

Preliminary study: immediate effect of moderate-intensity interval exercise on nitric oxide and other oxidative stress markers from stationary bicycling in healthy sedentary men

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Abstract

This study aimed to evaluate the immediate effect on nitric oxide (NO) and other oxidative stress parameters (malondialdehyde, MDA; glutathione, GSH; and protein hydroperoxide, PrOOH) from moderate-intensity interval exercise (MIIE) compared with moderate-intensity continuous exercise (MICE). This study was performed on 14 healthy sedentary males aged between 18 and 23 years with a mean age of 20.21 ± 1.47 years. All the participants were required to take MICE randomly at 60% of maximal oxygen consumption (VO_{2max}) for 30 mins, and MIIE in three cycles of between low- and moderate-intensity at 40% and 70% of VO_{2max} , respectively, for 37 mins with stationary bicycling. Before and immediately after exercise, blood of the participants was taken to evaluate oxidative stress markers. The results found that none of the parameters were statistically different before either MICE or MIIE exercises; NO (1.86 ± 0.68 and 1.76 ± 0.59 $\mu\text{mol/L}$), MDA (1.89 ± 0.59 and 1.81 ± 0.53 $\mu\text{mol/L}$), GSH (2.86 ± 0.65 and 2.75 ± 0.64 mmol/L), and PrOOH (1.47 ± 0.55 and 1.43 ± 0.55 $\mu\text{mol/L}$). After MICE, the NO (2.53 ± 0.58 $\mu\text{mol/L}$) and MDA (2.32 ± 0.52 $\mu\text{mol/L}$) increased, whereas GSH (2.39 ± 0.52 mmol/L) decreased significantly, the same as in the MIIE results regarding MDA (2.16 ± 0.54 $\mu\text{mol/L}$) and NO (2.80 ± 0.62 $\mu\text{mol/L}$). However, the GSH result was not significantly different after finishing the MIIE (2.56 ± 0.59 mmol/L). Neither exercise affected the PrOOH level. It was noticeable that the oxidative stress parameters between exercises were not statistically different, except for the NO in MIIE compared with MICE. Therefore, it can be concluded that MIIE in this preliminary study induces more release of NO and involves less antioxidant GSH when compared with MICE.

Keyword: Moderate-intensity continuous exercise, Moderate-intensity interval exercise. Nitric oxide, Oxidative stress..

Introduction

Exercise is a physical activity that maintains and enhances physical fitness (Porcari et al, 2015), which promotes health and prevents non-communicable diseases such as diabetes, obesity, and sarcopenia in aging people (Rueggsegger & Booth, 2018). Daily moderate-intensity exercise for 20 min three times a week is suggested by the American College of Sports and Medicine (ACSM) guideline (2018). Exercise training not only improves cardiopulmonary function by increasing gas exchange and ventilation for enhancing oxygenation within the muscle part (Heinonen et al, 2014), but also by increasing hormone and insulin activity (Williams et al., 2019; Wen et al., 2019; Cao et al., 2019). The theory of beneficial effects of regular exercise has been based on enhanced blood circulation in active muscles related to vessel vasodilation via nitric oxide (NO) function (Goto et al, 2007). Since 2008, interval exercise at high intensity for 4-12 weeks has been performed popularly. It improves aerobic capacity (Thompson 2019) with the highest oxygen consumption (VO_2), which enhances the aerobic oxidation pathway, the same as cardiopulmonary stimulation (Williams et al, 2019; Cao et al, 2019). In particular, the acute response to high-intensity interval exercise (HIIE) increases relative oxygen utilization significantly more than moderate-intensity continuous exercise (MICE) (Cabral-Santos et al, 2017). However, exercise at high intensity has been reported as an adverse event on the contracted muscle (Heinonen et al, 2014) and induced oxidative stress status (Kawamura & Muraoka, 2018). High free radical molecules are produced within the mitochondria during chain reactions in electron transportation; for example, superoxide (O_2^-), hydroxyl radical ($OH^\cdot$) by superoxide dismutase (SOD), and catalase function (Power & Jackson, 2008). These molecules affect the biological compartment through lipid peroxidation, protein oxidation, and DNA damage (Tofas et al, 2020). Therefore, antioxidant molecules, especially antioxidant glutathione (GSH), alpha-tocopherol (Vitamin E), and ascorbic acid (Vitamin C) are reduced because of their function on scavenging free radicals and non-radical molecules (Ji et al, 2016; Halliwell & Gutteridge, 2015). The effects of exercise related to NO

release are still controversial and discussed as to whether short or prolonged period training should be performed. A previous study found that the plasma NO level increased in athletes and sedentary subjects after acute ergometer exercise (Franco et al, 2001) and 3 hours after stopping the heavy-intensity exercise (Souza et al, 2019). In addition, moderate intensity is very challenging and safe as same as the interval training exercise for all people. Unfortunately, no evidence of NO or other oxidative stress markers during interval exercise at moderate intensity has been reported. Therefore, this preliminary study aimed to identify the effect of interval exercise at moderate intensity on the oxidative stress parameters, especially NO in male sedentary participants.

Methods

This study had a crossover experimental design for healthy sedentary men. Acute response to NO and other oxidative stress parameters from interval exercise at moderate intensity was compared with continuous exercise at moderate intensity. The study protocol was based on a previous study by Jamuratas et al (2018) on the acute responses to oxidative stress from high-intensity interval training (HIIT) with a cross-study design in 12 sedentary participants and approved by the Ethical Human Committee at the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand (AMSEC-64-EX-040). After calculating the sample size with the G*Power program and paired t-test under the malondialdehyde (MDA) level, a total of 14 participants were recruited into this study. The inclusion criteria for this study included sedentary and healthy male participants aged 18-23 years old, with normal body mass index (BMI) (18.5-24.9 Kg/m²) (ACSM, 2018), and without previous COVID-19 infection, fever (37.8 °C), or any symptoms such as cough, sore throat, dyspnea, vomiting, rash, or taste disorder before the 14-day study (Viner et al, 2020). The exclusion criteria comprised previous illnesses and hospitalization, reported unstable angina, chest pain, heart failure, myocardial infarction, acute pericarditis, arrhythmia, heart block, musculoskeletal disorder, hypertension, and communication or psychological impairment (Hillegeass 2011).

After the participants had signed their consent form, they randomly picked up a ball that had the number of each type of exercise on it; moderate-intensity continuous exercise (MICE) (numb.1) or moderate-intensity interval exercise (MIIE) (numb.2). Three appointment times were made for exercise testing by stationary ergometer in order to determine the direct maximal oxygen consumption (VO_{2max}) using the breath-by-breath technique, and the last two different exercises from the previously randomized selection were performed one-week apart (Souza et al, 2019). Heavy-intensity exercise, caffeine or alcohol consumption, and nutrient supplements were non-permitted before each test. Before each exercise and 10 min after completing the tests at the last two appointments, five milliliters of whole blood were taken from the anterior brachial vein by a medical technologist and kept in an ethylenediaminetetraacetic (EDTA) tube for NO and other evaluations of oxidative stress. If any participant had symptoms; for example, palpitation, vertigo, dyspnea, or leg fatigue during data collection, they were excluded from the study (Hillegeass 2011). All the participants were not permitted to indulge in moderate to heavy-intensity exercises or activities, or extra-vitamin or nutrient supplements during the study period.

Exercise Programs

Moderate-intensity continuous exercise (MICE): Warm-up exercise on a stationary ergometer without workload for 3 minutes was taken before 20 minutes of continuous exercise at 100 watts and 60% of VO_{2max}. Then, a 10-minute cool-down exercise was taken without workload. Thirty-three minutes of total exercise was performed under direct VO₂ determination using the breath-by-breath technique connected to a gas analyzer (CPX Ultima Gas Analyzer, St. Paul, Minnesota, USA) (Tsukamoto et al, 2016; Garber et al, 2011).

Moderate-intensity interval exercise (MIIE): Warm-up exercise on a stationary bicycle without workload was taken for 3 minutes before alternative three-cycle workloads of 40% and 70% of VO_{2max} for 3 and 4 minutes, respectively. Then, a 10-minute cool-down exercise was taken without workload. Therefore, 37 minutes of total exercise time was performed under direct VO₂ determination using the breath-by-breath technique connected to a gas analyzer (CPX Ultima Gas Analyzer, St. Paul, Minnesota, USA) (Wen et al, 2019; Tsukamoto et al, 2016; Garber et al, 2011).

Evaluation of oxidative stress parameters

A 400 µL volume of whole blood was taken from 5 mL and kept in an anticoagulant EDTA tube to analyze GSH by using 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) reagent (Ellman, 1959). Plasma was separated from residual whole blood by centrifuging at 3,000 rpm for 10 minutes. A 200 µL volume was processed immediately with Griess reagent to analyze NO (Grisham et al., 1996), whereas, 100 µL was taken to determine MDA by Thiobarbituric acid reactive substance (TBARs) (Zeb & Ullah, 2016). Then, a residual volume of 100 µL was taken for evaluating protein hydroperoxide (PrOOH) by 2,4-dinitrophenylhydrazine (DNPH) reagent (Weber et al, 2015). All parameters were laboratory evaluated by spectrophotometry (UV-VIS Shimadzu).

Statistical analysis

The normal distribution of all data was investigated statistically by the One-sample Kolmogorov-Smirnov test before presenting as mean and standard deviation. All of the parameters were analyzed statistically by the One-way ANOVA (2 types of exercises, 2 times) and Least Significant Difference (LSD) *post hoc* test. All statistical analyses were carried out using the statistical package for social science software (SPSS) version

11.0 (SPSS Inc. Chicago, IL, USA) for windows. All of the tests were used with significance at $p < 0.05$. In addition, the effect size (dz) of the results was investigated using G*Power 3.1.9.4 (Franz Faul, University Kiel, Germany).

Results

The results of all 14 male participants, aged 18-23 years old with normal BMI, direct VO_{2max} and VO_2 at 60%, 40%, and 70% of VO_{2max} , were evaluated by the breath-by-breath technique, as presented in Table 1. The results of the analyzed data distribution of all the oxidative stress parameters were normal ($p > 0.05$). Therefore, the data were represented by the mean and standard deviation (SD), and minimal and maximal values (Table 2).

Table 1. Characteristics and VO_2 of the participants

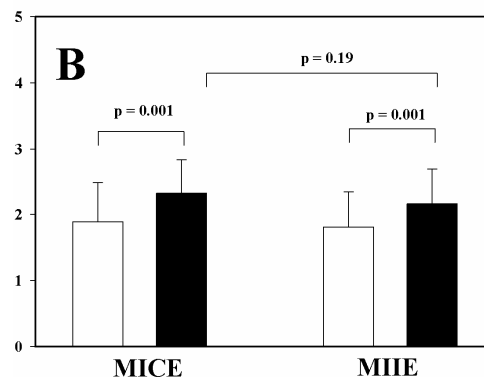
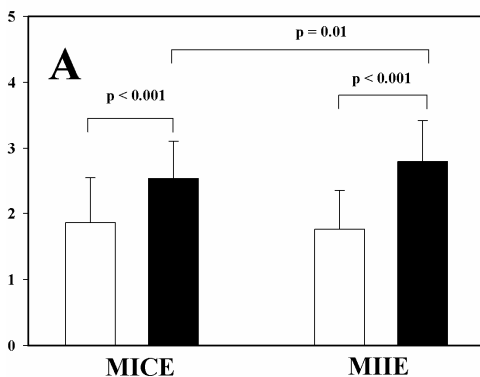
Parameters	Mean (S.D)	Min-Max
Characteristics		
Age (years)	20.21 (1.47)	18-23
Height (meters)	1.74 (0.05)	1.65-1.85
Weight (kilograms)	69.28 (4.51)	62-80
BMI ($Kg.m^{-2}$)	22.65 (0.90)	20.99-23.88
VO_2 (mL/Kg/min)		
VO_{2max} (mL/Kg/min)	35.24 (3.99)	29.50-42.20
VO_2 at 60% of VO_{2max}	14.09 (1.59)	11.80-16.88
VO_2 at 40% of VO_{2max}	21.15 (2.39)	17.70-25.32
VO_2 at 70% of VO_{2max}	24.67 (2.79)	20.65-29.54

The result of NO and other oxidative stress parameters at the baseline showed no statistical difference between exercises (NO = 0.196, MDA = 0.181, GSH = 0.441, and PrOOH = 0.291), with similar results after exercise (MDA = 0.19, GSH = 0.32, PrOOH = 0.196), except for NO ($p = 0.01$).

Table 2. Nitric Oxide (NO) and other oxidative stress parameters

Parameters	MICE		MIIE		dz
	Before	After	Before	After	
NO ($\mu mol/L$)	1.86 ± 0.68 (1.0-3.5)	2.53 ± 0.58 (1.5-3.6)	1.76 ± 0.59 (0.8-2.9)	2.80 ± 0.62 (1.8-3.9)	0.82
MDA ($\mu mol/L$)	1.89 ± 0.59 (1.1-2.9)	2.32 ± 0.52 (1.3-3.5)	1.81 ± 0.53 (1.1-2.7)	2.16 ± 0.54 (1.2-2.9)	0.36
GSH (mmol/L)	2.86 ± 0.65 (1.5-3.9)	2.39 ± 0.52 (1.4-3.2)	2.75 ± 0.64 (1.8-3.5)	2.56 ± 0.59 (1.5-3.4)	0.35
PrOOH ($\mu mol/L$)	1.47 ± 0.55 (0.5-2.3)	1.58 ± 0.47 (0.9-2.2)	1.43 ± 0.55 (0.5-2.2)	1.50 ± 0.53 (0.6-2.3)	0.37

Note: Data present the mean and standard deviation (min-max). MDA = malondialdehyde, GSH = glutathione, NO = nitric oxide, PrOOH = protein hydroperoxide, dz = effect size, MICE = moderate-intensity continuous exercise, MIIE = moderate-intensity interval exercise.



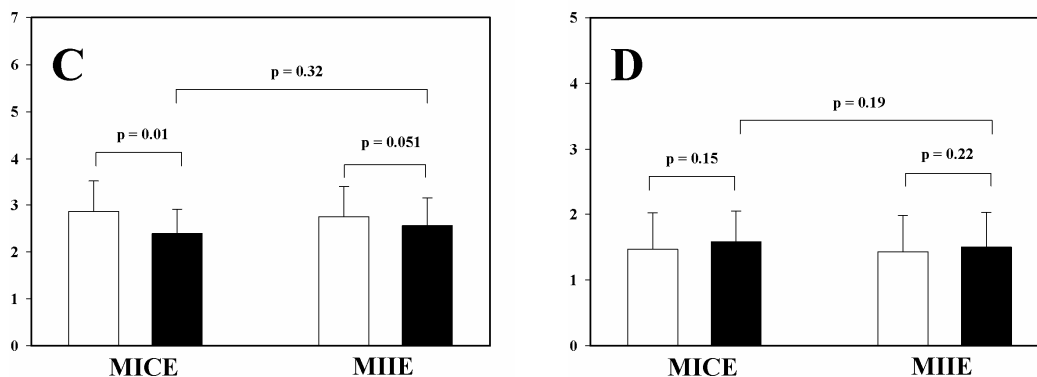


Figure 1. Oxidative Stress parameters; NO (A), MDA (B), GSH (C), and PrOOH (D) between before (white bar) and after exercise (black bar), and between MICE and MIIIE.

When comparing between before and after exercise of each type, the results were statistically different in NO (MICE, $p < 0.001$ & MIIIE, $p < 0.001$), MDA (MICE, $p = 0.001$ & MIIIE, $p = 0.001$), and GSH (MICE, $p = 0.01$ & MIIIE, $p = 0.051$), but not for PrOOH (MICE, $p = 0.155$ & MIIIE, $p = 0.22$) (Figure 1).

In addition, when comparing the analysis between types of exercise after completing the exercises, the results showed that NO from MIIIE was significantly higher when compared with that from MICE ($p = 0.01$) (Figure 1 A). Whereas, the MDA and GSH showed no statistical difference between types of exercise ($p = 0.19$ & 0.32) (Figure 1 B and C). Finally, there was no statistical difference in PrOOH between the types of exercise before they started and after their completion (Figure 1 D).

Moreover, the results of dz were calculated from t-tests (Means: the difference between two dependent means (matched pairs) showed a high dz (0.82) in NO, but low dz (0.3) in other parameters.

Discussion

This preliminary study aimed to investigate the effect of interval exercise on NO and other oxidative stress parameters (MDA, GSH, and PrOOH). Previous evidence reported that NO and other oxidative stress parameters correlated to exercise tolerance and physical function (Moncada et al, 1991). NO, especially, is not only generated within the endothelium but also a recently discovered NO synthase (NOS)-like enzyme residing in red blood cells (RCs), which increases blood flow and oxygen delivery to muscles during exercise (Simmonds et al, 2014). Thus, NO directly affects vascular vasodilation in active muscles (Gilligan et al, 1994).

Previous document reported that continuous exercise at heavy intensity for 30-60 min for 5 days a week improved physical endurance (ACSM et al, 2018) by under the blood flow in the active muscles and vasodilation from the NO mechanism (Goto et al, 2007), the same as in HIIIE (Thompson 2019). A previous document reported that continuous exercise at heavy intensity for 30-60 min, 5 days a week, improved physical endurance (ACSM et al, 2018) by blood flow. Improvement in maximal VO_{2max} was reported when compared with MICE (Cabral-Santos et al, 2017). In addition, it also has been claimed that heavy-intensity exercise creates higher oxidative stress in exercised muscles (Heinonen et al, 2014). Mitochondria is the main power source of free radical generation during exercise (Kawamura & Muraoka, 2018), the same as metmyoglobin and methemoglobin (Cooper et al, 2002). Free radical molecules such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), NO, and peroxy nitrite ($ONOO^-$) affect intracellular antioxidant compounds, especially GSH, Vitamin C and alpha-tocopherol (Vitamin E) (Ji et al., 2016; Halliwell & Gutteridge, 2015). Therefore, protein and lipid were the main targets of oxidation within the cellular compartment (Tofas et al., 2020).

The important NO marker is the main point in this study. The results found that NO increased after both the continuous and interval exercises were completed, which is consistent with previous evidence of acute effect from bicycling exercise (Franco et al, 2001). Both types of exercise were designed at moderate intensity (40-70% of VO_{2max}), which is consistent with previous evidence (Garcia, 2011). NO increased significantly after acute MIIIE by bicycling at 25%, 50% and 75% of VO_{2max} for 30 minutes (Garcia, 2011). Although NO levels increased after both types of exercise, there was a significant difference in MIIIE when compared with MICE. Therefore, interval exercise at moderate intensity possibly induces more NO release than continuous exercise. The reason for a higher NO level from interval exercise cannot be explained. There are various mechanisms; for example, acetylcholine neurotransmitter release at the neuromuscular junction (Dyakova, et al, 2015) and nuclear factor kappa B (NF- κ B) (da Silva Rossato et al, 2014). In addition, shear stress at the epithelial membrane from different blood flow velocities also is possible, thus inducing NO release from the function of endothelial nitric oxide synthase (NOx) (Nosarev et al, 2015), possibly the same as the mechanism related to calcium permeability (Sriram et al., 2016). A previous study showed an interesting result that interval exercise at

moderate intensity stimulated oscillatory shear index (OSI) more than continuous exercise (Lyall et al, 2019). Therefore, these mechanisms of NO generation during interval exercise at moderate intensity are possible.

Other parameters on oxidative stress status in this study were researched. Reports since 1978 have proposed that exercise can induce oxidative stress (Dillard et al, 1978). Bicycling exercise at 50% of VO_{2max} increased pentane from the lipid peroxidation process (Dillard et al, 1978; Chen & Yu, 1994), as in the result from Lovlin and co-workers (Lovlin et al, 1987). This idea is consistent with results in this study, in which increasing MDA and decreasing GSH levels were found, the same as in a previous study on acute aerobic exercise (Michailidis et al., 2007; Morillas-Ruiz et al., 2005). The result of antioxidant GSH from continuous exercise was shown to be against the mechanism of high oxidative stress. A previous study of boys and girls in high-intensity exercise found maximal velocity at 70-75%, but this study also presented maximal velocity in moderate-intensity exercise (Nikolaidis et al, 2007). This mechanism can be explained by the GSH function scavenging free radicals or lipid peroxide molecules in the body system (Kawamura & Muraoka, 2018). Whereas, the GSH result in the interval exercise with low and moderate intensities did not affect the GSH level within 20 minutes. Therefore, less oxidative stress can be expected. Unfortunately, this study did not evaluate free radical molecules directly, such as superoxide or hydroxyl radicals, thus, this hypothesis cannot be summarized exactly, and should be studied in the future. However, this study indicated that short-time-interval exercise at moderate intensity does not attenuate oxidative stress responses when compared with short-time high-intensity exercise training, as stated in a previous study (Bogdanis et al, 2013).

Finally, the result of PrOOH was no different in either group, thus the exact mechanism of exercise in protein oxidation cannot be concluded and is not explained clearly. However, previous evidence showed that protein oxidation was found in heavy-intensity exercise (Bogdanis et al, 2018) when compared to moderate-intensity exercise (Jamuratas et al, 2018). Therefore, the result of the non-significant change in PrOOH in both types of exercise can be explained by the lower intensity in this study.

This study found the dominant change of NO but unfortunately did not evaluate other parameters such as NOx, blood flow, shear index, neurotransmitters, or inflammatory markers that should be studied in the future. The GSH result, especially, found that interval exercise did not affect GSH oxidation when compared with continuous exercise as in the previous study (Heuks et al, 2000). Therefore, this can be confirmed the safety of interval exercise at moderate intensity.

Conclusion

Thus, this preliminary study can be concluded that MIE induces less oxidative stress and high NO generation when compared with MICE. However low dz with non-significant results from other MDA, GSH, and PrOOH parameters indicates that a larger sample size should be studied in the future. In addition, other gender and longer training, and specific markers such as NOx, neurotransmitters, and shear index are very interesting, and the beneficial effect of MICE on NO availability and distribution in the exercised muscle should be confirmed in the future.

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