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Lipid Peroxidation Level on Moderate-Intensity Interval Exercise on Non-Athlete Adolescent

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Abstract

Exercise is commonly perceived as a pleasurable and healthy activity. However, it is classified as a complex activity that potentially enhances the formation of free radicals and oxidative stress. This study aims to analyze lipid peroxidation, as the biomarker of free radicals, in interval exercise. A total of 16 trained non-athlete male adolescents, aged 20-21 years old, with normal blood pressure, normal resting heart rate, and gave good level of physical fitness voluntarily participated in this study. The participants were randomly separated into two groups, namely the K_1 (*n*=8, interval exercise with active resting medium intensity pedaling ergometer bike) and K_2 (*n*=8, interval exercise with resting without pedaling the ergometer bike). The interval exercise was carried out at 60-70% HR_{max} intensity for 35 minutes. The blood sample for Malondialdehyde (MDA) level analysis, as the lipid peroxidation biomarker, was taken using Thiobarbituric acid reactive substances (TBARS). The blood samples were collected before and after the interval exercise. The obtained data were analyzed using the one-way ANOVA test with a 5% significance level. Our results suggest no significant different level of MDA, a lipid peroxidation biomarker, between the interval exercise with resting through medium intensity pedaling ergometer bike and the interval exercise with resting with no pedaling ergometer bike. However, the interval exercise resting through pedaling an ergometer bike presented relatively higher lipid peroxidation than the interval exercise resting without pedaling an ergometer bike. Therefore, following the free radical concept, interval exercise with resting through pedaling an ergometer bike is more beneficial than the exercise without pedaling an ergometer bike resting.

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INTRODUCTION

Regular exercise is the first line of non-pharmacological treatment (Thomas et al., 2022) that is capable of preventing the formation of free radicals (Pan and Chen, 2021; Yosika et al., 2020). Therefore, exercise is deemed a beneficial non-pharmacological approach to maintaining the body's homeostasis toward the formation of free radicals and antioxidants (Seputra et al., 2022; Tofas et al., 2019). The moderate and physiological formation of free radicals is a valuable molecular signal to increase the adaptive regulation of increasing strength of muscle contraction (Thirupathi et al., 2021a). It also carries an essential role in the regulation of cell signaling and gene expression, required for muscle adaptation, as well as increasing antioxidant gene regulation (Erjavec et al., 2022). Therefore, exercise is an adaptation trigger to increase the antioxidant enzyme (Pan and Chen, 2021) and enhance antioxidants, preventing the negative effects of free radical reactivity (Lu et al., 2021). Thus, it prevents oxidative damage (Erjavec et al., 2022) and oxidative disease (Thirupathi et al., 2021b). Consequently, the formation of free radicals induced by exercise is highly beneficial in inhibiting a number of diseases and enhancing physical fitness, cardiorespiratory, capillary density, and synthesis of high-density lipoprotein (HDL) (Thomas et al., 2022). However, the exercise response and adaptation toward lipid peroxidation, a physiological biomarker of free radicals' formation, have not been extensively reviewed and analyzed. This subject is essential as it can be an opening point of the concept of exercise as a strategy to place exercise as a safe and profitable lifestyle that improves the metabolism and physiological health (McKeegan et al., 2021).

Exercise is a complex strategy to modulate free radicals, depending on its model, intensity, and duration (Ye et al., 2021). The formation of free radicals initiated by exercise can be beneficial in physiological conditions, to improve metabolism health (Simioni et al., 2018), as well as serving to be a vital signal in increasing antioxidant enzyme and muscle cell adaptation during the exercise (Erjavec et al., 2022). A previous study reported that continuous and medium-intensity exercise enhance the free radicals and formation of antioxidants, preventing oxidative stress and protecting from oxidative stress damage (Pan and Chen, 2021). However, high-intensity exercise can escalate the formation of non-physiological free radicals, increasing oxidative stress (Lu et al., 2021) caused by the imbalance between free radicals and antioxidant formation (Pan and Chen, 2021). Oxidative stress causes cell susceptibility and oxidative damage to the cellular components, inducing degenerative diseases, such as type 2 diabetes Mellitus, cardiovascular disease, neurodegenerative, and premature aging (Tofas et al., 2019). Comparatively, well-regulated exercise can be a trigger to increase antioxidant formation (Thirupathi et al., 2021b), precluding oxidative stress (Canals-Garzón et al., 2022).

Free radicals are regularly formed and physiological situations (Thirupathi et al., 2022). However, during exercises, the formation of free radicals tends to increase (Nobari et al., 2021), relying upon the exercise intensity, duration, and frequency (Thirupathi et al., 2021b). Previous research discovered that high-intensity acute exercise accelerated the formation of free radicals, which was not proportional to antioxidant formation (Simioni et al., 2018). Taherkhani et al. (2021) also reported that the exercises oriented to enhance physical performance and achievement significantly increase free radical formation. Additionally, studies also revealed that high-intensity exercise (Zeng et al., 2020) and anaerobic exercise substantially improve free radical formation (Tofas et al., 2019). Linearly, ultra-marathon running also expands the formation of free radicals, but it is not equal to the increase of antioxidants (Thirupathi et al., 2021a). However, other research discovered increasing free radical formation in medium-intensity workouts (Zhang, 2022) and aerobic exercise (Tofas et al., 2019). The exercise response toward free radical formation is highly varied, so exercise management is required to generate physiological free radicals (Lu et al., 2021). Therefore, this study examines the response of moderate intensity interval exercise toward free radical formation during the physiological condition to construct a strategy to prevent oxidative damage and increase metabolism health. This study used medium-intensity exercise with resting while pedaling the ergometer bike and resting without pedaling the ergometer bike on non-athlete trained participants. The formation of free radicals was detected using a lipid peroxidation biomarker by analyzing MDA levels with the TBARS method. It was essential to examine the exercise response toward the formation of free radicals in non-athlete trained participants. Later, our findings were expected to aid the construction of a strategy that minimized the oxidative stress that may result in tissue damage and free-radicals increase related diseases. Besides, the formation of a proper resting regulation that potentially prevents non-physiological free radicals increase is required. The resting regulation also potentially boosted exercise adaptation and physical performance.

METHODS

Research Design

This true experiment study aimed to analyze the response of medium-intensity exercise with resting intervals by pedaling an ergometer bike and moderate-intensity exercise with resting without pedaling toward the formation of free radicals. This study involved male non-athlete trained participants aged 19 – 22 years old with normal body mass index (BMI), excellent physical fitness, normal blood pressure, normal resting heart rate, and did not smoke. The participants consented to be involved in this study, as proven by the filling and signing of informed consent. A total of 16 participants were selected for this study. Those participants were divided into two groups G_1 (*n*=8, interval exercise with resting while pedaling the ergometer bike) and G_2 (*n*=8, interval exercise with resting without pedaling the ergometer bike but keeping sitting on the ergometer bike).

Mechanism of Exercise

The participants have explained the research purpose, research procedure, and other relevant provisions. The explanation included the procedure of the interval exercise using the ergometer bike (Ergocycle Technogym). The training was carried out at the same time, in the morning, from 06.00–10.00 a.m. The exercise was carried out at room temperature, as well as relatively comfortable humidity and air circulation.

The interval exercise was carried out by pedaling an ergometer (Ergocycle Technogym) bike for four minutes with 6 Measurement Equivalent Time (MET) intensity, 50-60 rpm paddle speed, and 100-watt power, followed by 4 minute resting interval. The exercise was completed with four working intervals and three resting intervals. The resting interval was carried out by keeping pedaling the ergometer (Ergocycle Technogym) bike with 2 MET (G_1) intensity and sitting on the ergometer bike resting interval without pedaling the bike. The comparison for the working and resting interval was 1:1, with a total of 24 minutes working interval and 12 minutes resting interval.

Procedure of Data Collection

The subject was selected using inclusion criteria and excellent physical fitness criteria (40–45 mL/kg/min). Physical fitness was measured using the Multi-Stage 20-m Shuttle Run Fitness Test (Sugiharto et al., 2022). Further, a 4 ml blood sample was drawn from the cubital vein prior to the interval exercise and 30 minutes after the exercise. Meanwhile, the free radical was determined using a lipid peroxidation biomarker by analyzing the MDA level using the Thiobarbituric acid reactive substances (TBARS) method (Seputra et al., 2022; Yosika et al., 2020; Spirlandeli et al., 2013) with nmol/mL unit (Erjavec et al., 2022). The test of free radicals was carried out in the Physiology Laboratory of the Faculty of Medical, Universitas Brawijaya Malang.

Data Analysis Technique

The obtained data were analyzed using Paired Samples t-Test and Independent Samples t-Test with a 5% significance level.

RESULT

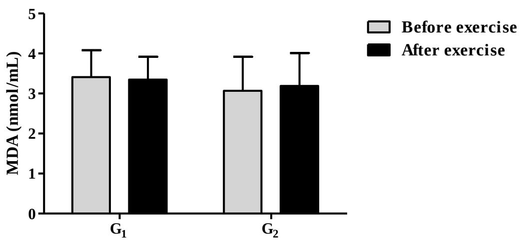
The statistical analysis results of the participants' characteristics showed no significant differences in each variable between the group members, as shown in Table 1.

Table 1. Results of Statistic Analysis on Each Subject's Characteristics from Two Groups

Variable	**G_1 (*n*=8)**	**G_2 (*n*=8)**
Age (yrs)	21.13±0.35	21.25±0.46
BH (cm)	173.13±2.77	171.29±3.13
BW (kg)	60.19±5.93	59.80±5.95
BMI (kg/m^2)	20.15±0.45	20.28±0.57

Description: BH: Body height; BW: Body weight; BMI: Body mass index.

The results of MDA level analysis on the effects of interval exercises with resting by keeping pedaling the ergometer bike and the interval exercise with resting without pedaling the ergometer bike toward the lipid peroxidation, as the free radical biomarker, are illustrated in Figure 1.



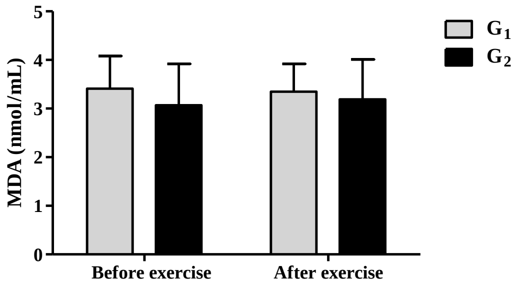


Fig. 1 Average and SD of MDA Level on Each Group

According to the research results presented in Figure 1, the average MDA level on the interval exercise with resting by pedaling an ergometer bike was lower than the MDA level for interval exercise with resting without pedaling the ergometer bike. During the interval exercise with resting by pedaling an ergometer bike, the MDA level tended to decrease, while in the interval exercise with resting without pedaling an ergometer bike, the MDA level tended to increase. Additionally, the results of the independent sample T-test showed no significant difference in MDA level between the groups ($p \geq 0.05$). Meanwhile, the results of a paired sample T-test showed the presence of significantly different MDA levels before and after the exercise in each group ($p \geq 0.05$).

DISCUSSION

The formation of free radicals is a physiological condition in normal metabolism, so it is inevitable during the metabolism processes (lu et al., 2021). However, during the resting condition, the free radical formation is relatively low and equal to the antioxidant formation (taherkhani et al., 2021). The physiological free radicals level becomes the molecule that acts as a signal in enhancing muscle adaptation and regulating the optimization of antioxidant function, both the enzymatic and non-enzymatic antioxidant (thirupathi et al., 2021b). Exercise carries the potential to increase physiological and non-physiological free radicals (thirupathi et al., 2021a). Our findings suggested a non-significant increase of lipid peroxidation, the biomarker of free radicals formation, in the non-athlete trained participants after the medium-intensity exercise with resting without pedaling a bike. Meanwhile, on the moderate intensity exercise with resting through pedaling an ergometer bike, the lipid peroxidation tended to decrease, as illustrated in figure 1. Interestingly, both responses of medium intensity exercise toward the free radical formation, those that tended to increase and decrease, show no significant difference. We estimated that exercise intensity, model, and level of the participants affect the response of free radical formation. Linearly, previous studies reported that the formation of free radicals is affected by the exercise intensity or distance, frequency, and individualization levels (gender, age, workout level (tryfidou et al., 2020), as well as workout types, model, and duration (prasertsri et al., 2022). This finding signifies that response to exercise is a complex physiological process involving all of the human systems, starting from the system in the cell to the molecular level (lu et al., 2021). The process induces physiological, hematological, and biochemical transformations, including the formation of free radicals and oxidative stress (erjavec et al., 2022). Our research results also confirm that exercise intensity seems to be the stimulus for the regulation of free radical biomarkers in both high and moderate intensity. Therefore, it positions governance of free radical formation during the exercise as a crucial and strategic approach (zeng et al., 2020). A number of previous research recorded that moderate intensity workout produces the moderate and physiological formation of free radicals (erjavec et al., 2022), decreases the production of reactive oxygen species (ros), and enhances the antioxidant capacity within the muscle cell (flensted-jensen et al., 2021), as well as preventing oxidative damage (thirupathi et al., 2021a).

Exercise potentially increases lipid peroxidation, but moderate-intensity exercise produces a lower formation of free radicals. Therefore, it confirms the vital role of exercise in physiological conditions that is capable of increasing free radicals, along with antioxidant formation, depending on the exercise's intensity, duration, and frequency (pan and chen, 2021). Another study revealed that response to exercise is proportional to exercise intensity and inversely proportional to someone's physical condition (tofas et al., 2019). Research on the animal test (rat) with 30 minutes of exercise for ten weeks uncovered a balance of free radical and antioxidant increase, which decreased pro-inflammatory cytokines (semeraro et al., 2022). Meanwhile, moderate-intensity aerobic exercise enhances the antioxidant capacity of the body (lu et al., 2021). Aside from the exercise intensity, the training level is also one of the factors that increase lipid peroxidation after the exercise (el abed et al., 2019a). The low level of lipid peroxidation in this study is probably caused by the factor of training level. Our participants were trained, but they were not-athlete who had regular physical training. Previous research reported that trained individuals have a better ability to adapt to regular or acute exercise while also presenting a more robust antioxidant defense, preventing the increase of lipid peroxidation (erjavec et al., 2022). Several studies also reported that a physically active person has lower oxidative stress and better antioxidant defense than people who are physically inactive (lu et al., 2021; powers et al., 2020). Additionally, hormesis theory explains that trained individuals and individuals who actively and regularly exercise have increased free radical formation, adaptive response, and active signal transduction pathways to improve antioxidant formation (tofas et al., 2019). Besides, the people trained with progressive exercise have a particular period for the increased defense regulatory mechanism to protect their bodies from oxidative damage (thomas et al., 2022). The trained individuals possibly have excellent adaptive skills toward exercise, enhancing their ability to regulate lipid peroxidation (el abed et al., 2019b) and antioxidant ability (erjavec et al., 2022) so that they can prevent excessive free radical formation and oxidative stress (tofas et al., 2019).

The results of our research suggest different lipid peroxidation levels, as the free-radical biomarker, between moderate intensity exercise with resting while pedaling a bike and without pedaling a bike. Impressively, in the medium intensity exercise with pedaling bike resting, the lipid peroxidation tended to decrease, while in the medium intensity exercise without pedaling resting, the lipid peroxidation tended to increase, as presented in figure 1. A study similar to this study using 30/30 intermittent running exercise reported that the exercise-induced greater lipid damage than the 15/15 continuous exercise. That study also revealed that continuous running stimulated a better balance of free radicals production and antioxidant defense compared to intermittent running (souissi et al., 2020). However, another study reported that continuous exercise with resting intervals significantly decreases the mda level (prasertsri et al., 2022). The different results may be caused by different exercise types and intensities. Different variations and types of exercises present different effects (thomas et al., 2022). The dynamic of mda responds as lipid peroxidation marker, the free radical biomarker, during the exercise is varied and not consistent, depending on the exercise types and intensity, as well as the training level (el abed et al., 2019b). The age factor and training level also affect the response and adaptation to free radicals (prasertsri et al., 2022). However, we presume the different lipid peroxidation tendency revealed in this study is caused by the redistribution of blood flow factor after the exercise. Redistribution of blood flow in the active cells, primarily on the skeletal muscle, also decreases the blood flow on the visceral organ, enabling hypoxia, followed by reperfusion in the concurrent condition. In time, it becomes other reactive oxygens, then a mediator for hydrogen peroxide formation (poblete aro et al., 2015). The different lipid peroxidation between the two exercises in this study can be caused by the different filling of oxygen consumption (souissi et al., 2020) and lactic acid accumulation, as well as lactic acid recovery. Referring to the lactic acid recovery model, resting while still actively doing light activities can decrease lactic acid faster (seputra et al., 2022), while lactic acid exposure can enhance the formation of free radicals (san-millan et al., 2022). Lactic acid and change of nadh/nadph ratio are correlated with the adaptive response of the tissue toward exercise. In contrast, the increase and decrease of lactic acid through light exercise enhances the nadh/nadph ratio, stimulating the antioxidant enzyme activity in scavenging free radicals (brooks, 2020). Interestingly, the tendency of lipid peroxidation increase is not significantly different from the lipid peroxidation before the workout, so exercise produces physiological lipid peroxidation. The insignificantly different lipid peroxidation may be induced by the exercise characteristics, including its intensity, type, and model (prasertsri et al., 2022) since exercise intensity and level also affect the adaptive response to exercise (radak et al., 2017). Previous research reported that light to moderate regular exercises reinforces the antioxidant defense mechanism of endogenous antioxidant defense and decreases oxidative stress level (ye et al., 2021).

The results of our findings also indicate that moderate-intensity interval exercise is secure for toxic lipid peroxidation without the risk of increased oxidative stress. Additionally, the increasing sod as the antioxidant enzyme within the body does not entirely remove the free radicals, but it maintains the body’s balance (lu et al., 2021). The physiological and proper lipid peroxidation act as the second intracellular that mediates metabolic stress adaptation and stimulates glucose transporter 4 (glut4) in the skeletal muscle fibers (henríquez-olguin et al., 2019). Consequently, the medium-intensity workout with resting while actively pedaling the bike and resting without pedaling can be recommended as a strategy for an active lifestyle and motivating people to exercise. This moderate-intensity interval exercise is easy to be carried out and enhances the feeling of comfort and security so that this exercise can be carried out by non-athlete trained people as well as the general society with a sedentary lifestyle (prasertsri et al., 2022). Our findings contribute to the development of molecular-based physiological exercise research, formulating exercises that carry optimum benefits in increasing people’s physical fitness and health. However, this study also has limitations that require follow-ups. First, this study used acute exercise, so it was unable to illustrate the adaptation of the exercise. Besides, we only involved a trained group. We did not compare our results with the untrained population, so our conclusion did not represent all groups. Additionally, this study only investigated the formation of free radical-induced by exercise using a lipid peroxidation biomarker, so future studies are suggested to use other biomarkers.

CONCLUSION

Our research findings suggest no significant increase in lipid peroxidation, the biomarker of free radicals, before and after the exercises. Besides, there was also no significant increase of lipid peroxidation between the moderate intensity exercise with interval resting by still pedaling a bike and moderate intensity exercise with resting without pedaling an ergometer bike. Therefore, the medium intensity with interval resting with pedaling a bike and without pedaling can be recommended as a nano-pharmacological approach and active lifestyle safe from lipid peroxidation increase and oxidative DAMAGE.

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