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## OXIDATIVE STRESS RESPONSE TO SHORT DURATION BOUT OF SUBMAXIMAL AEROBIC EXERCISE IN HEALTHY YOUNG ADULTS

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### Abstract

**Purpose**—The purpose of this study was to investigate the oxidative stress response to a short duration bout of submaximal exercise in a cohort of healthy young adults.

**Methods**—15 apparently healthy college age males and females completed a modified Bruce-protocol treadmill test to 75–80% of their heart rate reserve. Blood samples collected immediately before (pre-exercise), immediately after, 30, 60 and 120 minutes post-exercise were assayed for total antioxidant capacity (TAC), superoxide dismutase (SOD), thiobarbituric acid-reactive substances (TBARS), and protein carbonyls (PC).

**Results**—SOD activity was significantly increased from pre-exercise levels at 30 minutes (77%), 60 minutes (33%), and 120 minutes (37%) post-exercise. TAC levels were also significantly increased from pre-exercise levels at 60 minutes (30%) and 120 minutes (33%) post-exercise. There were no significant changes in biomarkers for reactive oxygen/nitrogen species (RONS) mediated damage (TBARS and PC) across all post-exercise time points.

**Conclusions**—In a cohort of healthy young adults, a short duration bout of submaximal aerobic exercise elicited increases in antioxidant activity/concentration, but did not evoke changes in oxidative stress-induced damage. These results may suggest that: (1) short duration bouts of submaximal aerobic exercise are sufficient to induce RONS generation; and (2) the antioxidant defense system is capable of protecting against enhanced RONS production induced by a short duration, submaximal exercise bout in healthy young adults.

### Keywords

Submaximal exercise; oxidative stress; antioxidants; free radicals

## INTRODUCTION

According to the latest physical activity recommendations, healthy adults under the age of 65 require moderate-intensity aerobic physical activity for a minimum of 30 minutes 5 days

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a week, or vigorous-intensity aerobic physical activity for a minimum of 20 minutes on 3 days each week to promote and/or maintain health (14). Perceived lack of time is a commonly cited barrier to physical activity (29); therefore, physical activity guidelines endorse the accumulation of exercise or physical activity in short bouts over the course of the day to make it easier for individuals to meet the daily recommendations (14). Empirical evidence supporting the splitting of exercise into several shorter bouts is beginning to accumulate, as changes in aerobic capacity, body composition, and blood pressure have been demonstrated to be similar between continuous and accumulated exercise training, though these results have not been consistently shown (21).

Oxidative stress is a condition characterized by an imbalance between reactive oxygen/nitrogen species (RONS) production and the antioxidant defense system whereby RONS generation exceeds the capacity of the antioxidant defense system to render RONS inactive. As a result of the RONS/antioxidant imbalance, oxidative cellular damage occurs as several macromolecules including lipids, proteins, and nucleic acids are subjected to oxidative modifications. Moderate and high intensity continuous acute aerobic exercise have been demonstrated to induce oxidative stress, most likely as a result of enhanced RONS generation (10). Therefore, a single bout of moderate to intense aerobic exercise may be viewed as a physiological stressor that promotes oxidative cellular damage including lipid peroxidation, protein damage, and DNA damage.

Initially, exercise-induced RONS generation was viewed as a detriment to exercise performance due to several studies linking oxidative stress to factors that can affect exercise performance including muscle damage (4), fatigue (2), impaired muscle force production (23), and reduced immune function (22). However, in recent years a new hypothesis based on the hormesis theory has emerged (24). According to this hypothesis, exercise-induced RONS generation is necessary during exercise for the initiation of adaptive processes. Mechanistically, it has been proposed that RONS-mediated signaling activates redox sensitive transcription factors, that in turn upregulate antioxidant and damage-repair enzymes. Consequently these adaptive processes may result in lower basal levels of RONS and a reduction in oxidative damage during exercise (24). In accordance with the hypothesis that RONS may be involved in training adaptation, it has been demonstrated that antioxidant supplementation attenuates mitochondrial biogenesis and endurance capacity in endurance-trained rats (13), prevents exercise-induced adaptation in the skeletal muscle of rats (12), and precludes the health-promoting effects of exercise in humans (26).

Several studies have demonstrated that exercise duration may be an important factor related to RONS production, as more prolonged bouts of aerobic exercise have been shown to elicit greater amounts of oxidative damage (3, 5, 25). In keeping with the hypothesis that exercise-induced RONS generation is a necessary physiological response, a shortened bout of moderate exercise, which would be prescribed as part of an accumulated exercise approach, could be detrimental to training adaptation and health promotion by limiting the amount of RONS that may accumulate in excess during more prolonged bouts of exercise. Therefore, the purpose of this study was to examine the oxidative-stress response to a single short-duration bout of submaximal aerobic exercise in a cohort of healthy young adults.

## METHODS

### Subjects

Young physically active adults between the ages of 18–25 were recruited through advertisements and word of mouth. All were apparently healthy and free of cardiovascular risk factors as assessed by completion of an extensive health history form during an initial laboratory visit. Exercise and physical activity patterns were assessed by questionnaire; only

subjects who engaged in regular exercise/physical activity 2 days/week were included in the study. College athletes, smokers, and women on birth control were excluded from the study. The study was approved by the Institutional Review Board of Temple University, Philadelphia, PA., and all qualified participants provided their written, informed consent.

## Procedures

Subjects were asked to refrain from non-steroidal anti-inflammatory drugs and vitamin supplements for 2 weeks prior to testing. Subjects were also asked to abstain from caffeine, alcohol, or exercise training for 24 hours prior to testing, and to fast for at least 10 hours the night before testing. Evidence has suggested that the hormone fluctuations during the menstrual cycle can influence oxidative stress responses to exercise (15). Therefore, all females were tested during days 1–5 of their menstrual cycle because hormone levels tend to be lowest early in the follicular phase.

On the morning of the study, height, weight, and blood pressure (BP) were measured, and a pre-exercise blood sample was collected immediately prior to exercising. Blood samples were collected into anticoagulant tubes (EDTA and Sodium-Heparin), centrifuged at 2000g for 20 minutes at 4°C, and then the plasma was frozen at –80°C until assay. A modified Bruce sub-maximal treadmill exercise test was then performed, with breath-by-breath gas samples collected and averaged over a 60-second period using a calibrated metabolic cart (Vmax Encore, SensorMedics, Yorba Linda, CA). Electrocardiogram was continuously monitored and the treadmill test was terminated when the subject reached 75–80% of their estimated heart rate reserve. Regression analysis using data collected by indirect calorimetry was used to predict  $\text{VO}_{2\text{max}}$  levels. Post-exercise blood samples were collected at the following time-points: immediately following exercise termination (within 2 minutes), 30, 60, and 120 minutes after exercise termination. All subjects remained in the lab area throughout the entire 2 hour post-exercise period in order to control for food and water intake. During this time, subjects were instructed to sit and read, or work on the computer, refrain from eating, and were only allowed to drink up to 1L of water. Any blood samples displaying evidence of hemolysis were discarded and not stored for assay. Subject data was only included if 80% of the blood samples were collected and free of hemolysis.

## Plasma Superoxide Dismutase (SOD)

SOD activity was measured using a commercially available kit according to manufacturer's instructions (Cayman Chemicals, Ann Arbor, MI). Plasma samples were diluted 1:5 in sample buffer (50mM Tris-HCl, pH 8.0). SOD activity was measured by utilizing a tetrazolium salt radical detector solution, diluted in assay buffer (50 mM Tris-HCl, pH 8.0, containing 0.1 mM diethylenetriaminepentaacetic acid and 0.1 mM hypoxanthine), to detect superoxide radicals generated by hypoxanthine and xanthine oxidase. One unit of SOD activity is defined as the amount of enzyme needed to exhibit a 50% dismutation of the superoxide radical. Absorbance was read at 450 nm using a SpectraMax Microplate Reader (Molecular Devices, Sunnyvale, CA). The detection limit was 0.025 U/ml. To control for inter-assay variability, plasma samples from pre-exercise, immediately post-exercise, 30 minutes post-exercise, 60 minutes post-exercise, and 120-minutes post exercise for each subject were assayed on the same plate in duplicate. Inter-assay and intra-assay coefficients of variation were 5.9% and 12.4 % respectively.

## Total Antioxidant Capacity (TAC)

TAC was measured using a commercially available kit according to manufacturer's instructions (Cayman Chemicals, Ann Arbor, MI). Plasma samples were diluted 1:20 in Assay buffer (5mM potassium phosphate, pH 7.4, containing 0.9% sodium chloride and 0.1% glucose). TAC measurement was based on the ability of antioxidants in the plasma to

inhibit the oxidation of ABTS<sup>®</sup> (2,2'-Azino-di- to ABTS<sup>®+</sup> by metmyoglobin). The capacity of the antioxidants in plasma to prevent ABTS<sup>®</sup> oxidation is compared with that of a water-soluble vitamin E analogue, Trolox. Absorbance was read at 750 nm using a SpectraMax Microplate Reader (Molecular Devices, Sunnyvale, CA), and TAC activity quantified as millimolar Trolox equivalents. The detection limit was 0.044 mM. To control for inter-assay variability, plasma samples from pre-exercise, immediately post-exercise, 30 minutes post-exercise, 60 minutes post-exercise, and 120-minutes post exercise for each subject were assayed on the same plate in duplicate. Inter-assay and intra-assay coefficients of variation were 6.7% and 9.2% respectively.

### Protein Carbonyls (PC)

Average plasma protein levels were determined to be 6 g/dL by using the Bradford Protein Assay prior to the measurement of PC. PC formation was determined with the Oxiselect<sup>™</sup> Protein Carbonyl ELISA Kit according to manufacturer's instructions (Cell Biolabs, Inc., San Diego, CA). Absorbance was read at 450 nm using a SpectraMax Microplate Reader (Molecular Devices, Sunnyvale, CA). The detection limit was 0.375 nmol/mg. To control for inter-assay variability, plasma samples from pre-exercise, immediately post-exercise, 30 minutes post-exercise, 60 minutes post-exercise, and 120-minutes post exercise for each subject were assayed on the same plate in duplicate. Inter-assay and intra-assay coefficients of variation were 5.5% and 7.8% respectively.

### Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidation was assessed by the measurement of TBARS in the plasma as previously described (32). Briefly, the assay involves the reaction of malondialdehyde (MDA), contained in the sample, with thiobarbituric acid (TBA) under high temperature and acidic conditions to form a MDA-TBA complex that can be quantified colorimetrically. On the day of assay, plasma samples were mixed with sodium dodecyl sulfate solution and TBA reagent (530 mg thiobarbituric acid solubilized in a mixed solution containing 50 ml of sodium hydroxide and 50 ml acetic acid). Absorbance was read at 535 nm using a SpectraMax Microplate Reader (Molecular Devices, Sunnyvale, CA). All reagents were obtained from Cayman Chemical (Ann Arbor, MI). To control for inter-assay variability, plasma samples from pre-exercise, immediately post-exercise, 30 minutes post-exercise, 60 minutes post-exercise, and 120-minutes post exercise for each subject were assayed on the same plate in duplicate. Inter-assay and intra-assay coefficients of variation were 12.9% and 15.1% respectively.

### Statistics

Data are presented as means  $\pm$  SE and significance was set at  $p < .05$ . The distribution of all variables was examined using the Shapiro-Wilk test for normality, and homogeneity of variances was determined using Levene's test. All data were normal. Paired-samples t-tests were used to compare pre-exercise values to each post-exercise time point. One-way repeated measures ANOVA was conducted to assess whether there was a significant effect of time. Statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL).

## RESULTS

Fifteen young adults (3 females; 12 males) participated in this study. The entire group had a mean age of  $21 \pm 0.4$  years, BMI  $25.8 \pm 1.1$  kg/m<sup>2</sup>, and BP  $123/78 \pm 2/2$  mmHg. The total duration of the exercise protocol was  $14:23 \pm 00:19$  min:sec.  $VO_{2peak}$  and estimated  $VO_{2max}$  were  $37.3 \pm 1.3$  ml/kg/min and  $45.1 \pm 1.9$  ml/kg/min respectively.

### SOD Responses

Figure 1 shows SOD activity for the entire study group over time. SOD activity was significantly increased from pre-exercise levels ( $4.5 \pm 0.4$  U/ml) at 30 minutes ( $7.7 \pm 0.7$  U/ml, 77% increase from pre-exercise;  $p < .001$ ), 60 minutes ( $6.6 \pm 0.8$  U/ml, 33% increase from pre-exercise levels;  $p < .01$ ), and 120 minutes ( $6.8 \pm 1.3$  U/ml, 37% increase from pre-exercise levels;  $p < .05$ ) post-exercise. There was also an increase in SOD activity immediately post-exercise ( $5.4 \pm 0.4$  U/ml, 20% increase from pre-exercise levels), however this difference failed to reach statistical significance ( $p = .08$ ). Repeated measured analyses showed a significant time effect for SOD activity ( $p < .05$ ).

### TAC Responses

Figure 2 shows TAC levels for the entire study group over time. TAC levels were significantly increased from pre-exercise levels ( $1.7 \pm 0.2$  mM) at 60 minutes ( $2.2 \pm 0.3$  mM, 30% increase from pre-exercise;  $p < .05$ ) and 120 minutes ( $2.4 \pm 0.2$  mM, 33% increase from pre-exercise levels;  $p < .01$ ) post-exercise. No significant time effect was found in repeated measures analyses.

### TBARS Responses

Figure 3 shows TBARS levels for the entire study group over time. No significant time effect was found as plasma TBARS levels were similar across all time points when compared to pre-exercise levels.

### PC Responses

Figure 4 shows PC levels for the entire study group over time. There was no significant time effect as plasma PC levels were similar across all time points when compared to pre-exercise levels.

## DISCUSSION

To the best of our knowledge, the present study is the first to examine the 2-hour time-course oxidative stress response to a short duration bout of submaximal exercise in a cohort of healthy young adults. Although numerous studies have determined the effects of acute exercise on oxidative stress, many of them have measured blood oxidative stress biomarkers in response to exercise protocols that are 30 minutes or longer in duration (11). Therefore, the present study may add to the existing acute exercise-oxidative stress literature by providing information regarding the exercise-induced oxidative stress response to an exercise bout that is similar in intensity and duration to a bout that would be performed as part of an accumulated exercise approach.

The short half-life, small concentration, and strong ability to react make RONS hard to directly quantify in human blood samples. More often, RONS-mediated damage of lipids, proteins, or DNA molecules are used to assess oxidative stress. In the present study we measured TBARS and PC as markers of lipid peroxidation and free radical-damaged proteins respectively. We show that a short duration bout of submaximal exercise does not elicit changes in these biomarkers of RONS mediated-damage in healthy young adults. Similarly, Dayan et al (8) showed that a maximal treadmill exercise test lasting 8–12 minutes was not sufficient to elicit lipid peroxidation in a group of healthy male volunteers. Likewise, Miyazaki et al (19) showed that a short-duration cycle ergometer exercise test to exhaustion did not produce changes in PC levels in a group of untrained young male adults. Taken together, the present and previous study findings may suggest that a short-duration bout of aerobic exercise does not elicit oxidative stress induced cellular damage, irrespective of exercise intensity, in healthy adults. When considering that both submaximal and



maximal bouts of aerobic exercise of at least 30 minutes in duration have typically been shown to elicit RONS-mediated damage (11), the magnitude of oxidative modifications to macromolecules from an acute bout of aerobic exercise may be duration-dependent. In support of this hypothesis, Bloomer et al (5) have shown that plasma PC concentrations increase in a duration dependent fashion with submaximal aerobic exercise in aerobically trained men and women. As such, these findings lend support to the present study findings that short duration submaximal exercise may be a suitable exercise prescription to prevent exercise-induced oxidative stress damage.

Antioxidant enzymatic activity or concentration may also be quantified and used to measure RONS. The increased production of antioxidant enzymes may indicate increased RONS generation, as the body attempts to neutralize an excess of RONS by increasing antioxidant synthesis and/or enzymatic activity. In the present study, we measured two biomarkers of the antioxidant defense system: SOD, an enzyme that is the first line of defense against the free radical superoxide; and TAC, which quantifies the sum of all antioxidants. Our results show that a short duration bout of submaximal exercise elicited a significant increase in SOD activity when compared to pre-exercise levels 30, 60, and 120 minutes post-exercise; and a significant increase in TAC levels 60 and 120 minutes post-exercise. These findings may indicate that a short duration bout of submaximal aerobic exercise is sufficient to induce RONS generation in healthy young adults. When considering that there were no changes in the biomarkers of free radical mediated-damage despite the changes in SOD activity and TAC, our results may suggest that the antioxidant defense system is capable of protecting against RONS production induced by a short duration, submaximal exercise bout in healthy young adults.

Previous studies investigating the acute submaximal exercise response of antioxidant enzymatic activity or concentration, however, can neither confirm nor refute this hypothesis as conflicting results have been reported (6, 16, 17, 27, 28, 30, 31). For SOD activity, some studies have reported no change after submaximal aerobic exercise (16, 27, 28), while other studies have shown an increase (6, 9). Likewise, previous findings on the post-exercise response of TAC after submaximal exercise have also yielded inconsistent results. TAC has been shown to remain unchanged following submaximal bouts of aerobic exercise in some studies (28, 30, 31), but has been reported to increase in others (17). It has been suggested that the antioxidant defense system is temporarily decreased immediately following an acute bout of exercise, as antioxidants are used to neutralize the free radicals produced during exercise. Thereafter, antioxidant levels increase above pre-exercise levels during the recovery period as a result of the excess in free radicals (11). Considering these time-dependent changes in antioxidant levels, it is probable that the conflicting findings in SOD activity and TAC following a bout of submaximal aerobic exercise could be confounded by the lack of multiple time samples. In a time-course study in which 10 serial blood samples during a 24-hour period after an acute bout of aerobic exercise were collected, Michalidis et al (18) concluded that the optimal post-exercise time point for blood collection and measurement of TAC is 2-hours post-exercise as their results showed that TAC peaks 2-hours post-exercise, remains elevated 3-hours post-exercise, and declines thereafter. Of the studies that have reported no change in SOD activity and TAC following submaximal aerobic exercise, only three obtained more than one sample after the immediate post-exercise time point sample, at 20-minutes (30), 1 hour (31), or 24-hours post-exercise (28). Thus, these studies may have missed the time-dependent changes in SOD activity and TAC post-exercise that were observed in the present study.

It must be noted that there are some limitations to our study. First, our sample size is small but this was due to the exclusion of college athletes, diabetics, smokers, and women on birth control. This was intentionally done in order to create as homogenous group as possible and

to ensure lack of confounding variables that may influence oxidative stress measures. Considering the small sample size, we have conducted power analyses and found that for the biomarkers SOD, TAC, TBARS, and PC the statistical power ranged from 78–99% with an alpha level of 0.05. Second, diet was not controlled leading up to the study, but we felt it was not necessary because recent research has shown that in subjects of similar age and health status, no day to day differences exist in macronutrient ingestion (1, 7). Third, almost all oxidative stress biomarkers have been criticized for their reliability. For example, the lack of specificity of the TBARS assay for MDA has led to some reservations about its validity (20). In general, every assay has its advantages and disadvantages. Given that 2 biomarkers were measured for indices of both RONS-mediated damage and antioxidant activity/concentration, and considering that similar trends for RONS-mediate damage (no change in both TBARS and PC) and antioxidant activity/concentration (increased post-exercise); it is apparent that our study was capable of describing exercise-induced oxidative stress to some extent. Fourth, the incremental nature of the graded exercise protocol used as our exercise stimulus is not a common exercise protocol used by the general public, thus the generalizability of our study findings may be limited. Finally, the present study investigated the oxidative stress response to a single bout of aerobic exercise. Though we found no evidence that a pro-oxidant state persists 2-hours post exercise, it is possible that subsequent bouts of exercise that would occur with an accumulated exercise approach could elicit a different response.

In conclusion, in a cohort of healthy young adults, a short duration bout of submaximal aerobic exercise elicited increases in antioxidant activity/concentration 30–120 minutes post-exercise, suggestive of increased RONS-generation, but did not elicit oxidative stress-induced damage. Since the hormetic effect of exercise is predicated off of the notion that too much generation of RONS is harmful due to the resultant oxidative damage to macromolecules, while modest generation is apparently beneficial to upregulating antioxidant defense mechanisms against oxidative stress, it would appear that shortened bouts of submaximal aerobic exercise could be sufficient for adaptive processes. Thus, short duration bouts of submaximal aerobic exercise, as part of an accumulated exercise approach, could provide an added benefit beyond improving exercise compliance by preventing undue oxidative stress, while permitting a sufficient exercise stimulus to elicit exercise adaptation. Future studies, however, will be needed to confirm this hypothesis.

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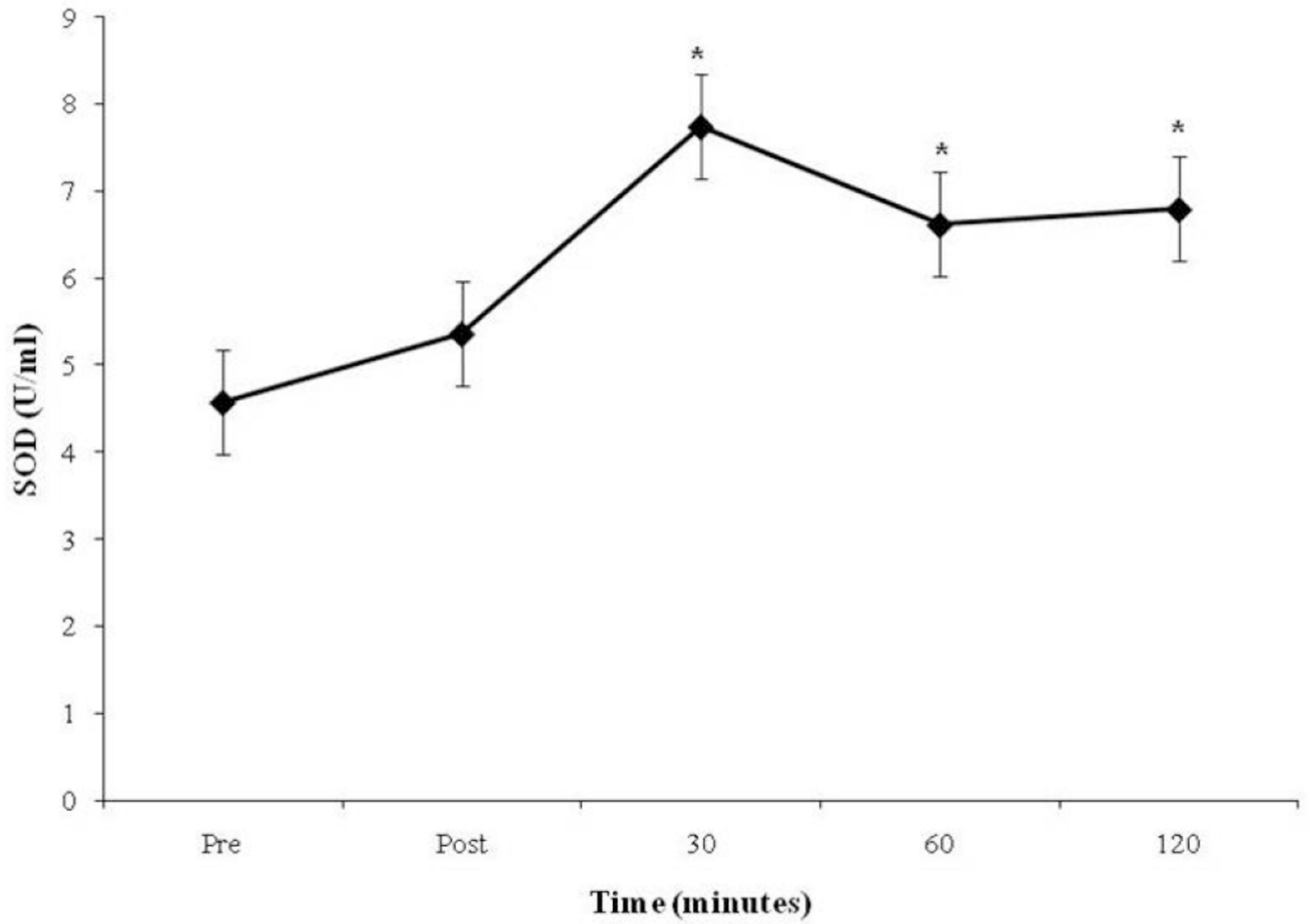
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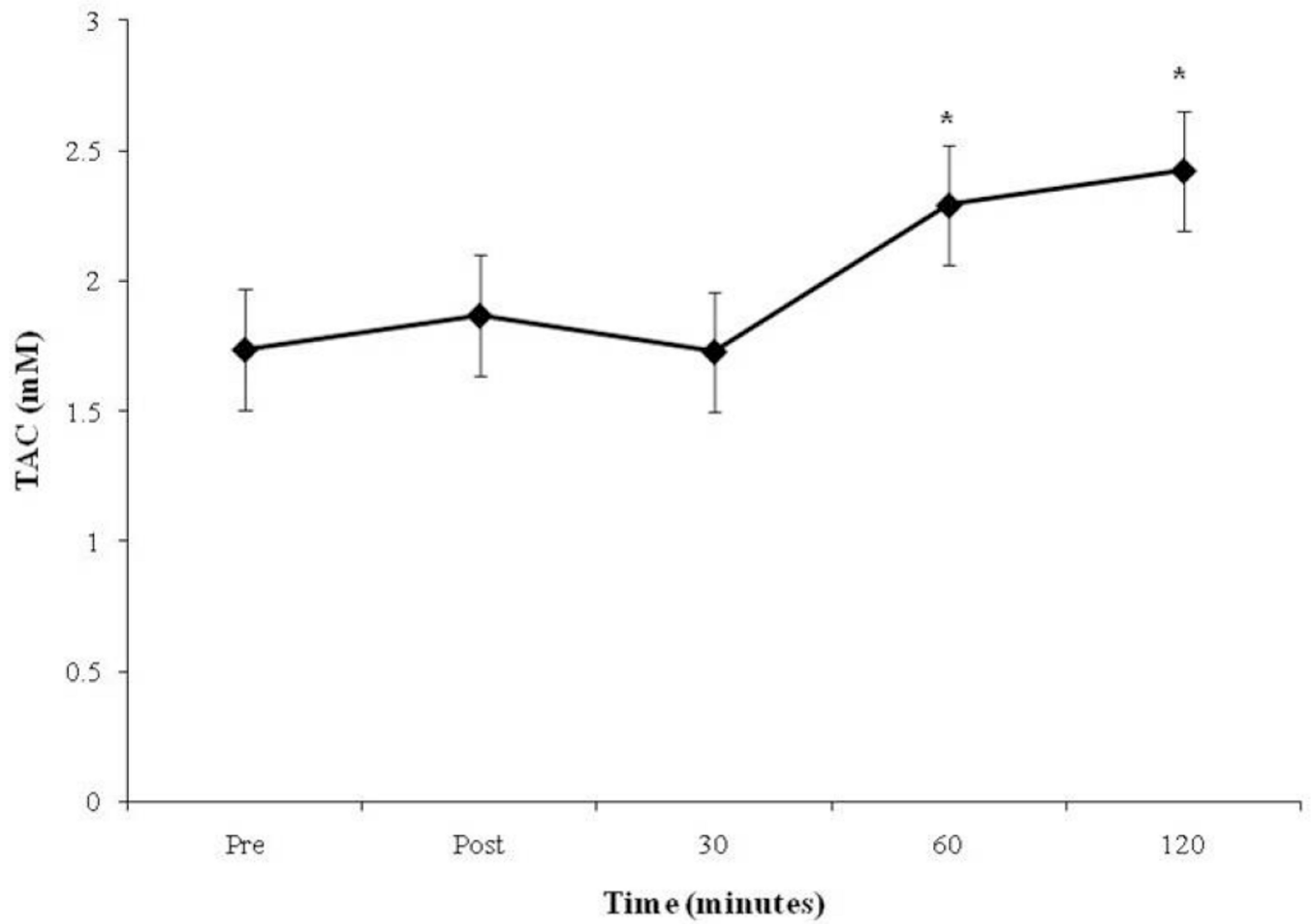
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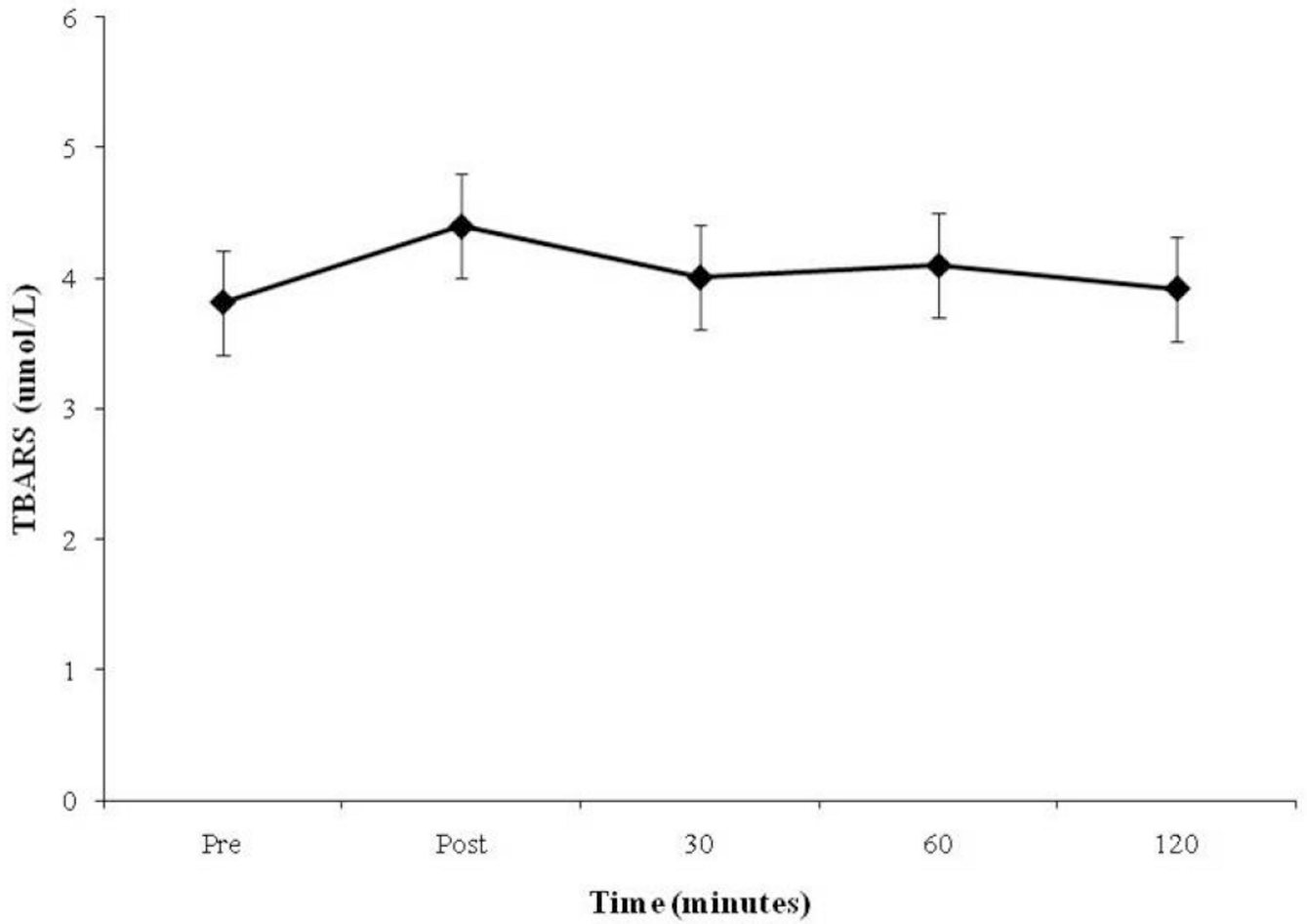
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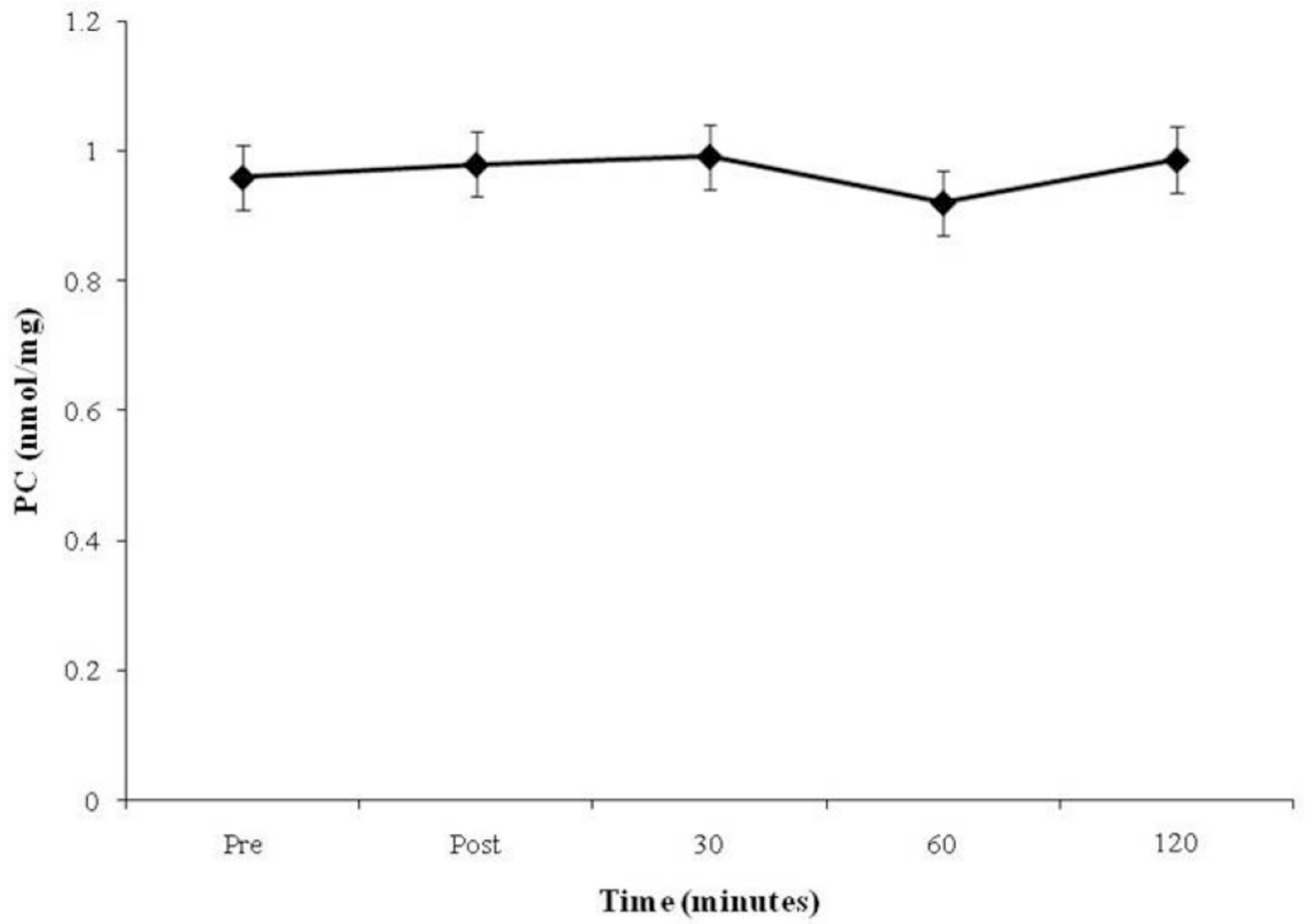
**Figure 1.** SOD activity for the entire study group. Values are means  $\pm$  SE. \* Denotes significant difference from pre-exercise levels.



**Figure 2.** TAC levels for the entire study group. Values are means  $\pm$  SE. \* Denotes significant difference from pre-exercise levels.



**Figure 3.** TBARS levels for the entire study group. Values are means  $\pm$  SE.



**Figure 4.**  
PC levels for the entire study groups. Values are means  $\pm$  SE.