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PRODUCTION OF FREE RADICALS AND CATALASE ACTIVITY DURING ACUTE EXERCISE TRAINING IN YOUNG MEN

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Abstract. Reactive oxygen species (ROS) are constantly produced by cells that promote cellular oxidative damage and are neutralized by an antioxidant system including superoxide dismutase, glutathione, peroxidase and catalase. Male volunteers were exercised for 20 minutes, three days (60, 70 and 80% of maximum heart rate). Catalase activity and plasma malondialdehyde concentration were measured. The mean age of the volunteers was 25±7 years, with body mass index of 24.03±4.32 kg/m^2. Acute exercise training produced an increase of malondialdehyde concentration that was exercise intensity-dependent in young volunteers. However, catalase activity shows a great variability at baseline and the percentual of reduction was exercise intensity-independent in this particular population. Therefore, our study shows that acute cycling exercise promotes an increase of oxidative stress that was exercise intensity-dependent in young volunteers. Furthermore, the antioxidant system measured by catalase activity was effective to counterbalance the ROS production showing a saturation behavior at an intensity of 70 % of maximum heart rate.

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Key words: Catalase - Malondialdehyde - Oxidative stress - Acute exercise - Reactive oxygen species

Introduction

Reactive oxygen species (ROS) such as peroxynitrite, superoxide, and superoxide of hydrogen are constantly produced by cells that promote cellular oxidative damage. In normal conditions, mitochondrial aerobic metabolism is an important source of ROS production and these free radicals are neutralized by an

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elaborate antioxidant system including superoxide dismutase, glutathione, peroxidase and catalase [14]. Additionally to enzymes, non-enzymes antioxidant agents play a key role to detoxify ROS from our body including vitamins A, E and C [19]. Evidence has shown that pathological conditions and exhaustive exercise lead to an excessive production of ROS which in turn, promotes severe cellular damages [4,15]. On the other hand, several lines of evidences have shown that shear stress induced by moderate physical exercise promotes an up-regulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase. These enzymes are scavengers of reactive oxygen species causing an increase of NO bioavailability to the vascular smooth muscle and enhancement of endothelium-dependent vasodilatation [5,6,7,15]. However, a previous study failed to find any alterations in SOD or catalase levels in thoracic aortic homogenates in trained rats [9]. Thus, the aim of this work was to evaluate the relationship between intensity of acute cycling exercise and concentration of antioxidant enzyme, catalase and the rate of oxidative stress by measurement of malondialdehyde in young men.

Materials and Methods

Participants: Male volunteers were recruited by advertisement on the Campus of Catholic University of Brasilia (UCB). To be included in the study, the volunteers had to be sedentary, non-smokers who were currently not taking any medications. The exclusion criteria were individuals with cardiovascular (angina, valvular disease, stroke), pulmonary, neurological or psychiatric diseases, diabetes, arthritis, and VO_2 max greater than 40ml/kg/min. To detect medically excluding factors, the volunteers underwent a medical evaluation performed by a medical doctor. This study was approved by Ethical Committee of the UCB and the eligible subjects were informed of the procedures and risks of the study, and signed a written informed consent. Only 20 participants fulfilled the inclusion criteria and were included in this study.

Experimental procedure: Prior to exercise protocol design the volunteers performed one incremental maximal load test as previously described by Golding, Myers and Sinning [8]. Briefly, the test consisted in a progressive increment of workload by pedaling on a cycle ergometer (Monark, Sao Paulo, SP, Brasil). The pedaling rate started at 50 rpm, with a load of 25 W, and the incremental workload was changed by every three minutes up to exhaustion. This test was carried out to obtain the maximum heart rate of each volunteer.

Exercise protocol design consisted of three sessions, for 20 minutes, during

three days at an intensity of 60%, 70% and 80% of maximum heart rate. Heart rate was monitored using a heart rate monitor (Polar A3, Kempele, Finland). The intensity of exercise training was randomly attributed for each volunteer. All the volunteers performed the exercise protocol in the afternoon, and they were instructed to arrive at the laboratory in a rested and to avoid strenuous exercise 48 hours before the exercise protocol.

Enzymatic determinations: Venous blood samples were taken from the antecubital vein after overnight fasting before and after exercise protocol. Plasma was separated by centrifugation and stored in freezer at -80°C.

In order, to verify the effect of exercise protocol we determined the antioxidant enzyme, catalase, and the lipid peroxidation rate by measurement of malondialdehyde.

Catalase activity was measured by spectrophotometric method based on the decomposition of H_2O_2 [1].

Plasma malondialdehyde concentration was measured by spectophotometry by using thiobarbituric acid (TBARS) assay [18].

Statistical analysis: Data were analyzed using one way ANOYA for repeated-measures followed by Bonferroni post test. A P value <0.05 was considered as statistically significant.

Results

Table 1
Concentration of malondialdehyde (nmol/ml) and catalase activity in incremental workload test (WT), and during acute exercise training at intensity of 60, 70 and 80% of maximum heart rate in young male volunteers

Time	WT	60%	70%	80%
		Malondialdehyde concentration		
Baseline	73.6 ± 44	73.6 ± 44	65.6 + 49.1	6 1.6 ±47.4
After exercise	119 ±60*	105.4 + 51.8*	93.5 ±48.7*	97.6 ±53.6*
%	61.6	36.2	42.4	58.2
		Catalase activity		
Baseline	19.39 ±0.1	16.28 + 0.05	18.26 ±0.10	19.94 ±0.49
After exercise	15.20 ±0.09*	14.28 + 0.80*	15.05 + 0.75*	16.97 ±0.68*
%	21.56	9.15	20.29	14.89

Data are means ± SEM for 20 volunteers; % of increment after and before exercise training; *significantly different from basal values

The mean age of the volunteers was 25±7 years, weighting 74.4±12.97 kg, height of 176.03±6.20 centimeters with body mass index of 24.03±4.32 kg/m^2.

Acute exercise training produced an increase of malondialdehyde concentration that was exercise intensity-dependent in young volunteers (Table 1). However, catalase activity shows a great variability at baseline and the percent of reduction was exercise intensity-independent in this particular population (Table 1).

Discussion and Conclusion

Our study shows that acute physical exercise at an intensity of 60, 70 and 80 % of maximum heart rate produces an increase of oxidative stress rate measured by lipid peroxidation that was exercise intensity-dependent, but this increment was not accompanied by parallel changes in catalase activity in young men.

Oxidative stress is part of metabolic process in all mammalian cells generating free radicals. Physical activity increases the generation of free radicals by several pathways including increasing of catecholamines release and oxygen uptake by mitochondria mainly in skeletal muscle [19]. Additionally, several investigators have studied the mechanisms by which physical exercise modulate the expression antioxidant systems and further characterize the relationship between oxidative stress and antioxidant enzyme activity in skeletal muscle either in man [2,16] or laboratory animals [3,12,13]. However, no conclusive data exist regarding to this association. Our findings show a direct relationship between intensity of cycling exercise and oxidative stress rate measured by lipid peroxidation in young man which was not accompanied by a parallel increase in catalase activity concentration. The reason for that could be due to the high production of ROS in response to physical exercise exceeds the detoxification capacity of antioxidant enzymes. In fact, our results show that at an intensity of 80% of maximum heart rate catalase activity concentration is lower than at intensity of 70% showing a saturation of this antioxidant enzyme. Interestingly, most of studies have been shown that only moderate physical exercise (55 to 70% of intensity) promotes health beneficial effects in the cardiovascular and neuroendocrine system [10,11,17].

The results showed in this study that acute cycling exercise promotes an increase of oxidative stress that was exercise intensity-dependent in young volunteers. Furthermore, the antioxidant system measured by catalase activity was effective to counterbalance the ROS production showing a saturation behavior at an intensity of 70 % of maximum heart rate.

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