralize ROS relatively more, so it appears the elevated levels of SOD enzyme.

Based on the results of physical exercise with a rest interval of active or passive leads to increased formation of ROS, but also increased the enzyme activity of SOD to neutralize ROS are formed. Based on the results of research exercises have a risk of increased ROS, therefore it is necessary restructuring exercise program is good, correct, regular and measurable by prioritizing SMARTer principle (Specific, Measurable, Achievable, Realistic, Time frame, Exciting and Recorded), to improve the coping mechanisms on the body. To increase the meaningfulness of exercise on the body is still needed further research using a concept based on molecular physiology, while to uncover patterns of exercise response to the homeostatic systems of the

body in physical exercise should be repeated with the study of time series models.

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