Effect of Exercise Intensity on Antioxidant Enzymatic Activities in Sedentary Adults

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Abstract
This study was designed to investigate the influence of relative exercise intensity on antioxidant enzymatic activities in sedentary healthy adults after cycling at different exercise intensity (50%VO₂ max, 60%VO₂ max and 70%VO₂ max) for 10 minutes. Venous blood were collected pre- and immediately post-exercise from 24 sedentary healthy adults (mean age= 20.83 ± 2.32 years, VO₂ max =2.15 ± 0.36 liter/min, BMI= 21.01 ± 4.27 kg/m²). Erythrocyte enzymes, i.e catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured as indirect measures of reactive oxygen species (ROS) production. (Subject cycled on the cycle ergometer with the estimated workload that corresponds to the measured maximal oxygen uptake (VO₂ max) that assigned or given to exercise intensity at the speed of 50 to 60 revolutions per minute (rpm) for 10 minutes.) Heart rate (HR) and VO₂ were monitored every minute during exercise test to ensure the subject was exercising at the relative exercise intensity given. Data were analysed with repeated measured ANOVA and paired t-tests. SOD increased significantly (p<0.05) by 25.3%, 55.8%, and 49.6% immediately post-exercise at 50%VO₂ max, 60%VO₂ max and 70%VO₂ max respectively, with a significant exercise intensity effect. On the other hand, 24.1% increment in CAT activity at 50%VO₂ max, and 38.1% and 10.0% decrement at 60%VO₂ max and 70%VO₂ max were also significantly affected by exercise intensity (p<0.05). No significant changes in GPx activity were noted. These data suggested that ROS such as superoxide anions (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (OH⁻) production increased as the exercise intensity increased. The changes in SOD, CAT and GPx activity were closely related to exercise intensity. The decrement observed in CAT and GPx activity levels would indicate the presence of oxidative stress.

Keywords: exercise intensity, reactive oxygen species (ROS), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT).

Introduction
Cells continuously produce reactive oxygen species (ROS) as part of the metabolic processes. Among the earliest biochemical reactions found were hydrolysis of fatty acid from membrane phospholipids, production of biologically active eicosanoids, and peroxidation of lipids with formation of ROS. The latter reaction will produce agents which are responsible for cellular damage. Superoxide (O₂⁻), ferryl, and hydroxyl anions are common reactive compounds that caused lipid peroxidation. Under normal conditions, superoxide (O₂⁻) anions are generated during mitochondrial electron transport. There is a balance between antioxidants and oxidants produced by aerobic cellular systems. These ROS were neutralized by an elaborate antioxidant defense system consisting of enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and numerous nonenzymatic antioxidants including vitamins A, E, and C, glutathione, ubiquinone, and flavonoids.

During normal respiration, the human body produces oxygen free radicals (ROS). When ROS interact with the polyunsaturated fatty acids in membranes or lipoproteins, the process of lipid peroxidation begins. Although countervailing biologic mechanisms normally controls these peroxidation reactions, severe oxidative stress produces reactive oxygen free radicals and induces uncontrolled lipid peroxidation. Because the cell membranes consist primarily of lipids, the uncontrolled lipid peroxidation can cause cell injury and death via DNA strand breakage and membrane damage.

Skeletal muscle cells continuously generate ROS, a cascade of diffusible, low-molecular-weight molecules that derive from diatomic oxygen. The parent molecule in the ROS cascade is the superoxide anion (O₂⁻) radical. Skeletal muscles produce O₂⁻ at low rate, which is dramatically accelerated during contractile activity. Several intracellular sources may contribute to O₂⁻ anions synthesis. O₂⁻ anions are by-products of mitochondrial electron transport, accounting for approximately 3% of total oxygen consumption by the organelle. O₂⁻ may also be synthesized enzymatically by xanthine oxidase, a pathway stimulated by loss of calcium homeostasis. Other potential sources include prostanoit catecholamine autooxidation, prostanoit metabolism, NAD(P)H oxidase.

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activity, and the nitric oxide (NO) synthases. However, any contributions from these latter sources, are poorly characterized in skeletal muscle.

Physical exercise involved muscular contraction; therefore the ability of muscle to increase its mitochondrial oxygen consumption is substantial in order for the muscle to undertake co-ordinated body movement. As part of the process for delivery of energy supplies, oxygen generally undergoes four-electron reduction to water, catalysed by cytochrome oxidase complex in mitochondria. This process has been claimed to account for 95%-98% of the total oxygen consumption of tissues, but the remainder may undergo one electron reduction with the production of the superoxide radical (O$_2^-$). Furthermore, one electron reduction of superoxide radical (O$_2^-$) produces hydrogen peroxide (H$_2$O$_2$), which will be released by mitochondria. Therefore, theoretically aerobic exercise could lead to an increased production of superoxide radicals because of the vastly increased electron flux through the mitochondrial electron transport chain. Mitochondria has well-developed systems for protection against accumulating oxygen radical with the presence of a specific mitochondrial superoxide dismutase.

However, if ROS production surpasses the protection and repair mechanisms, the net effect is oxidative stress, an identified causative factor in several disease states and proposed as a factor in the cellular physiological attrition indicative of aging. Such states are primarily achieved through damage, by ROS, to macromolecules such as DNA, lipids, and protein. Exercise-induced oxidative stress has been specifically associated with disruptions in cellular homeostasis, e.g., muscle fatigue, muscle contractile dysfunction, and after severe exercise, cellular apoptosis and muscle damage.

There are a few studies which showed aerobic exercise leads to release of mitochondria-derived superoxide radicals (O$_2^-$) from the muscle cells. Reid et al [2] found that superoxide (O$_2^-$) produced in contracting myocytes was released into the interstitial fluid.

During exercise, superoxide radicals (O$_2^-$) are generated within mitochondria during the contractile activity. Superoxide radicals (O$_2^-$) and H$_2$O$_2$ are subsequently lost from the muscle cells into interstitial fluid. In the extracellular fluid, OH$^-$ is then generated from the released superoxide or H$_2$O$_2$ by an iron-catalysed system. Therefore increased radical species concentration in the extracellular fluid following exercise is expected. O$_2^-$ crosses cell membranes slowly, unless there is a specific channel for O$_2^-$ such as erythrocyte membrane that has an anion channel through which O$_2^-$ can move [1]. However, generated O$_2^-$ crosses cell membranes easily and could penetrate inside the cell and cause OH$^-$ to be formed. O’Neill et al [3] reported an increased of OH$^-$ production by the contracting skeletal muscle. The OH$^-$ production during exercise appeared to be related to the tension developed by the muscle.

Exercise also increases oxygen consumption relative to basal levels, particularly in skeletal muscle and heart. Oxygen uptake is greatly increased during intense exercise. According to Davies et al [4] and Sjödin et al [5], muscle oxygen utilization during strenuous exercise can increase as much as 100-200 times than at rest. Paradoxically, this may be associated with increased production of ROS such as superoxide (O$_2^-$), H$_2$O$_2$, and the hydroxyl radical (OH$^-$), although it has been suggested that absence of massive oxidative damage during intense periods of cellular respiration may, in part, be due to a reduction in ROS leak across mitochondria. In other words, physical exercise could induce peroxidation of lipid in cellular membranes and increased level of thiobarbituric acid reactive substance (TBARS) in plasma is the consequence of peroxide leakage from tissues especially from muscle.

Oxygen radicals might be produced in excess during physical exercise and these substances are responsible for some of the deleterious effects of excessive or unaccustomed exercise. Oxygen radicals could be produced in excess under certain exercise regimens, but this was not an inevitable consequence of all forms of exercise and did not always initiate cellular damage or degeneration. If the production of ROS is excessive as observed during strenuous aerobic exercise or if the antioxidant defence mechanisms are impaired, the balance between pro-oxidants and antioxidants is lost, leading to oxidative stress. Thus, there is an apparent paradox between the benefits of moderated and strenuous aerobic exercise.

There is a general agreement that single bout of exercise can change the status of some antioxidants in tissues. Such responses, however, are highly dependent on numerous factors including exercise types, duration, intensity, previous exercise exposure, subject age, subject species, nutritional status, tissue and fibre type examined, sampling time, and assay technique employed. Research by Jenkins et al [6] found antioxidant levels in skeletal muscle increased above rest in proportion to the energy demands of the exercise.

Although it has been established that there is an expected accumulation of antioxidant enzymes and an increased oxidative stress status, but whether intensity do play a major role in determining the level of oxidative stress is still undetermined. The information in this area is very much lacking. Therefore, present study was undertaken to determine whether the intensity of exercise affect erythrocyte antioxidant enzymes activity.

Experimental Procedures

Subjects

24 healthy volunteers aged 19-30 years old were recruited through flyers, posters and word of mouth in Kuala Lumpur area for this preliminary study. There
were no significant differences in age, height, body mass and haemoglobin concentration between subjects. This study is carried out in accordance to the specifications approved by the Ethical Committee of Universiti Kebangsaan Malaysia (UKM). The volunteers were given a health-screening test, which include a physical examination and respiratory function test to determine their health status. Individuals with cardiovascular disease, respiratory problems, cancer, diabetes mellitus and those who smoke and drink alcohol were excluded from the study. Written informed consent was obtained from all subjects. Physical characteristics of subjects at baseline are presented in Table I.

Criteria for subject participation

Inclusion criteria for participation in the study included healthy non-smoking status, age 19-30 years, and a peak VO$_2$ of a sedentary. To more accurately document small differences in physical fitness, healthy inactive/sedentary people status was determined by predicted and measured peak VO$_2$ as recommended by Wasserman et al [7] and sedentary criteria by Dunn et al [8] and Phil and Jurimae [9] as the subject eligibility assessment. The subject is classified as sedentary if measured peak VO$_2$ less than predicted peak VO$_2$ and performed any physical activity at least 3 days per week for 20 min or more each time. Potential participants with the following characteristics were excluded: antioxidant supplements (e.g. vitamin C, vitamin E, selenium, carotenoids etc.), other supplements (performance enhancing or herbal-type products), abnormal cholesterol, triglyceride, or glucose levels, vegetarian or other restrictive dietary requirements, presence of self-reported history of myocardial infarction, suffer from any of known cardiopulmonary disease, have abnormal cardiopulmonary function, under psychological treatment, take any medication that might interfere with the test, anemia, hemoglobin level <12, consumes alcohol, systolic blood pressure ≥140mmHg or diastolic blood pressure ≤90mmHg.

PreScreening

Baseline information obtained includes VO$_2$ max, body composition assessment, general health screening questionnaire, physical activity screening questionnaire, food-frequency-questionnaire and physical examination by a qualified doctor.

Peak Oxygen Uptake

Peak oxygen uptake (peak VO$_2$) was determined using maximum incremental exercise test (symptom-limited) on cycle ergometer in which, subjects exercised on a cycle ergometer while measurements of gas exchange data was obtained breath by breath using Cortex Metamax 3B, Germany. Heart rate (HR) was recorded using a Polar Heart rate Monitor (Polar Electro, Inc., Woodbury, NY, USA).

The exercise protocol used is one-minute incremental protocol [7]. The work rate increment, W per minute, depended on the height, age, gender and health of the subject. The work rate increment to reach the subject’s estimated peak VO$_2$ in 8-12 minutes was calculated.

Work rate (W) increment per minute = (*Peak VO$_2$, ml/min - VO$_2$ unloaded, ml/min)/ 100

*VO$_2$ unloaded in ml per minute = 150 + (6 X Weight, kg)

Peak VO$_2$ in ml per minute = (height, cm - age, years) X 20 for sedentary men

Initially, subject cycled for 3 minutes of unloaded pedalling. Then, increment was added at the start of each minute. The subject was encouraged to continue and maintain the cycling frequency between 50 to 60 rpm as long as safely possible. When the cycling frequency cannot be maintained or when the subject decided to terminate the incremental exercise, the cycle is returned to the unloaded setting.

Testing Schedule

All subjects visited the lab 4 times inclusive the maximal oxygen uptake test. This was a repeated measures study, in which each subject acted at his/her own control. All groups were pre-tested and post-tested, and no control group was exposed to the intervention. All subjects were acclimated to the use of the cycle ergometer. Correct execution of each exercise test was supervised by the investigator. During the first visit, physical examination by a qualified medical officer, body fat analysis, ECG test, blood pressure measurement and maximal exercise test were conducted. During visits 2 till 4, qualified subjects were asked to cycle for 10 minutes on the cycle ergometer at the given workload that correspond to their relative exercise intensity (individually) in random order.

Exercise bouts

From the results of Maximal Exercise Test, the test workload that correspond to the their relative exercise intensity (%VO$_2$ max) and HR$_{max}$ was determined. HR$_{max}$ during maximal graded exercise testing was used. The Exercise Target Heart Rate (HR$_{test}$) for each subject was calculated using the formula (HR$_{test}$ = Exercise Intensity X HR$_{max}$ X 1.15). The VO$_2$ and HR were monitored every minute to ensure the subject is exercising at the intensity given.

The exercise task consisted of 3 minutes warm-up on a cycle ergometer, immediately followed by the exercise tests at the given workload. Subjects cycled with the estimated workload for a given duration while VO$_2$ and HR was measured using Cortex Metamax 3B, Germany and polar heart monitor. Gas exchange data was obtained breath-by-breath and was recorded. Calibration of the system was performed before each test. HR were recorded
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continuously using Polar heart rate monitor. The subjects were encouraged to continue and maintain the HR at the HR_{rest} at the duration given.

To ensure that the observation represent differences due to the effect of different intensity and duration of exercise instead of the training effects, the subjects were given at least 3 days of rest after maximal exercise test, and in between exercise test. Furthermore to allow the oxidative stress levels to return to baseline, at least 3 days were required [10].

Subjects were required to maintain activity level throughout the experimental period. The subjects were requested to refrain from exhaustive physical activity and eating, drinking for at least 2 hours prior to testing and to dress in attire suitable for all tests including maximal exercise test.

Blood samples were collected pre- and immediately post-exercise trial for measurement of antioxidant enzymes activity and stored at -80°C until the analysis.

**Blood sampling and haematological markers**

Blood samples were obtained from a forearm vein of each subject in heparinized and EDTA tubes before and immediately after each exercise bouts. Blood was immediately centrifuged at 3000rpm for 10 minutes at 4°C to separate plasma from red blood cell pellets. Plasma and red blood cells samples were immediately frozen and stored at -80°C until analysis.

**Erythrocyte Antioxidant Enzymes Analysis**

Erythrocyte enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were used as intracellular antioxidant markers. SOD activity in erythrocyte was determined according to Beyer and Fridovich [11]. One unit of SOD was designated as the amount of haemoglobin that inhibits the rate of nitro blue tetrazolium reduction by 50%. All samples were performed in duplicate.

CAT activity in erythrocyte was quantified using spectrophotometer based on the method previously described by Aebl [12]. The decomposition of H$_2$O$_2$ by CAT enzyme was followed directly by the decrease in absorbance at 240nm. The difference in absorbance per unit time was measured as CAT activity. All samples were performed in duplicate.

GPx activity in the erythrocyte was determined spectrophotometrically by the method of Paglia and Valentine [13]. H$_2$O$_2$ was added to a medium containing phosphate buffer, EDTA, NaN3, NADPH, GSH and erythrocyte hemolysate, where the change in absorbance of the system was followed at 340nm. All of these antioxidant enzymatic activities were expressed relative to the hemoglobin concentration.

**Statistical Analysis**

All data were expressed in mean ± SEM. Data were analysed for normality distribution using Shaphiro-Wilks test. The data sets were found to be normally distributed. Therefore, further analysis was performed using parametric tests. Analysis of variance (ANOVA) with repeated measures was performed for all exercise bouts, with time (pre- and post-exercise), exercise intensity and exercise duration as the within-subject factors. Homogeneity of covariance or sphericity assumption of the data was checked using Mauchly test. The sphericity assumptions for SOD, CAT and GPx data sets were maintained p <0.001.

Since the sample size for each level of factor were equal, therefore concern about the homogeneity of variances was eliminated. When significant intensity by time interactions occurred, simple main effects were assessed using paired-t tests. Post hoc comparisons were made by comparison-contrast tests. A probability level of 0.05 were set for significance. All statistical analysis were performed using Statistical Packages for Social Sciences (SPSS 12 for Windows; SPSS, Chicago, IL).

**Results**

**Subject Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>20.83 ± 2.32</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.89 ± 12.19</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.35 ± 5.66</td>
</tr>
<tr>
<td>BMI (kg/m-2)</td>
<td>21.01 ± 4.27</td>
</tr>
<tr>
<td>VO$_2$ max (liter/min)</td>
<td>2.15 ± 0.36</td>
</tr>
<tr>
<td>VO$_2$ max (ml/min/kg)</td>
<td>36.42 ± 1.19</td>
</tr>
</tbody>
</table>

Mean ± SD: n =24, all males

All 24 subjects completed all the exercise trial for 10 minutes duration. Table I summarizes the characteristics of subjects in this study. Based on the Panel on Energy, Obesity and Body Weight Standards [14], the body mass indexes (BMI) of 21.01 ± 4.27 kg/m-2 is in the desirable range for adult men. Subjects were matched across age and aerobic fitness (using VO$_2$ max normalized to body weight as the criterion). Based on U.S. Department of Health and Human Services [15], cycling VO$_2$ max values of 36.42 ± 1.19 ml/min/kg indicate that the subjects are healthy, but sedentary.
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Antioxidant Enzyme Activities (SOD, CAT, GPx)

Table II: Effect of (10 minutes) exercise at (3 diff exercise) intensities on erythrocyte antioxidant enzyme levels

<table>
<thead>
<tr>
<th>Exercise Intensity (%VO₂ max)</th>
<th>50% VO₂ max</th>
<th>60% VO₂ max</th>
<th>70% VO₂ max</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Mean ± SEM)</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>SOD (unit/mgHb)</td>
<td>0.83±0.05</td>
<td>1.42±0.09**</td>
<td>0.86±0.09</td>
</tr>
<tr>
<td>CAT (u/s/mgHb)</td>
<td>0.29±0.02</td>
<td>0.36±0.02**</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>GPX (umol/min/mgHb)</td>
<td>0.0058 ± 0.0002</td>
<td>0.0056 ± 0.0001</td>
<td>0.0055 ± 0.0002</td>
</tr>
</tbody>
</table>

*p<0.001 relative to pre-exercise; ** p<0.05 relative to pre-exercise

Table II showed the effect of three different exercise intensities at 10 minutes duration on the antioxidant enzyme activities.

From the bar graph in Figure 1, the SOD activity for the three different exercise intensity levels for 10 minutes increased significantly after exercise compared to before exercise at all exercise intensity levels (p<0.05). Figure 2 showed the exercise intensity induced changes in SOD activity (ΔSOD). ΔSOD (0.59 ± 0.09 unit/mgHb at 50%VO₂ max vs 0.48 ± 0.11 unit/mgHb at 60%VO₂ max and 0.56 ± 0.08 unit/mgHb at 70%VO₂ max) were found to be statistically significant different between exercise intensity level (P<0.05). However, changes in SOD activity (ΔSOD) were not found statistically different between 60%VO₂ max and 70%VO₂ max.

From the Bar Graph (Figure 3), CAT activity increased significantly (p<0.05) after exercised at 50%VO₂ max, however decreased significantly (p<0.001) after exercised at 60%VO₂ max, and 70%VO₂ max for 10 minutes. Figure 4 showed changes in CAT activity; ΔCAT (0.07±0.029 50%VO₂ max vs -0.16±0.034 60%VO₂ max and -0.10±0.023 u/s/mgHb 70%VO₂ max) were found to be statistically significant different between exercise intensity level (P<0.05). However, changes in CAT activity (ΔCAT) were not found to be statistically different between 60%VO₂ max and 70%VO₂ max.

![Figure 1: Superoxide Dismutase Activity (unit/mgHb) Pre- and Post-exercise at Exercise Intensity of 50%VO₂ max, 60%VO₂ max, and 70%VO₂ max for 10 minutes.](image1)

Mean ± SEM; N=24; *p<0.001 relative to pre-exercise; ** p<0.05 relative to pre-exercise

![Figure 2: Changes in SOD Activity; ΔSOD (Post - Pre) at Exercise Intensity of 50%VO₂ max, 60%VO₂ max, and 70%VO₂ max for 10 minutes (unit/mgHb)](image2)

Mean ± SEM; N=24; values with different letters (a,b,c) are significantly different between exercise intensity levels, a:50% & 60%, b:60% & 70%, c:50% & 70%, p<0.05.

1 - 50%VO₂ max, 2 - 60%VO₂ max, 3 - 70%VO₂ max.

![Figure 3: Catalase Activity Pre- and Post-Exercise at Exercise Intensity of 50%VO₂ max, 60%VO₂ max, and 70%VO₂ max for 10 minutes (u/s/mgHb)](image3)

Mean ± SEM; N=24; *p<0.001 relative to pre-exercise; ** p<0.05 relative to pre-exercise
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There were no significant difference in GPx activities after exercise at all exercise intensity for 10 minutes (Table II and Figure 5). However, from the bar graph in Figure 5, it showed that there's a trend of decrement in GPx activities after exercise. In addition, this study also revealed that changes in GPx activities (∆GPx) were not statistically different between exercise intensities compared.

![Figure 4: Changes in CAT activity; ∆CAT (Post - Pre) at Exercise Intensity of 50%VO2max, 60%VO2max, and 70%VO2max for 10 minutes (μmol/min/mgHb)](image)

Mean ± SEM; N=24; values with different letters (a,b,c) are significantly different between exercise intensity levels, a:50%&60%, b:60%&70%, c:50%&70%, *p<0.05.
1 - 50%VO2max, 2 - 60%VO2max, 3 - 70%VO2max.

There were no significant difference in GPx activities after exercise at all exercise intensity for 10 minutes (Table II and Figure 5). However, from the bar graph in Figure 5, it showed that there's a trend of decrement in GPx activities after exercise. In addition, this study also revealed that changes in GPx activities (∆GPx) were not statistically different between exercise intensities compared.

![Figure 5: Glutathione Peroxidase Activity Pre- and Post-Exercise at Exercise Intensity of 50%VO2max, 60%VO2max, and 70%VO2max for 10 minutes (umol/min/mgHb)](image)

Mean ± SEM; N=24; values with different letters (a,b,c) are significantly different between exercise intensity levels, a:50%&60%, b:60%&70%, c:50%&70%, *p<0.05.
1 - 50%VO2max, 2 - 60%VO2max, 3 - 70%VO2max.

There were no significant difference in GPx activities after exercise at all exercise intensity for 10 minutes (Table II and Figure 5). However, from the bar graph in Figure 5, it showed that there's a trend of decrement in GPx activities after exercise. In addition, this study also revealed that changes in GPx activities (∆GPx) were not statistically different between exercise intensities compared.

![Figure 6: Changes in GPx activity; ∆GPx (Post - Pre) at Exercise Intensity of 50%VO2max, 60%VO2max, and 70%VO2max for 10 minutes (umol/min/mgHb)](image)

Mean ± SEM; N=24; values with different letters (a,b,c) are significantly different between exercise intensity levels, a:50%&60%, b:60%&70%, c:50%&70%, *p<0.05.
1 - 50%VO2max, 2 - 60%VO2max, 3 - 70%VO2max.

In other words, changes in SOD activity across time depended on the exercise intensity level. Tests of within-subjects contrasts also showed that there was a significant interaction effect of I x t (F= 10.87, P=0.003). This result implies that the pattern of changes in SOD activity over the time differed between exercise intensity levels. In other words, the exercise intensity effect occurred.

The different mean SOD activity after exercise between three different exercise intensity levels being compared was significant. Therefore, there was a difference in the mean SOD activity between exercise intensity levels of exercise being compared.

Repeated measures ANOVA test for CAT activity also revealed statistically significant differences for Time; t (F= 11.47, P=0.003), and the interaction effect t x I (F=22.34, P=0.000). These results showed that the changes in CAT activity over time was statistically significant and the interaction effect of I x t was also significant. In other words, changes in SOD activity across time depended on the exercise intensity level. Tests of within-subjects contrasts also showed that there was a significant interaction effect of I x t (F=20.52, P=0.000) and effect by time (F=11.47, P=0.003). This result implies that the pattern of changes in CAT activity over the time differed between exercise intensity levels and the CAT activity post-exercise was significantly different from pre-exercise.

However, intensity effect (F=0.456, P=0.636) was not significant, which means that this result occurring by chance is more than 1 in a 1000. Therefore, the changes of CAT activity after exercise across exercise intensity level is not significant. In other words, the changes in the mean CAT activity after exercise did not depended upon exercise intensity.
For GPx activity, the Greenhouse-Geisser adjustment is used, and the results from within-subjects tests revealed a statistically significant different for time (F= 6.44, P=0.018), and intensity (F= 13.488, P=0.000), but not for interaction effect of I x t (F= 0.547, P=0.583). These results show that the changes in GPx activity over time and across intensity were statistically significant. However, the interaction effect of I x t was not significant. In other words, changes in SOD activity across time did not depended on the exercise intensity level.

Tests of within-subjects contrasts showed that there was no significant interaction effect of I x t (F=.841, P=0.369). This result showed that the pattern of changes in GPx activity over the time was not different between exercise intensity levels. However there was a significant effect of intensity (F=8.586, P=0.008) and time (F= 6.449, P=0.018). The GPx activity was significantly different between exercise intensity and the GPx activity post-exercise. The GPx activity was significantly different for time (F= 6.44, P=0.018), and intensity (F= 13.488, P=0.000), but not for the interaction effect of I x t (F= 0.547, P=0.583). These results show that the changes in GPx activity over time and across intensity were statistically significant. However, the interaction effect of I x t was not significant. In other words, changes in SOD activity across time did not depended on the exercise intensity level.

**Discussion**

Many studies have reported that acute aerobic exercise contributes to oxidative stress, especially when performed at high intensity levels [16, 17]. The two mechanisms linking acute aerobic exercise and oxidative stress are increased pro-oxidant activity via a mass action effect when VO$_2$ is elevated 10- to 15-fold above rest and inadequate antioxidant activity relative to pro-oxidants. Antioxidant enzymes may be activated selectively during an acute bout of exercise depending on the oxidative stress imposed on the specific tissues as well as the intrinsic antioxidant defense capacity. Skeletal muscle may be subjected to a greater level of oxidative stress during exercise than liver and heart due to marked increase in ROS production. Conversely, SOD, CAT and GPx provide primary defense against ROS generated during exercise.

The high reactivity of free radicals makes their direct detection in human difficult. Consequently, majority of studies have examined markers of lipid peroxidation, protein oxidation, cellular redox status and changes in antioxidant enzyme activity as indirect measures of free radical involvement in tissue damage.

Antioxidant protection against pro-oxidant activity is indicated by SOD, CAT and GPx activity as intracellular antioxidant markers. Despite the different and complex metabolic stressors imposed by a different exercise intensity levels, different intensity generated different response of antioxidant enzymes activities. Post-exercise antioxidant enzymes activities at a different intensity level may be related to several factors including oxygen consumption during exercise, dietary antioxidant intake, and plasma fat content. In order to investigate effect of exercise intensity on antioxidant enzymes activity, several factors that may contribute to the oxidative stress level have been controlled. Therefore, subjects involved in the present study were limited to healthy sedentary adults and not taking any supplement.

Exercise induced changes in antioxidant enzymes activities. During exercise, 10-fold or more increased in total body oxygen consumption can easily occur. As the intensity of exercise increased, the energy demand and the heat production increased. According to Sies [18] the increment in energy demand and heat production may strained the ability of ETS to completely reduce oxygen to water, resulting in the formation of ROS O$_2^·$, H$_2$O$_2$ and OH. Conversely, the increase in exercise intensity will elevate the oxygen consumption and produces more ROS.

Within most cell types, including erythrocytes, the enzymes SOD, CAT and GPx are the most predominant antioxidant enzymes. SOD catalyses the formation of O$_2^·$ to H$_2$O$_2$. SOD is considered the first line antioxidant defense system against cellular oxidants while CAT compliments SOD antioxidant function. CAT compliments the SOD antioxidant defense process by converting any remaining oxidant species to non-reactive H$_2$O. GPx is likely the cell’s subsequent response mechanism when SOD and CAT fail to meet the body’s cellular antioxidant needs such as during high intensity exercise.

Few studies have investigated either the acute or chronic effects of exercise on antioxidant enzymes, but no study has ever investigated the effect of exercise intensity specifically. In the present study, subjects performed exercise at 3 different intensities corresponding to their maximal oxygen consumption (VO$_2$max). The protocol used in the present study was controlled for exercise time and absolute exercise intensity (calculated exercise workload) that corresponds to the relative intensity (50%VO$_2$max, 60%VO$_2$max and 70%VO$_2$max).

In the present study, exercise at intensity of 50%VO$_2$max, 60%VO$_2$max and 70%VO$_2$max increased SOD activities significantly by 25.3%, 55.8%, and 49.6% pre- to immediately post exercise respectively. SOD is the first line antioxidant defense system against cellular oxidants. Therefore the increase in SOD activity in the erythrocytes might be a major factor in order to avoid an expected elevation of lipoperoxidation due to exercise. This finding showed that the reactive oxygen species generated increased as the exercise intensity increased.

The results of repeated measures ANOVA also revealed the changes of SOD activity after exercise significantly depended on exercise intensity. The increase in SOD activity (P<0.05) associated with 60%VO$_2$max and 70%VO$_2$max was about twice the increase (P<0.05) with 50%VO$_2$max. However there is no significant difference between 60%VO$_2$max and 70%VO$_2$max. The increase in erythrocyte SOD activity after exercise at the higher intensity is in accordance with the proposed mechanism of enhanced catabolism of O$_2^·$. This increase, in turn,
depends on the metabolic rate, and is associated with the production of ROS specifically O$_2^-$. Higher SOD activities may have been partly responsible for attenuating ROS. This finding is supported by Tauler et al [19] that found amateur cyclists performing submaximal test, at 80% of maximal oxygen uptake showed increased erythrocyte SOD activity by 25%, but no changes seen after maximal test.

During strenuous exercise, the aerobic metabolic rate in the skeletal muscle is raised up to 10 times the resting levels, enhancing leakage of superoxide anion (O$_2^-$) from the mitochondria to the cytosol [20]. The subsequent reactions give rise to other reactive oxygen species such as reactive hydroxyl radical. These reactive oxygen species have been shown to induce damage in all cellular macromolecules, such as lipid, protein, and DNA. Therefore, an increase in the generation of reactive oxygen species during exercise has been considered to be an oxidative stress.

Previous studies found SOD, CAT and GPx activities elevated after exercise [20, 21, 22]. Similarly, previous reports have shown that levels of SOD activity increased after an acute bout of exercise in red blood cells [23, 24]. Ji [25] has proposed the activation of SOD to be caused by increased O$_2^-$ production during exercise according to in vitro SOD kinetics. SOD is partially occupied by its substrate (O$_2^-$), and its catalytic activity increases with increasing O$_2^-$ concentration within a wide range [26].

Results from this study are in contradiction with those of Vider et al [27] that found exercise until volitional exhaustion (exercise-induced oxidative stress) did not change SOD activity significantly. High levels of H$_2$O$_2$ have been shown to inhibit SOD invitro [28]. Because of its kinetic properties, assays of SOD are usually based on indirect methods involving the inhibition of a reaction in which O$_2^-$ is generated by using a variety of electron donors [29]. The aforementioned studies have used a variety of exercise protocols and subject populations. However, many of these studies have not employed a specific exercise intensity and duration as the exercise stimulus.

CAT activity in response to a single bout of exercise is variable. In this study, the erythrocyte CAT activity increased by 24.14% pre- to post-exercise significantly (p<0.05) after exercising at 50%VO$_2$ max, however decreased significantly (p<0.05) after exercising at 60%VO$_2$ max, and 70%VO$_2$ max for 10 minutes by 38.1% and 10.0% respectively. This seems to agree with Tauler et al, 2005 who also found erythrocyte CAT activity decreased by 12% after submaximal test at 80%VO$_2$ max performed by amateur cyclists, but no changes seen after the maximal test.

Furthermore, present study also found the effect of exercise intensity on the CAT activity being significant. The increase in CAT activity after exercise implied an activation of the CAT due to the increased H$_2$O$_2$ production. O$_2^-$ production increased with exercise, and the SOD catalyses the formation of O$_2^-$ to H$_2$O$_2$. Therefore the H$_2$O$_2$ concentration will also increase with exercise. The decomposition of H$_2$O$_2$ to form water and oxygen is accomplished in the cell by catalase. As a result, increased in CAT activity would convert any remaining H$_2$O$_2$ to non-reactive H$_2$O. According to Chance and colleagues, (1979), CAT activity increased enormously with an increase H$_2$O$_2$ concentration.

The increment in exercise intensity elevates oxygen consumption and the production of ROS specifically O$_2^-$. Therefore H$_2$O$_2$ formation increased as the intensity of exercise increased. Thus, as the intensity of exercise increased, CAT activity should be increased. However, CAT activity decreased after exercise at 60%VO$_2$ max, and 70%VO$_2$ max. This is probably due to the CAT enzyme could not compensate for the remaining H$_2$O$_2$ produced from the reaction catalyse by SOD. This results were supported by Aguilo et al [30] that also found a decrease in erythrocyte catalase activity of nearly 20% in trained cyclists, following a submaximal exercise for 90 minutes.

A few of previous studies revealed no significant alteration in CAT activity with acute exercise. Rokitzki et al [31] found no difference in erythrocyte catalase activity levels following marathon running and Marzatico et al [32] also reported that sprinters did not alter erythrocyte catalase activity after performing a sprint-type exercise. However, study by Vider et al [27] found serum CAT increased after exercised at maximal intensity or until volitional exhaustion. Ji and Fu [33] also found CAT activity increased significantly after an acute bout of exercise to exhaustion in the deep portion of vastus lateralis muscles in rats. It is possible that the observed increase in muscle CAT activity reflected an activation of mitochondrial CAT due to the increased H$_2$O$_2$ production. Allesio and Goldfarb [34] found that catalase activity is increased after an acute bout of exercise in muscle. The above studies have used a variety of exercise protocols and subject populations, tissues, sampling time and assay techniques. Thus, these variations could lead to research findings for CAT activity response to exercise to vary widely.

GPx is another antioxidant enzyme with a much greater affinity for H$_2$O$_2$ at low concentrations compared to catalase. This means that at low concentrations, GPx plays a more active role in removing H$_2$O$_2$ from the muscle cell. GPx oxidizes reduced glutathione (GSH) to its oxidized form (GSSG) while reducing hydroperoxide (ROOH) and H$_2$O$_2$ to ROH and H$_2$O, respectively.
GPx is the cell’s response mechanism when SOD and CAT fail or unable to meet the body’s cellular antioxidant needs such as during high intensity exercise. Therefore, the exercise duration of 10 minutes in the present study did not elicit significant changes in GPx activity between pre- and post-exercise at intensity 50% VO2 max, 60% VO2 max and 70% VO2 max (p<0.05). However GPx activity showed a decrease immediately post-exercise. Thus either the erythrocyte GPx does not respond to exercise, or the exercise duration of 10 minutes used was insufficient to trigger a GPx response. According to Child et al [35] more intense exercise, known to elevate serum lipid peroxides, could affect erythrocytes GPx activity. On the other hand, H2O2 was produced at high concentration during exercise for GPx to remove it. As a result GPx activity decreased immediately after exercise.

The increment in the exercise intensity increased the oxygen consumption, and thus the ROS production also increased. Therefore, the changes of GPx activity pre to immediately post-exercise were expected to increase as the intensity of exercise increased. However, in this study the GPx activity changes pre to immediately post-exercise is not significantly different between exercise intensity levels. According to Tiidus et al [36], an increase in oxygen consumption during exercise activates the enzyme GPx to remove H2O2 and organic hyperperoxides from the cell. Results from this study concurred with other previous studies that showed no significant changes in GPx activity after exercise. Vider, et al, 2001 also found no significant changes in erythrocyte GPx activity after exercised until volitional exhaustion. Rokitzi et al [37] and Tauler et al [38] showed little to no difference in pre versus post race measures of erythrocyte GPx activity in the athletes of marathon running and cycling. Marzatico et al [32] found an increase in erythrocyte GPx activity after a sprint-type exercise, but no change when an endurance exercise was performed.

GPx activity has been shown to demonstrate varied responses to an acute bout of exercise in the various types of tissues. Some studies showed no significant changes in GPx in skeletal muscle after acute exercise [39, 40, 41]. But studies by Ji and Fu [33] and Ji et al [17] have reported that GPx activities in both erythrocyte and skeletal muscle increased in response to maximal exercise. The above studies used a number of different exercise protocol, different subjects population and different methods to identify GPx activity, which makes it difficult to compare the study results.

Collectively, the results of the present study combined with other reported data can be integrated into a working hypothesis on the interrelationships among exercise and antioxidant status. The increment in the maximal oxygen uptake as the intensity of exercise increased during the present study may have led to the generation of more ROS. The increased in SOD as one of the primary enzymatic antioxidant defences against superoxide radicals reflects an increase in the generation of the ROS specifically O2-. As the intensity of exercise increased, more O2^- produced, and more H2O2 will be produced than the GPx and CAT can scavenge. The decrease in GPx and CAT activity as the intensity of exercise increased also reflects an increase in the generation of the ROS to levels greater than the GPx and CAT can handle.

It is assumed that in sedentary healthy adult, the maximal oxygen uptake increased as the intensity of exercise increased. Therefore, more O2^- produced as the intensity increased, as well as H2O2 formation as a consequence of SOD action. As intensity of exercise increased, the SOD will increased but GPx and CAT will be decreased. However, at low exercise intensity of 50% VO2 max, the H2O2 generated from O2^- is sufficiently high enough to activate CAT scavenging mechanism, which implies low oxidative stress status. It showed that for 10 minutes duration of exercise at the low intensity of 50% VO2 max, oxidative stress level would not be increased in sedentary healthy adults.

These findings suggested that by exercising at lower intensity, there was a lower production of reactive oxygen species (ROS) such as superoxide anions (O2-), hydrogen peroxide (H2O2), and hydroxyl radicals (OH-), with a lower request for antioxidant defence such as SOD, CAT and GPx. The decrement observed in CAT and GPx levels would indicate that the presence of oxidative stress was able to modify blood antioxidant profiles. The changes in erythrocyte SOD, CAT and GPx activities were closely related to exercise intensity. In other words, the ROS production increased as the intensity of exercise increased.

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References


