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The Effect of Exercise Intensity and Duration on Oxidative Stress and Antioxidant Enzymes Activity among Sedentary Healthy Adults: A Repeated Measures Study

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Abstract

Background Aerobic exercise can increase oxidative stress, but it can produce the necessary stimulus for physiological adaptation of exercise. However, the effects of intensity and duration of exercise on oxidative stress status are unclear. This study aimed to compare the effects of exercise intensity (I) and duration (D) on the oxidative stress [malondialdehyde (MDA)], and the responses of the antioxidant enzymes [catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), antioxidant enzymes ratio (AE)] among sedentary adults.

Methods In a randomized crossover design, 25 sedentary adults, performed nine cycling exercise sessions with a constant load of 50%, 60% and 70% VO_2peak for 10-, 20- and 30- minutes duration. Plasma MDA, CAT, GPx and SOD activity were measured before exercise (baseline) and immediately after each session (post).

Results The interaction effect of intensity and duration was significant for percentage changes of MDA (F_{IxD}=3.59, df=4, p<0.05) and CAT activity (F_{IxD} =3.38, df=2.146, p<0.05). Repeated Measures ANOVA analysis revealed that intensity is the major controlling factor for MDA (F_I =54.24, df=2, p<0.05 vs F_D=8.62, df=2, p<0.05), and CAT responses (F_I =14.24, df=1.619, p<0.05 vs F_D = 5.96, df=1.347, p<0.05). However, the main determinant factor for SOD (F_D =11.82, df=1.166, p<0.05 vs F_I =5.58, df=1.289, p<0.05) and AE (F_D =11.63, df=1.201, p<0.05 vs. F_I =3.035, df=1.32, p>0.05) is exercise duration.

Conclusions These findings suggest that exercise intensity was an essential factor of acute oxidative stress and antioxidant enzyme responses compared with the duration of exercise.

Introduction

Regular exercise has beneficial effects on health [1, 2] and increases the production of reactive oxygen species (ROS). Although ROS primarily act as messengers in signal transduction for the regulation of various cellular functions [3], imbalance in the equilibrium of pro-oxidant and antioxidants is proven to cause higher levels of oxidative stress [4, 5]. Previous studies showed that acute exhaustive exercise impaired these health benefits via the increment of ROS dan free radical production [6, 7].

The level of markers due to oxidative damage to lipids [8], proteins [9, 10], and DNA [9] are often said to be caused by the high intensity of aerobic exercises. Oxidative stress instigated by aerobic exercise is due to the rise in oxygen consumption (VO_2) by 10 to 15 fold above the resting level, leading to the increase in oxygen flux by 100 fold higher than the resting value in the contracting skeletal muscle muscles [11]. The steep rise in the oxygen flux is followed by the overproduction of ROS [12]. Subsequently, such a high concentration of ROS overwhelm the antioxidant capacity and affecting redox balance [6, 10, 11].

Oxidative stress associated with exercise could lead to a disruption in cellular homeostases, such as muscle fatigue [13, 14, 15], and after performing a high-intensity exercise, cellular apoptosis [16] and muscle damage ensue [17].

Therefore, unaccustomed aerobic exercise triggers changes in the oxidative status, which is most commonly studied in the blood plasma and erythrocytes. Free radicals in the blood are known to play a pivotal role in tissue damage and have adverse effects on erythrocytes. Exercise-induced oxidative stress has been proposed as one of the different factors that play a role in non-foot strike intra-vascular hemolysis during regular or single bouts of exercise while exercising or during recovery periods [18].

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Even though acute exercise increases oxidative stress, exercise also produces the necessary stress to stimulate chronic adaptations that are beneficial [10, 19], including the reduction of the inflammatory response [20, 21] and oxidative stress by enhancing the antioxidant system [22]. These exercise-mediated responses seem to be dependent on several factors, such as exercise intensity [23, 24], duration [25], nutritional intake [26], training status [27, 28] and the muscle mass involved [29, 30].

The relationship between exercise and oxidative stress is highly complex and not only depending on the mode, intensity, and duration of the exercise. Other contributing factors include age, gender [31], fitness level, psychological stress and eating [32]. Thus, attempting to evaluate the interaction between intensity and duration of exercise with oxidative stress needs to consider age, body composition, fitness level, and nutrient intake to minimize the variance between recruited subjects.

The evidence is still limited, particularly on establishing the interaction effect of low to moderate intensity and duration of exercise on the oxidative stress level, especially among sedentary adults. Therefore, this study aimed to investigate the acute effect of intensity and duration of exercise on oxidative stress biomarkers and antioxidants activity. All the confounding factors contributing to oxidative stress levels, including gender, age, nutritional status, fitness level, and type of exercise, were controlled.

Methods

Study Design and Population

A repeated-measure within-subject design was used in this study to investigate the effect of exercise intensity and its duration on oxidative stress levels. Inclusion criteria were male, aged 20–22 years old and those categorized as sedentary. The latter employed the criteria by Pihl and Jurimaë¨ [33] based on performing any physical activity between 1–2 times per week of less than 20 minutes duration in each. Those with any known co-morbidities such as cardiorespiratory conditions, metabolic diseases and other pre-existing diagnoses warranting regular prescription of medications, actively smoking and consuming alcohol regularly were excluded from the study. Eligible subjects were interviewed and measured for demographic information, basic body parameters, total nutrient intake and four primary outcomes, as explained in the following section. Nutrient intake was analyzed using a modified Food Frequency Questionnaire by *Komposisi Nutrisi Makanan Malaysia* and Diet 4 software.

A total of sixty (60) male subjects aged 20 to 22 years old were screened for eligibility, and 31 of them fulfilled the criteria. As presented in Table 1, the measured VO_2pk (36.6 ± 1.17 ml/min/kg) significantly lower than the predicted VO_2pk (48.1 ± 1.10) at $p < 0.05$, indicating subjects are sedentary [34]. Only 25 subjects completed the exercise program, in which this figure fulfilled the calculated sample size based on the power of $1 - \beta = 0.95$. Subject dropout was caused by a loss of interest to continue and the inability to perform the exercise with a workload given.

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Table 1
Characteristics of all subjects (n = 25)

Characteristic	Mean ± SE	
Age	20.8 ± 0.45 years	
Body weight	58.8 ± 2.39 kg	
Height	167.5 ± 1.12 cm	
BMI	20.9 ± 0.84 (kg/m^{-2})	
Fat Mass	10.8 ± 1.20 kg	
Body Fat	17.4 ± 1.25 %	
Measured VO_2pk	36.6 ± 1.17 (ml/min/kg)	
Predicted VO_2pk	48.1 ± 1.10* (ml/min/kg)	
Nutrient level	*Pre-training*	*Post-training*
Calorie	1853 ± 70 kcal	1771 ± 85 kcal
Protein	112 ± 3 g	118 ± 2 g
Carbohydrate	281 ± 18	272 ± 16
Fat	38 ± 3	39 ± 2
Retinol	197 ± 30 µg	193 ± 32 µg
Carotene	1511 ± 423 µg	1482 ± 512 µg
Ascorbic Acid	83 ± 12 mg	88 ± 13 mg

N=25; BMI: Body Mass Index; VO_2pk: Peak Oxygen Consumption; * measured VO_2pk significantly different from predicted VO_2pk at $p<0.05$.

Table 1 summarizes the characteristics of all subjects. The body mass index (BMI) ranged between 18.5 to 24.9 kg/m^{-2}. The mean percentage of body fat (17.4 ± 1.25%) of subjects was consistent in the fair category [35]. The total intake in calories, protein, carbohydrate, fat, retinol, carotene and ascorbic acid before and three months after the nine (9) exercise bouts did not differ significantly.

Outcome variables

Each subject was assessed for four primary outcomes: 1) peak aerobic capacity, 2) oxidative stress status, and 3) erythrocyte antioxidant enzymes analysis. Peak aerobic capacity was assessed at pre-exercise and post-exercise with a graded exercise cycling test based on calculated Peak Oxygen Uptake (VO_2pk) from measured oxygen consumption (VO_2). A peripheral blood sample was obtained from the forearm vein of each subject into a BD Vacutainer® tube coated with K_2EDTA before and immediately after each exercise bout. Blood samples collected were immediately centrifuged at 3000 rpm for 10 minutes at 4°C to separate plasma from red blood cell pellets. Resultant plasma and red blood cells samples were immediately frozen and stored at -80°C.

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Plasma malondialdehyde (MDA) was used as an oxidative stress status marker, and the levels were determined according to Pilz et al. [36]. The intracellular antioxidant markers were measured using erythrocyte antioxidant enzymes activity includes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Activity for one unit of SOD was designated as the amount of haemoglobin that inhibits the rate of nitro blue tetrazolium reduction by 50% [37]. GPx activity was determined spectrophotometrically by the method of Paglia and Valentine [38]. H_2O_2 was added to a medium containing phosphate buffer, EDTA, NaN_3, NADPH, GSH and erythrocyte hemolysate, followed by measuring the change in absorbance of the system at 340nm. These antioxidant enzymatic activities were expressed relative to the haemoglobin concentration. CAT activity was quantified using a spectrophotometer based on the method previously described by Aebi [39]. The decomposition of H_2O_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.

Intervention

Following the adequate screening, eligible subjects completed a graded exercise cycling test to measure peak aerobic capacity (VO_2pk) for determining the subsequent workloads for a total of nine exercise bouts. $\dot{V}O_2$pk was measured with a cycle ergometer (Ergometrics 900, Ergoline, Germany) through a one-minute incremental exercise protocol as previously described [40]. While exercising, Cortex Metamax 3B (Germany) measured the gas exchange while heart rate was recorded with Polar Electro, Inc., Woodbury, NY, USA heart monitor. For determining the workload based on the $\dot{V}O_2$pk, each subject cycled with unloaded pedalling for 3 minutes, followed by exercising at the incremental workload. The subject was encouraged to maintain cycling exercise until the subject could not sustain the workload for more than 30 seconds, or the cycling frequency between 50–60 rpm cannot be maintained, or when the subject decided to terminate the exercise. Additional criteria of having a respiratory exchange ratio of more than 1.1 were used to ensure that maximal O_2 uptake was reached. Results were used to determine the corresponding workload for 70%, 60% and 50% of $\dot{V}O_2$ peak for each subject.

A total of nine exercise sessions were carried out using randomized crossover design protocol; either the 50% VO_2pk for 10-mins; 60% VO_2pk for 10-mins; 70% VO_2pk for 10-mins; 50% VO_2pk for 20-mins; 60% VO_2pk for 20-mins; 70% VO_2pk for 20-mins; 50% VO_2pk for 30-mins; 60% VO_2pk for 30-mins; or 70% VO_2pk for 30-mins. As the study's main objective was to identify the effect of intensity and duration of aerobic exercise on oxidative stress, subjects performed exercise at a constant predetermined work rate for each intensity throughout the duration without an increase in VO_2. The exercises in this study were performed in a temperature-controlled laboratory between 18^o to 20^{o}C to ensure the measured VO_2 for each exercise bout can be maintained.

A constant load exercise was used in this study. In general concept, constant load exercise is an exercise performed at a constant workload throughout the exercise duration without an increase in VO_2 [41]. The exercise workload for corresponding exercise intensity (%VO_2pk) was based on target HR (HRtarget) using the results of the maximal HR in graded exercise testing (HRmax). Hence, the target was calculated using the formula of exercise intensity x HRmax × 1.15 [42]. The VO_2 and HR were monitored each minute to ensure that subjects were exercising at the given intensity.

In order to minimize the residue effect of exercise bouts and ensuring that the findings observed are due to the effect of different exercise intensity and duration and not others, subjects were given three days of rest (wash-out period) after the maximal exercise test and three days interval in between exercise bouts. The usual level of physical activity and nutritional intake was maintained throughout the experimental period. This study was conducted according the following the specifications approved by the Ethical Committee of

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Universiti Kebangsaan Malaysia, Kuala Lumpur (Ref. no.: UKM 1.5.3.5/244/PPP2). All subjects had agreed to participate in this study by providing written informed consent.

Statistical Analysis

All data are expressed in mean ± standard error (SE). Data were analyzed for normality distribution using the Shaphiro-Wilks test. Analysis of variance (ANOVA) with repeated measures was performed for all exercise bouts by all subjects, with time (pre-exercise and post-exercise), exercise intensity and exercise duration as the within-subject factors. Homogeneity of covariance or sphericity assumption of the data was confirmed using the Mauchly test. Homogeneity of variances was tested for every level's sample size. The sphericity assumptions for SOD, CAT and GPx data sets were maintained at $p < 0.05$. All statistical analysis was performed using Statistical Packages for Social Sciences (SPSS 22 for Windows).

Result

Table 2 summarises the mean measured VO_2 and HR for each exercise duration and intensity. There were no significant differences in measured VO_2 and HR between the 10-, 20- and 30- minutes for each exercise intensity.

Table 2
Oxygen usage and Heart Rate (HR) Responses at different Intensity and Duration of exercise

Duration (Mins)	Intensity (%)	Measured VO_2 (mean ± SE)	Exercise HR (mean ± SE)
10	50%VO_2pk	20.30 ± 1.088	127.56 ± 3.586
20		20.31 ± 0.983	126.36 ± 3.298
30		20.37 ± 1.030	126.28 ± 3.246
10	60%VO_2pk	24.15 ± 1.001	148.68 ± 3.718
20		24.05 ± 0.926	148.08 ± 3.577
30		24.35 ± 1.004	149.56 ± 3.663
10	70%VO_2pk	27.58 ± 1.125	166.64 ± 4.206
20		27.88 ± 1.147	167.64 ± 4.233
30		27.90 ± 1.056	169.32 ± 3.945

% VO2pk % of peak oxygen consumption, N number of subjects=25, % changes percentage changes, * Significantly different from pre–exercise at $p<0.05$

Oxidative Stress and Antioxidant Enzymes Responses

The results of MDA concentration and erythrocytes' antioxidant enzyme activities are presented in Table 3. Paired t-test analysis denotes MDA concentration was shown to increased significantly immediately after exercise at all intensity levels and durations. SOD activity was also found to increase immediately after completing all exercise bouts, except it decreased significantly at 70% VO_2pk for 30 minutes. CAT activities also increased significantly after

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exercise at 50% VO_2pk for 10 and 20 minutes but decreased after exercise at 60% VO_2pk for 30 min duration. As exercise intensity intensified to 70%VO_2pk, the CAT activities significantly reduced after exercise at all durations.

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Table 3
Changes in antioxidant enzymes activities and MDA after exercised at different intensity and duration.

I	D		SOD	CAT	GPx	AE	MDA
% VO_2pk	mins		(unit/mgHb)	(u/s/mgHb)	(unit/min/mgHb)	(unit/min/mgHb)	(nmol/ml)
		(mean ± SEM)					
50%	10	Pre	0.83 ± 0.049	0.29 ± 0.019	6.009 ± 0.0002	3.172 ± 0.3333	9.23 ± 0.357
		Post	1.41 ± 0.082*	0.36 ± 0.018*	5.621 ± 0.0001	4.009 ± 0.2914	10.28 ± 0.300*
		% changes	93.3 ± 22.02	44.6 ± 14.62	-4.5 ± 3.00	61.52 ± 21.804	12.7 ± 1.81
60%	10	Pre	0.91 ± 0.096	0.40 ± 0.022	5.507 ± 0.0001	2.447 ± 0.2873	8.41 ± 0.381
		Post	1.28 ± 0.044*	0.41 ± 0.021	5.112 ± 0.0001	3.248 ± 0.1839*	9.97 ± 0.322*
		% changes	139.3 ± 47.45	4.9 ± 4.77	-4.3 ± 4.95	147.51 ± 51.332	21.5 ± 3.79
70%	10	Pre	1.14 ± 0.048	0.34 ± 0.012	7.281 ± 0.0004	3.413 ± 0.1889	10.01 ± 0.360
		Post	1.68 ± 0.069*	0.31 ± 0.018*	6.491 ± 0.0004	5.679 ± 0.4152*	12.98 ± 0.356*
		% changes	52.6 ± 8.72	-8.6 ± 3.49	-2.9 ± 8.27	72.07 ± 11.272	32.1 ± 3.91
50%	20	Pre	1.18 ± 0.052	0.45 ± 0.027	6.941 ± 0.0002	2.842 ± 0.2218	9.10 ± 0.265
		Post	1.54 ± 0.092*	0.49 ± 0.029*	6.058 ± 0.0001*	3.324 ± 0.3163	10.31 ± 0.289*
		% changes	34.7 ± 8.74	12.8 ± 3.79	-10.4 ± 3.75	21.59 ± 7.984	14.7 ± 3.54
60%	20	Pre	1.19 ± 0.082	0.41 ± 0.019	6.755 ± 0.0001	2.994 ± 0.2171	8.58 ± 0.383
		Post	1.54 ± 0.051*	0.42 ± 0.027	6.303 ± 0.0001*	4.189 ± 0.4255*	10.57 ± 0.255*
		% changes	44.8 ± 12.16	3.8 ± 6.17	-5.7 ± 2.69	49.34 ± 12.552	30.4 ± 7.45
70%	20	Pre	1.15 ± 0.060	0.54 ± 0.031	6.573 ± 0.0002	2.308 ± 0.1951	9.37 ± 0.341
		Post	1.51 ± 0.073*	0.49 ± 0.037*	6.079 ± 0.0003*	3.496 ± 0.3218*	13.20 ± 0.249*
		% changes	40.5 ± 10.79	-9.7 ± 3.77	-7.1 ± 3.32	59.59 ± 13.852	44.6 ± 4.88

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I % VO_2pk	D mins		SOD (unit/mgHb)	CAT (u/s/mgHb)	GPx (unit/min/mgHb)	AE (unit/min/mgHb)	MDA (nmol/ml)
		(mean ± SEM)					
50%	30	Pre	1.01 ± 0.067	0.40 ± 0.023	7.363 ± 0.0001	2.617 ± 0.1938	8.49 ± 0.277
		Post	1.21 ± 0.066*	0.41 ± 0.026	6.995 ± 0.0001*	3.070 ± 0.2187	10.16 ± 0.261*
		% changes	30.8 ± 10.38	4.9 ± 5.38	-4.1 ± 2.26	28.35 ± 10.470	21.3 ± 3.26
60%	30	Pre	1.73 ± 0.112	0.37 ± 0.024	5.948 ± 0.0001	5.319 ± 0.5969	9.06 ± 0.324
		Post	1.99 ± 0.134*	0.34 ± 0.021*	5.336 ± 0.0001*	6.373 ± 0.5307*	11.88 ± 0.365*
		% changes	20.2 ± 8.07	-6.5 ± 2.93	-10.3 ± 1.88	32.55 ± 10.239	32.3 ± 2.99
70%	30	Pre	1.17 ± 0.053	0.33 ± 0.019	8.031 ± 0.0002	3.835 ± 0.3066	8.36 ± 0.369
		Post	0.87 ± 0.039*	0.29 ± 0.017*	7.610 ± 0.0001*	3.076 ± 0.1973*	13.85 ± 0.302*
		% changes	-22.7 ± 4.10	-7.8 ± 3.81	-4.8 ± 1.31	-11.53 ± 7.246	72.5 ± 7.41

n=25; * Significantly different from pre–exercise at p<0.05

Similarly, GPx activity decreased significantly after 20 and 30 minutes at all intensity levels (Table 3). The ratio of activities of antioxidant enzymes was calculated using $AE = \frac{SOD}{GPx + CAT}$ [43]. In contrast, the antioxidant enzyme activity ratio only increased after exercise at 60% and 70% VO_2pk for all durations, except it decreased at 70% VO_2pk for 30 minutes.

A significant interaction effect of intensity and duration were only detected for percentage changes of MDA concentration (F_{IxD} =3.59, df = 4, $p < 0.05$) and CAT activity (F_{IxD} =3.38, df = 2.146, $p < 0.05$). Percentage increment of MDA continued to increase (Fig. 1a and Fig. 1b) but the percentage increment of CAT decreased in response to the increment of both intensity and duration of exercise (Fig. 2a and Fig. 2b). The percentage changes of CAT activities were positive across all exercise intensity and duration except at 70% VO_2pk for all durations and at 60% VO_2pk for 30 minutes. As for GPx, the percentage changes in GPx are low and did not varied after exercise at different intensities and duration (Fig. 3a and Fig. 3b).

Differ from CAT, the percentage changes of AE and SOD exhibit a similar pattern, with positive percentage changes after all exercise bouts except negative percentage change after exercising at 70% VO_2pk for 30 minutes (Fig. 4a, Fig. 4b, Fig. 5a and Fig. 5b). Furthermore, the highest percent increase of SOD and AE is after exercise for 10 minutes of exercise at all intensity 50%, 60%, and 70% VO_2pk. Even though the SOD and AE increased following exercise, the percentage changes of SOD and AE decreased with exercise duration but increased with exercise intensity (Fig. 4a, Fig. 4b, Fig. 5a and Fig. 5b).

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Table 4 summarises the interaction effect of exercise intensity and duration on percentage changes of SOD, CAT, GPx, AE and MDA levels. Higher F value suggested that exercise intensity is the major controlling factor for MDA (p = 0.000) and CAT (p = 0.000). This implies exercise intensity is a more substantial stimulus than exercise duration to induce MDA concentration and CAT responses. Conversely, the F value of exercise duration is higher than exercise intensity for SOD (p = 0.001) and AE (p = 0.001). This indicates that the primary determinant factor of SOD and AE is exercise duration.

Table 4
Interaction effect of intensity and duration for percentage changes of antioxidant enzymes activities and MDA

Parameters	Intensity x Duration	Intensity	Duration
MDA	F = 4.28*	*F = 54.24**	F = 8.62*
	df = 2.989	df = 2	df = 2
SOD	F = 1.68	F = 5.58*	*F = 11.82**
	df = 2.055	df = 1.289	df = 1.166
CAT	F = 3.38*	*F = 14.24**	F = 5.96*
	df = 2.146	df = 1.619	df = 1.347
GPX	F = 0.524	F = 0.152	F = 0.65
	df = 1.99	df = 1.48	df = 1.49
AE	F = 2.117	F = 3.035	*F = 11.63**
	df = 1.982	df = 1.32	df = 1.201

* significant at p<0.05, N number of subjects=25

Discussion And Conclusion

Acute bouts of aerobic exercise can induce a state of oxidative stress. Previous studies have reported increased oxidative stress for both healthy and diseased subjects following single bouts of exercise [44, 45]. This is consistent with the present study that found oxidative stress increased as measured by MDA immediately following each submaximal exercise bout. An increase in oxidative stress following exercise is accompanied by increased antioxidant responses [46, 47] that were also discovered in the present study–particularly CAT and SOD. Activities of SOD and CAT increased following exercise to counteract the rise in ROS production [6, 46, 48, 49, 50].

Even though aerobic exercise can cause oxidative stress, it appears that this stimulus is required to allow for an increased endogenous antioxidant defences and improved cardiorespiratory fitness. The degree of oxidation is proportional to the amount of oxidant production contributed by exercise mode, intensity, and duration [51]. Furthermore, the American College of Sports Medicine recommends that the appropriate exercise intensity for improving cardiorespiratory fitness in young adults be 4.8 to 7.1 METs, which is equivalent to 50–85 percent of VO2max and 60–90 percent of age-predicted HRmax [52]. As a result, the current study wants to determine whether oxidative stress levels and enzymatic antioxidant status are more affected by intensity or duration of exercise.

In this study, the effects of exercise intensity and duration on oxidative stress (measured through MDA and

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ed systematically. This study employed nine exercise bouts with different

intensities and duration prescribed explicitly for each healthy and sedentary young adult. The exercise was delivered using submaximal aerobic cycling in which the moderate intensity (50%, 60%, and 70% VO_2pk) and duration (10, 20, and 30 mins) were manipulated.

The main findings of this study revealed that the percentage increment of MDA was dependent on the exercise intensity and duration. This is in agreement with Ammar et al. [47], Ribeiro-Samora et al. [53], El Abed et al. [54], and Boukhris et al. [55], which reported that the oxidative stress level is dependent on the intensity and duration of the exertion. Previous research showed that aerobic exercise performed at high intensity [8, 14, 29, 56, 57], particularly prolonged aerobic exercise [48], is associated with increased oxidative stress. A study by Johnson et al. [58], McAllister et al. [59], and McClean et al. [23] revealed that the increase in oxidative stress among trained subjects occurred following moderate intensity and short duration of exercise.

On the other hand, Bloomer et al. [9] found that the MDA was not increased after cycling for 30 minutes at an intensity of 70% VO_2max among ten trained male athletes. This might be stipulated by active participants or trained athletes possessing greater protection against ROS oxidation than sedentary participants [12]. A high level of protection against ROS is shown to reduce lipid peroxidation (MDA). Leeuwenburgh and Heinecke [60] and Perrone et al. [61] also suggested that the oxidative stress produced from exercise depends on the capacity and the adaptability of the antioxidant defence in the body.

The magnitude of the oxidative damage due to exercise depends on oxygen consumption, ROS production rate, and the balance between antioxidants and ROS [11]. According to Sakellariou et al. [62], aerobic exercise results in a 1 to 3-folds increased of superoxide during muscle contraction. When the intensity and duration of exercise are increased, oxygen consumption [63], metabolic stress [64], mechanical stress [65, 66] and metabolism rate [67] will escalate, leading to higher production of ROS [68]. Inadequate protection by antioxidants causes cellular damage to increase due to increased lipid peroxidation and oxidative stress [61, 69].

This study has demonstrated a decrease in the percentage changes of CAT, SOD, and AE in response to exercise duration and intensity. The reduction in the SOD and CAT percentage instigated the rise in the MDA percentage. Furthermore, the percentage changes of AE, SOD, and CAT were dependent on the exercise intensity and duration. This is consistent with a study by He et al. [6] that proved the magnitude of SOD activities' magnitude depends on the intensity and duration.

The study design employed in this study permitted us to determine that intensity is the major determinant factor for MDA as indicated by through ANOVA analysis. This suggests that exercise intensity resulted in more significant responses of blood oxidative stress than the duration of exercise. This is in line with the previous studies which have observed that exercise intensity is the key determinant of oxidative stress following aerobic exercise [53, 58]. Earlier studies by He et al. [6] had discovered that for the same total energy expenditure, oxidative stress was also found to increase after exercise at a higher intensity but not after a longer duration. Intensity of exercise intensifies the respiration rate [70] leading to amplification of oxygen consumption and electron transport chain reaction [11]. It is estimated that the whole body oxygen consumption may increase up to 10–20 fold [72, 72] while staggering figure up to 100–200 fold is seen in the exercising muscles [73]. Despite such rise, only 0.15% of the utilised oxygen produce ROS.

Previous studies by Johnson et al. [58] and McAllister et al. [59] reported an increase in oxidative stress among trained subjects following 30 and 60 min of moderate aerobic exercise. Such finding is in concordance with the present study, where we have discovered moderate intensity and short duration of aerobic cycling exercise increased

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the oxidative stress in sedentary healthy young adults. Thus, we can conclude that the threshold intensity for stimulating an increased response of oxidative stress following aerobic exercise is between 50 and 70% VO_2pk in sedentary young adults. Johnson et al. [58] have found that the duration required for aerobic exercise with threshold intensity of 50–70% VO_2pk in trained subjects is between 20 and 60 minutes.

This study has discovered different determinant factors for each antioxidant enzyme. Here, exercise duration is the major determinant factor for SOD and AE. This finding implies that an increase in exercise duration would activate more highly oxidative muscle fibres – type I and type IIa muscle fibres leading to a more significant increase in the SOD activity [75]. Thus, excessive H_2O_2 produced from the dismutation reaction of superoxide inhibited SOD and CAT enzymes by changing the redox condition in the cell and changes in the antioxidant enzyme's catalytic centre [76, 77]. Subsequently, an increase in exercise duration reduces AE.

On the other hand, exercise intensity is the main determinant factor for CAT. It is stipulated that the increase in blood glucose inhibits CAT [78]. The exponential rise of adrenalin and noradrenalin hormones with intensity was observed to be much faster than the increment seen with exercise duration. The latter only showed a linear relationship [79]. Both adrenalin and noradrenalin hormones stimulate β-adrenergic receptors on the pancreas and increase glucagon secretion [80]. Resultant high glucagon in plasma increases the blood glucose level through the mobilization of free fatty acid (FFA) from adipose tissue, mobilization of glucose from the liver, and an increase of gluconeogenesis [81].

This study also revealed that the intensity of 70% VO_2pk generated the highest stressor stimulus for pro-oxidative than the lower intensity exercises for all 10, 20 and 30 min exercise duration. Exercise at 70% VO_2pk has affected the redox balance by producing more ROS and subsequently increased the SOD activity. An increase in glucose concentration in the blood could also inhibit the CAT enzyme [78]. Accumulation of H_2O_2 (hydrogen peroxide) produced following exercise at 70% for 30 minutes has also inhibited SOD and decreased its activity. Thus, this study discloses that exercise intensity above 60% VO_2pk is more effective in controlling the response of antioxidant enzymes. This study also suggests that antioxidant enzymes appear to be selectively activated during exercise depending on the amount of ROS produced.

It appears that the sensitivity of cells to free radicals depends on the equilibrium between the formation of hydrogen peroxide from superoxide in the dismutation reaction catalyzed by SOD and its degradation by GPx and CAT, rather than on the activities of individual antioxidant enzymes [82]. In this study, the AE was observed to increase significantly only after exercise at 60% and 70% VO_2pk for 10-, 20-, and 30-minutes, except for 70% VO_2pk intensity in 30-minutes duration where the ratio was reduced. These results are primarily in agreement with a previous study by Georgakouli et al. [83] that observed a significant elevation of plasma total antioxidant capacity among healthy individuals after 30 min at 50–60% of the heart rate reserve on a cycle ergometer. The increment in plasma MDA with intensity and duration found in this study suggests that the balance of oxygen metabolism is compromised during exercise. However, we did not observe any relationships or associations between the percentage changes in AE and the oxidative stress markers, MDA. Similarly, this finding supports the hypothesis of exercise-induced oxidative stress among sedentary adults [8, 47, 84, 85].

SOD is sensitive to the overproduction of superoxide and hydrogen peroxidase [86], and this fact is reflected in this study whereby the AE significantly decreased after exercising at 70% VO_2pk for 30 minutes. According to Garaiová et al. [43], AE changes the equilibrium between the formation of hydrogen peroxide from superoxide dismutation and its decomposition by other enzymes (GPx, CAT) in erythrocytes. The reduction of AE and the increase of MDA in this study showed that exercising at 70% VO_2pk for longer than 20 minutes creates oxidative stress. These findings

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indicate the probability that these results may support the theory that the contribution of antioxidant enzyme disequilibrium from oxidative stress during exercise is secondary to limited CAT activity and most likely due to an insufficient increase in the GPx activity. In other terms, oxidative stress is initiated by an imbalance in the activities of antioxidant enzymes, SOD against GPx and CAT. It can be postulated that exercising at a higher intensity and for a longer duration is associated with the overproduction of free radicals (Fig. 6).

Moreover, these indicate that free radicals produced during exercise at 70% VO_2pk for 30 minutes have exceeded the antioxidant enzymes' capacity. Although 70% VO_2pk is classified as moderate intensity, exercising for more than 20 minutes can be conceived as a high exercise dose for sedentary young male adults, hence triggering ROS and subsequent oxidative stress. This finding supports the suggestion [87] for accumulating at least 30 minutes of exercise at moderate intensity each day to maintain cardiovascular fitness and reduce potential risks of non-communicable diseases. This finding is also incoherent with previous studies demonstrating increased oxidative stress after moderate-intensity exercise among young, healthy male subjects [23]. Moderate-intensity exercise is thought to confer beneficial effects, but prolonged exercise leads to elevated ROS production at higher exercise intensities [58, 88]. However, a significant elevation of plasma total antioxidant capacity was observed in a healthy untrained male adult after cycling for 30 minutes at 70% of maximum workload [89].

For any exercise to deliver the expected health benefit, there should be an optimal level of ROS produced during exercise that may induce favourable adaptations following repeated exposure [19], including increased expression of antioxidant enzymes over time such as superoxide dismutase and catalase [90]. However, the increase of oxidative stress above the optimal level may compromise health and performance. Too much ROS might impair antioxidant defence capacities leading to substantial cell damage [91, 92]. Prolonged and irreparable oxidative damage could predispose to diseases such as neurodegeneration and cardiovascular [88].

These findings further emphasize the need to achieve optimal exercise intensity and duration and provide physical trainers, exercise enthusiasts, and clinical practitioners with practical settings. Here, conducive and optimal exercise intensity and duration bring beneficial health outcomes. However, categorizing intensity is not straightforward because the functional capacities of each individual vary widely, especially for different age groups and fitness levels. Hence, future research should propose intensity categories based on age group, fitness level, and gender. Concurrently, works should evaluate the long-term effect of the optimal intensity and duration presented in this study. An investigation should be carried out to determine the chronic state of oxidative stress and antioxidant enzyme responses concerning exercise intensity and duration. Existing literature is yet to demonstrate the physiological mechanism that underpin the oxidative process during exercise, especially when intensity or duration are increased. Here, remains the opportunities for future research to evaluate the differences in production pathways of ROS and free radicals based on different exercise intensity and duration.

List Of Abbreviations

I, intensity; D, duration; VO_2pk, peak oxygen consumption; VO_2, oxygen consumption; HR, heart rate; ROS, reactive oxygen species; BMI, body mass index; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; MDA, malondialdehyde; AE, the ratio of antioxidant enzymes activity.

Declarations

Ethics approval & consent to participate: The ethical approval of this study was obtained from the Universiti Kebangsaan Malaysia Research Ethics Committee, with reference no.: UKM 1.5.3.5/244/PPP2. All

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participants provided written informed consent to participate in this study.

Consent to publish: All authors read and approved the final manuscript for publication.

Availability of data and materials: The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication: Not Applicable.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: DMAD - contributes to conception and design, involves in data acquisition, data analysis and interpretation, drafting manuscript and give final approval for publication; FA - contributes to conception and design, revising and proof reading manuscript, and give final approval for publication; DMPB - contributes to conception and design; and give final approval for publication; TFTK - contributes to conception and design, revising manuscript and give final approval for publication; WZWN - contributes to conception and design and give final approval for publication.

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Figures

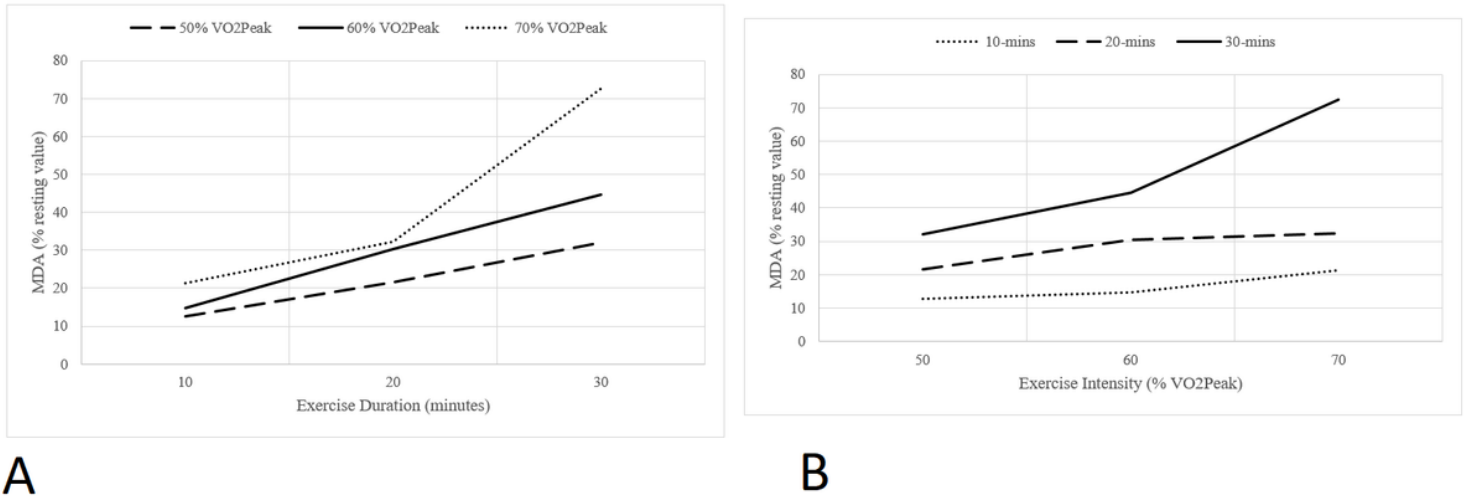

Figure 1

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a Percentage MDA changes at 10, 20, and 30 minutes during exercise. b Percentage MDA changes at 50%, 60% and 70% VO2peak during exercise.

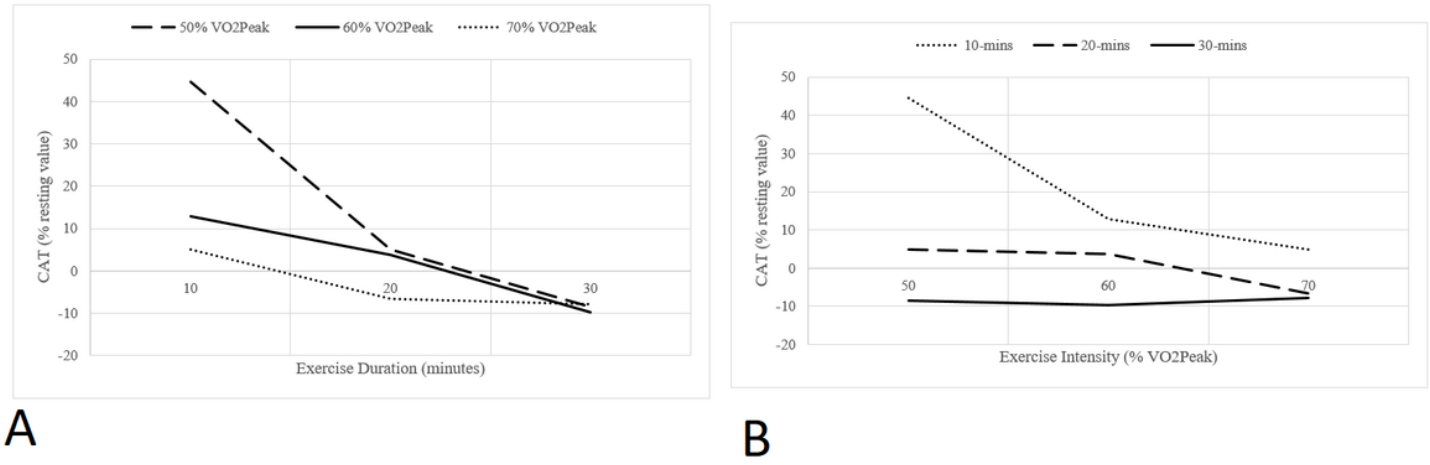

Figure 2

a Percentage CAT changes at 10, 20 and 30 minutes during exercise. b Percentage CAT changes at 50%, 60% and 70% VO2peak during exercise.

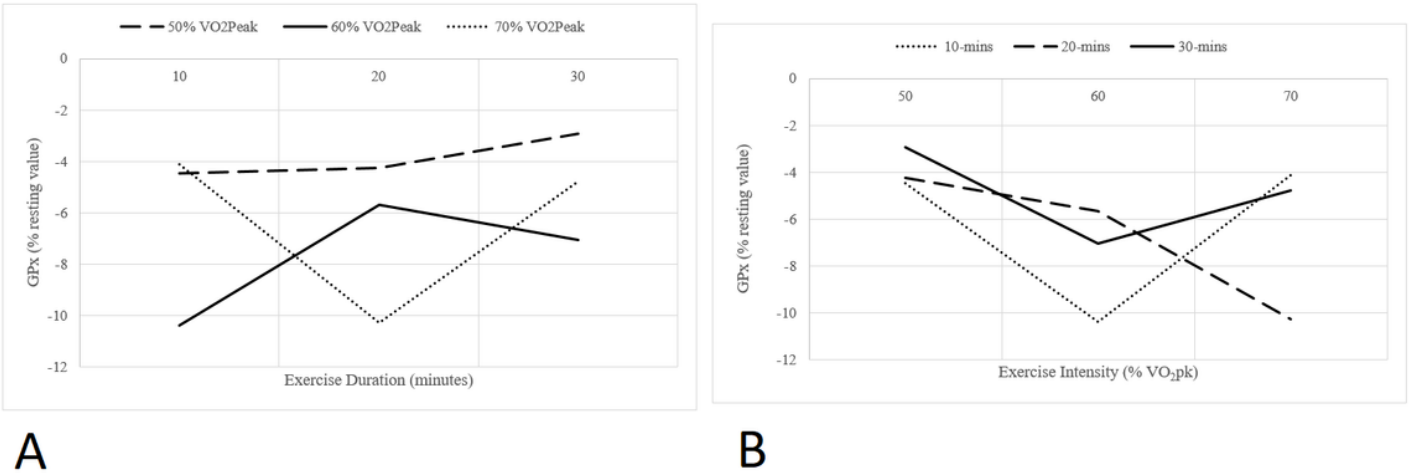

Figure 3

a Percentage GPx changes at 10, 20 and 30 minutes during exercise. b Percentage of GPx changes at 50%, 60% and 70% VO2peak during exercise.

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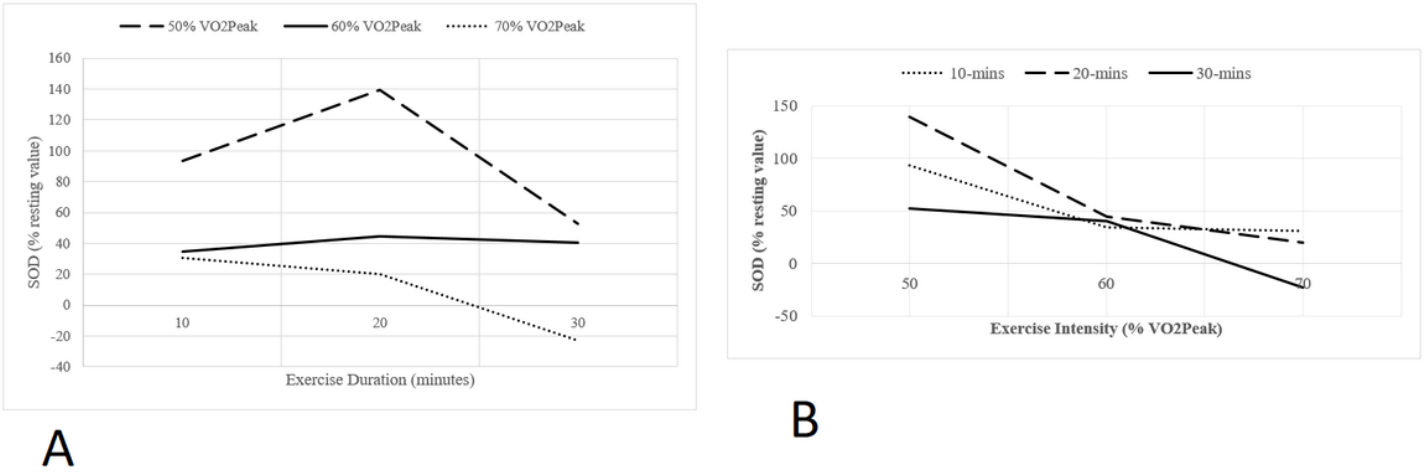

Figure 4

a Percentage SOD changes at 10, 20 and 30 minutes during exercise. b Percentage of SOD changes at 50%, 60% and 70% VO2peak during exercise.

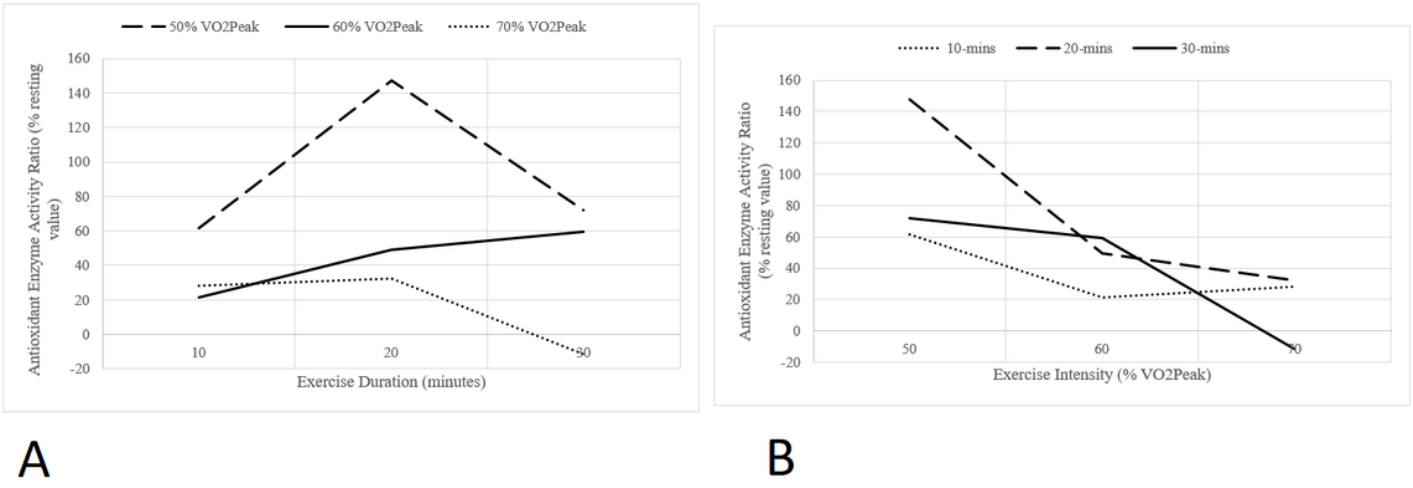

Figure 5

a Percentage AE changes at 10, 20 and 30 minutes during exercise. b Percentage of AE changes at 50%, 60% and 70% VO2peak during exercise.

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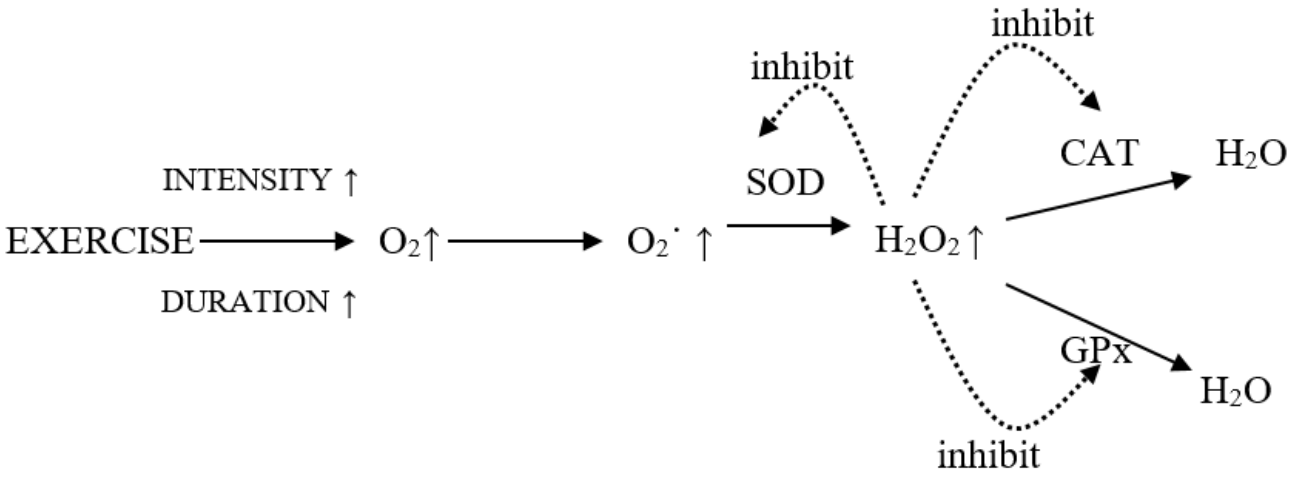

Figure 6

Relationship between intensity and duration of exercise with Reactive Oxygen Species (ROS) production.

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