Oxidative Stress: Comparing Sub-Maximal Physical Activities as a Self-Reminder for Human Health System

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Abstract. Numerous prospective epidemiological studies have investigated the effects of exercise or estimates of physical activity can increase oxidative stress. This research aims to provide an overview of the difference of oxidative stress after having sub-maximal physical activity in the morning-afternoon and as a self-reminder for human health system. This is an experimental laboratories research with pre-post-test design. The sub-maximal physical activities in this research is riding ergo-cycle with 80% HRmax for 6 minutes. The study revealed there is a significant improvement of MDA plasma degree and decreased activity of enzyme SOD before and after having sub-maximal physical activities in the morning and afternoon. Oxidative stress and the subsequent oxidation of LDL are considered a major contributor to the lifestyle-related diseases, impairment of endothelial function and the development of atherosclerotic lesions. For this reason, it is essential to make body as a self-reminder for human health system by considering the time and the intensity for having physical activities.

1. Introduction
Physical activity or exercise exerts numerous favorable effects on general health [1] and appear to have a positive long-term influence on all selected diseases, will have live longer and enjoy greater quality of life [2]. Habitual leisure-time physical activity (PA) improves cardiorespiratory and reduced health outcomes such as cardiovascular disease and mortality [3, 4]. Habitual PA has also been shown to lower resting heart rate (HR), improve heart rate recovery (HRR) [5], and lower resting blood pressure (BP) [6]. However, physical activity is strongly associated with increased oxidative damage and it depends on other factors such as mode and intensity of physical activity, and site of free radical production [7].

The first indications that physical activity results in tissue damage caused by means of free radicals appeared in 1978, so the remaining three decades witnessed a significant increase in knowledge on that particular matter. It is well known that both active and inactive skeletal musculature produce reactive oxygen and nitrogen species though it still remains unclear the exact place of origin of oxidants during exercise [8].

Aerobic exercise is a physical activity that meets the energy provision via the oxidative energy system during the exercise [9]. The aerobic exercise linked to the increase in oxygen that resulted in an increase formation of oxidant compounds followed by oxidative stress. Oxidative stress is a condition characterized by an imbalance between reactive oxygen/nitrogen species (RONS)
production and the antioxidant defense system whereby RONS generation exceeds the capacity of the antioxidant defense system to render RONS inactive [10].

Oxidative stress in anaerobic exercise is accompanied with ischemic reperfusion of muscles and xanthine oxidase activity in addition to electron leakage that happens during aerobic training regime [11]. Also, it has been shown that anaerobic training regime has a positive effect on oxidative status and lower muscular damage was observed compared with aerobic regime [12]. Increased aerobic metabolism during physical activity is a potential source of oxidative stress. Also, anaerobic physical activity and oxidative stress are interrelated because the intense anaerobic activity leads to damage proteins, lipids and nucleic acids in muscle cells and blood [8].

Most of people are likely doing physical activity in the morning because of the air pollution lower than in the afternoon, and the temperature is cooler. Exposure to air pollutants can increase the formation of radicals in the body that can lead to oxidative stress [13]. Based on some research found that in the human body happened some increase activity during physical activity include heart rate, some enzyme activity gastrointestinal, as well as body temperature, oxygen consumption and increased levels of catecholamine which is higher in the afternoon [14].

However, little is known regarding patterns for having physical activity to prevent oxidative stress. In addition, given the potential effect of oxidative stress during physical activity, especially regarding the time or the exercise session. Therefore, it is essential to reveal an overview of the difference of oxidative stress after having sub-maximal physical activity in the morning-afternoon and as a self-reminder for human health system.

2. Materials and Methods

2.1. Subject
Eight man volunteers by using purposive random sampling who were all healthy, man between the ages 20 – 25 years, have normal body mass index. Self-administrated questionnaires established participants to be nonsmoking, free from diabetes, hypertension, and coronary heart disease, and they were not taking medications. Subjects are instructed to not to engage in any exhausting physical activities more than 50 % VO2max in two days before treatment, eat two hours before doing sub-maximal physical activity and the subject asked to riding ergo cycle until 80% HRmax maintained for 6 minutes. Hereinafter, to measure the sub-maximal physical activities in the afternoon the subjects asked to recovery their physical condition for a week.

2.2. Instrumentation and Procedure
Peripheral venous blood was collected from the antecubital vein of the subjects. Blood samples were transported and stored in the biochemistry laboratory where analyses were performed strictly following international guidelines [15]. The following parameters were measured for oxidative stress: malondialdehyde (MDA) and superoxide-dismutase (SOD).

2.2.1. Procedure Measurement malondialdehyde (MDA). MDA has been identified as the product of lipid peroxidation that reacts with thioobarbituric acid to give red species absorbing at 535 nm. All blood samples inserted into the sample rack and sorted according to the colour of the blood sample. 1 ml of patient or control serum was combined with 2 ml of Trichloroacetic acid (TCA) - Thioobarbituric acid (TBA) – Hydrochloric acid (HCL) solution and mixed thoroughly, when heated for 15 minutes in boiling water bath. After cooling, the precipitate was removed by centrifugation at 3000 rpm for 10 minutes. The absorbency was determined at 535 nm against reagent blank, which was containing the entire reagent minus the serum.

2.2.2. Procedure Measurement superoxide-dismutase (SOD). Antioxidant status was determined by measuring the SOD levels in the blood. SOD activity was measured using a commercially available
kit according to manufacturer’s instructions. Plasma samples were diluted 1:5 in sample buffer (50mM Tris-HCl, pH 8.0). SOD activity was measured by utilizing a tetrazolium salt radical detector solution, diluted in assay buffer (50 mM Tris-HCl, pH 8.0, containing 0.1 mM diethylenetriaminepentaacetic acid and 0.1 mM hypoxanthine), to detect superoxide radicals generated by hypoxanthine and xanthine oxidase. One unit of SOD activity is defined as the amount of enzyme needed to exhibit a 50% dismutation of the superoxide radical. Absorbance was read at 450 nm using a Spectra Max Micro plate Reader (Molecular Devices, Sunnyvale, CA).

2.3. Statistical Analysis
Descriptive statistics were determined for each variable recorded. Data are presented as mean±SD. Normality test to measure whether data obtained has a normal distribution so it can be used in parametric statistics (inferential statistics). Paired t-test and independent t-test was applied for Statistical evaluation of the data generated using SPSS (Statistical package for Social Studies) Version 19.0 Software. The Statistical significance level was put at ‘p’ value < 0.05 with Confidence Interval (CI) of 95%.

3. Results
Generally, the average levels of HRmax in the morning and afternoon before having sub-maximal physical activities were 67.63±5.31 bpm and 74.88±4.15 bpm. The average of the characteristics research according to age, height, and weight as follow as: 21±0.46 years old, 165.13±7.16 cm, 61±11.69 kg (Table 1). Normality test data are analyzed using the Kolmogorov-Smirnov test. Based on table 2 obtained $p_{value}$ > 0.05, it means that all of data for MDA and SOD are normally distributed.

| Characteristic of the subject       | Mean | SD (±) |
|------------------------------------|------|--------|
| Age                                | 21   | 0.46   |
| Height                             | 165.13 | 7.16    |
| Weight                             | 61   | 11.69  |
| HR in the morning                   | 67.63 | 5.31    |
| HR in the afternoon                 | 74.88 | 4.15    |

| Variabel              | Morning (p) | Afternoon (p) |
|-----------------------|-------------|---------------|
|                       | Before      | After         | Before    | After         |
| MDA (nmol/ml)         | 0.67        | 0.95          | 0.57      | 0.74          |
| SOD (%)               | 0.81        | 0.99          | 0.87      | 0.68          |

**Malondialdehyde (MDA) response:** Figure 1 shows an overview data of MDA changes before and after having sub-maximal physical activities in the morning and afternoon. Both of MDA average level in the morning and afternoon before and after increase rapidly as follow as: 5.48±3.24 nmol/ml and 8.09±3.05 nmol/ml; 7.31±2.92 nmol/ml and 12.78±2.89 nmol/ml.

**Superoxide-dismutase (SOD) response:** Figure 2 shows that not only SOD average change level in the morning which has decreased but also in the afternoon before and after having sub-maximal physical activities as follow as: 83.37±9.24%; 73.61±11.10%; 79.81±75.96%.
Figure 1. Average of MDA changes before and after having sub-maximal physical activity in the morning and afternoon

![MDA changes](image1)

Figure 2. Average of SOD changes before and after having sub-maximal physical activity in the morning and afternoon

![SOD changes](image2)

Analysis paired t-test of oxidative stress parameters before and after having sub-maximal physical activities for MDA in the morning $p_{value}$ 0.002 (CI: 1.34 – 3.86) and afternoon $p_{value}$ 0.000 (CI: 4.15 – 6.77); SOD in the morning $p_{value}$ 0.027 (CI: 1.51 – 18.00) and afternoon $p_{value}$ 0.001 (CI: -5.62 – -2.17) with confidence level 95%. It shows that there is significant improvement of MDA plasma degree and decreased activity of enzyme SOD before and after having sub-maximal physical activities in the morning and afternoon at table 3.
Table 3. Statistical results of paired t-test: comparing before and after having sub-maximal physical activities in the morning and afternoon

| Variable          | SD before having sub-maximal physical activity | SD after having sub-maximal physical activity | \( p \text{value} \) | (95% CI)         |
|-------------------|-----------------------------------------------|-----------------------------------------------|-------------------|-----------------|
| MDA (nmol/ml)     |                                               |                                               |                   |                 |
| Morning           | 3.24                                          | 3.05                                          | 0.002             | 1.34 – 3.86     |
| Afternoon         | 2.92                                          | 2.89                                          | 0.000             | 4.15 – 6.77     |
| SOD (%)           |                                               |                                               |                   |                 |
| Morning           | 9.24                                          | 11.10                                         | 0.027             | 1.51 – 18.00    |
| Afternoon         | 8.90                                          | 9.81                                          | 0.001             | -5.62 – 2.17    |

Referring to statistical results for independent t-test table 4 there is a significant difference in the levels of plasma MDA (\( p \text{value} = 0.002 \)) and no significant difference in activity of enzyme SOD after having sub-maximal physical activities in the morning and afternoon (\( p \text{value} = 0.119 \)).

Table 4. Statistical results of independent t-test: comparing before and after having sub-maximal physical activities in the morning and afternoon

| Variable          | Mean difference before-after having sub-maximal PA | \( p \text{value} \) | (95% CI)         |
|-------------------|---------------------------------------------------|-------------------|-----------------|
| MDA (nmol/ml)     |                                                   |                   |                 |
| Morning           | 2.61                                              | 0.002             | -4.51 – -1.21   |
| Afternoon         | 5.47                                              |                   |                 |
| SOD (%)           |                                                   | 0.119             | -13.55 – 1.72   |
| Morning           | -9.76                                             |                   |                 |
| Afternoon         | -3.85                                             |                   |                 |

4. Discussion

Several manifestations of physical activity or exercise provided health benefits in healthy and functional muscles and bones, increased strength and endurance, angiogenesis and neurogenesis, reduced risk for chronic disease such as overweight and diabetes, improved self-esteem and psychological well-being, and finally higher levels of subjective and psychological well-being as well as reduced stress, anxiety and depression [16]. Regardless of its positive benefits, exercise had a negative impact, for example, it forms lactic acid and free radicals, because exercise could be a stressor for the body that can affect all systems [17]. Lactic acid is formed as the result of training activities and exercises with high intensity for a long time (prolonged exercise). As for the free radicals are formed by the peroxidation (auto oxidation) fats which are characterized by the formation of Reactive Oxygen Species (ROS), and such formation could cause oxidative stress.

In this study, the researchers compared the difference of oxidative stress after having sub-maximal physical activity in the morning-afternoon. Indirect assessment of oxidative stress involved the measurement of the more stable molecular products formed via the reaction of RONS with certain biomolecules [18]. MDA is one of the ways to measure oxidative stress though it should not be the first choice in research since it is only a secondary product. This substance originates in the course of autooxidation of fatty acids. It is common to be measured through its reaction with thiobarbituric acid [11].

The results of the analysis of plasma MDA before physical activity in the morning 5.48 nmol/ml and in the afternoon 7.31 nmol/ml. MDA is one of the indicators used to measure oxidative stress [19]. MDA plasma levels were higher in the afternoon than in the morning because of pollutants and the ambient temperature in the afternoon was higher than in the morning. This would result in the increase
of the inhalation of pollutants in the afternoon which is also can increase RONS. Overproduction of RONS due can result from a variety of stressors, such as exposure to environmental pollutants [20] excessive nutrient intake [21] or physical activity or exercise [22]. In fact, any situation in which the consumption of oxygen is increased, as during physical activity, could result in an acute state of oxidative stress.

The average of SOD before having physical activity in the morning was 83.37% while in the afternoon was 79.80%. In normal conditions, body will produce antioxidant as a body’s defense system due to the improvement of production of free radicals. Antioxidants are needed by the body as one of the protection systems of the cellular level. Materials and chemical reactions that can negate free radicals are called antioxidants. In contrast, those materials and chemical reactions can cause free radical called prooxidant. In physiological conditions, the balance between prooxidant and antioxidant substances is kept slightly in favor of prooxidant products, thus favoring a mild oxidative stress [23].

The paired t-test revealed that there was a significant improvement of MDA plasma degree and decreased activity of enzyme SOD before and after having sub-maximal physical activities in the morning and afternoon ($p_{value} < 0.05$) as a contributor factor in the incidence of oxidative stress. In addition, there were no significant differences found in sub-maximal physical activities in the morning and afternoon. Physical activity is related to the production of free radicals in terms of intensity and duration of exercise as well as the physical condition of a human body. Therefore, adjusting the intensity of physical activity was essential to prevent oxidative stress [14].

Moving on to the independent t-test, it showed that there was a significant difference in the levels of plasma MDA and no significant difference in activity of enzyme SOD after having sub-maximal physical activities in the morning and afternoon. Psychiatric conditions and circadian rhythms were higher in the afternoon than in the morning. This happened because of the ambient temperature and circadian rhythms, and secretions in the afternoon were higher than in the morning. It is important to note that the increase of rectal temperature by physical activity is greater in the early morning than in the afternoon and late evening [24]. With regard to this, it is suggested for people who want to do physical activity to have good time management as it has profound positive effect for health.

5. Conclusion
Physical activity is very important for human health. This is due to the notion that physical activity or exercise can improve your health and reduce the risk of developing several diseases like type 2 diabetes, cancer and cardiovascular disease, improve physical, and mental health. Physical activity can have immediate and long-term health benefits. Most importantly, regular activity can improve your quality of life. This research found out that having sub-maximal physical activity in the morning and afternoon could increase oxidative stress by measuring the MDL and SOD. Oxidative stress and the subsequent oxidation of LDL are considered a major contributor to the lifestyle-related diseases, impairment of endothelial function and the development of atherosclerotic lesions. This might happen because our body has a limited capacity to increase its antioxidants and control free radicals. Some researchers believe that long, intense exercise (especially endurance training, untrained persons) may cause more oxidative stress than humans can handle. For this reason, it is essential to make body as a self-reminder for human health system by considering the time and the intensity for having physical activities.

References
[1] Warburton D E, Nicol C W, Bredin S S 2006 Health benefits of physical activity: the evidence. can med ass j 174 (6) 801–809
[2] Miriam Reiner, Christina N, Darko J and Alexander W 2013 Long-term health benefits of physical activity – a systematic review of longitudinal studies BMC Public Health 13 813-822.
[3] Haskell W L, Lee I M, Pate R R, Powell K E, Blair S N, Franklin B A, Macera C A, Heath G
W. Thompson P D and Bauman A 2007 Physical activity and public health: updated recommendation for adults from the American college of sports medicine and the American heart association Med Sci Sports Exerc 39 1423–1434

Kesaniemi Y K, Danforth E Jr, Jensen M D, Kopelman P G, Lefèbvre P, Reeder B A 2001 Dose-response issues concerning physical activity and health: an evidence-based symposium Med Sci Sports Exerc 33 S351–S358

Yaylali Y T, Fındıkoglu G, Yurtdas M, Konukçu S and Senol H 2015 The effects of baseline heart rate recovery normality and exercise training protocol on heart rate recovery in patients with heart failure The Anatolian Journal of Cardiology 15(9) 727-734

Murray A, Delaney T and Bell C 2006 Rapid onset and offset of circulatory adaptations to exercise training in men Journal of human hypertension 20(3) 193-200

Martinović Jelena, Violeta Dopsaj, Jelena Kotur-Stevuljević, Milivoj Dopsaj, Aleksandra Stefanović, Goran Kasum and Ana Vujović 2010 Are oxidative stress and antioxidant defense status associated with energy expenditure in athletes of various sports? Serbian Journal of Sports Sciences. 4 (2): 75–81.

Powers S K and Jackson M J 2008 Exercise induced oxidative stress: cellular mechanisms and impact on muscle force production Physiological Reviews 88 1243–1276.

Basavarajaiah S, Boraita A, Whyte G, Wilson M, Carby L, Shah A, Sharma S 2008 Ethnic differences in left ventricular remodeling in highly-trained athletes relevant to differentiating physiologic left ventricular hypertrophy from hypertrophic cardiomyopathy. J Am Coll Cardiol 51 2256-2262

Marija Stanković and Dragan Radovanović 2012 Oxidative stress and physical activity Sport Logia, 8 (1) 1-11

Finaud J, Lac G and Filaire E 2006 Oxidative stress Sports medicine 36(4) 327-358

Selamoglu S, Turgay F, Kayatekin B M, Gonenc S and Yslegen C 2000 Aerobic and anaerobic training effects on the antioxidant enzymes in the blood Acta Physiologica Hungarica 87 267-273

Prahalad A K, Inmon J, Dailey L A, Madden M C, Ghio A J, Gallagher J E 2001 Air pollution particles mediated oxidative DNA base damage in a cell free system and in human airway epithelial cells in relation to particulate metal content and bioreactivity Chem Res Toxicol Jul 14 (7) 879-887

Mohhebbi and H Azizi H 2011 Maximal fat oxidation at the different exercise intensity in obese and normal weight men in the morning and evening Journal of Human Sport and Exercise 6 (1) 49-58

Banfi G and Dolci A 2003 Preanalytical phase of sport biochemistry and haematology The Journal of Sports Medicine and Physical Fitness 43, 223-230

Trevor Archer 2014 Health benefits of physical exercise for children and adolescents J Nov Physiother 4 (2) 1-4

Willmore J H and Costill D L 2008 Physiology of sport and exercise USA Human Kinetics 216-236.

Mohammad S, Najmul I, Zamirullah K, Fauzia K and Mohammad M H 2014 Oxidative stress in sports persons after a bout of intense exercise: a cross sectional study Biomedical Research 25 (3) 387-390

Halliwell B and Gutteridge J M C 2007 Free radicals in biology and medicine Oxford: Oxford University Press P: 33

Halliwell B 1991 Reactive oxygen species in living systems: source, biochemistry, and role in human disease Am J Med 91 14-22

Sies H, Stahl W, Sevanian A 2005 Nutritional, dietary and postprandial oxidative stress J Nutr. 135 969-972

Vollard N B, Shearman J P, Cooper C E 2005 Exercise-induced oxidative stress: myths, realities and physiological relevance Sports Med 35 1045-1062
[23] W Dröge 2002 Free radicals in the physiological control of cell function *Physiological Reviews* **82** (1) 47–95

[24] Aldemir H, Atkinson G, Cable T, Edwards B, Waterhouse J, and Reilly T 2000 A comparison of the immediate effects of moderate exercise in the early morning and late afternoon on core temperature and cutaneous thermoregulatory mechanisms *Chronobiol Int.* **17** 197–207