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Racial Differences in the Time-Course Oxidative Stress Responses to Acute Exercise

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ABSTRACT

Fairheller DL, Diaz KM, Sturgeon KM, Williamson ST, Brown MD. Racial Differences in the Time-Course Oxidative Stress Responses to Acute Exercise. **JEPonline** 2011;14(1):49-59. African Americans have disproportionate levels of cardiovascular disease and oxidative stress. The purpose of our study was to examine racial differences between African American and Caucasian adults in time-course oxidative stress responses to a treadmill test. After a 12-hr fast, 18 participants (9 of each ethnic group; 21 ± 0.4 yrs) completed a submaximal treadmill test and underwent serial blood draws: Pre, Post (within 2 min), 30, 60, and 120 min after exercise. At each time-point, superoxide dismutase (SOD, U/mL), total antioxidant capacity (TAC, mM), protein carbonyls (PC, nmol/mg), and thiobarbituric acid-reactive substance (TBARs, μmol/L) were measured. We found no difference between groups for blood pressure, BMI, or exercise capacity (as measured by volume of oxygen consumed, VO₂ max). African Americans had significantly ($p < 0.05$) higher SOD (Pre: 5.45 ± 0.4 vs. 3.69 ± 0.69; 60 min: 8.99 ± 0.7 vs. 4.23 ± 0.6; 120 min: 9.69 ± 1.6 vs. 3.52 ± 0.7), TAC (Pre: 2.31 ± 0.25 vs. 1.16 ± 0.3; Post: 2.39 ± 0.2 vs. 1.34 ± 0.2; 30 min: 2.29 ± 0.2 vs. 1.09 ± 0.2), and PC (Pre: 1.09 ± 0.1 vs. 0.82 ± 0.1; Post: 1.14 ± 0.1 vs. 0.81 ± 0.1; 30 min: 1.13 ± 0.1 vs. 0.85 ± 0.1; 60 min: 1.06 ± 0.1 vs. 0.81 ± 0.05), but not TBARs. Between groups, only SOD exhibited a different time-course response: levels for African Americans rose steadily throughout the 120 min, while levels for Caucasians peaked at 30 min and by 120 min had returned to pre-exercise levels. Race had a greater effect on oxidative stress responses than submaximal exercise did. African Americans had significantly higher TAC, SOD, and PC levels compared to Caucasians.

Key Words: African Americans, Sub-maximal Exercise, Antioxidants, Nitric Oxide, Superoxide Dismutase, Total Antioxidant Capacity

INTRODUCTION

African Americans exhibit disproportionate levels of hypertension (HT), higher incidence of cardiovascular and renal disease, and elevated levels of oxidative stress when compared to other ethnic groups, in particular Caucasians. Additionally, studies in un-stimulated endothelial cell culture have shown that these racial differences also exist in vitro by reporting heightened oxidative stress in African American cells compared to Caucasian cells (17).

The effects of submaximal acute exercise on the oxidant/antioxidant balance over a post-exercise time period are not well known. Inconsistencies are found in results from one study to the next due to differences in exercise protocol, training status, and gender. It is also not well known whether a disparity exists between races in the oxidative stress response to exercise. Since exercise is often prescribed as a non-pharmacologic treatment of chronic diseases like HT, and given that African Americans tend to have higher levels of oxidative stress, it becomes critical to understand the appropriate exercise intensity that will not elicit an exaggerated oxidative stress response.

Oxidative stress is an imbalance between the production of free radicals and the antioxidant system's ability to buffer the oxidative damage. Exercise causes an increase in oxygen consumption and, therefore, the production of reactive oxygen species (ROS), ultimately leading to increased oxidative stress if the antioxidant system's buffering ability is insufficient. This oxidative stress response to exercise varies by biomarker. It is understood that proteins are expressed in response to exercise, their expression levels peak at different times, and the amount of time that it takes to return to baseline expression levels varies by marker. Classic pre-post exercise protocols collect the post-exercise sample immediately following the exercise bout, and only a few studies exist where more than 2 blood samples have been collected to explore time-course responses to acute exercise. Along these lines, in 2007 Michailidis et al. (22) investigated the time-course responses of several oxidative stress markers during a 24-hour period after one session of 45 minutes of treadmill exercise at 70 to 75% VO_2 max. They studied 11 untrained men and found different response times for the oxidative stress markers. However, to the best of our knowledge, this type of study has not been done to examine potential racial differences.

The purpose of the present study was to examine racial differences between African American and Caucasian adults in the time-course oxidative stress responses to an acute exercise bout. Because exhaustive exercise to volitional fatigue is not commonplace for most exercise sessions among the general public, we sought to determine whether responses to a submaximal exercise test differed by race.

METHODS

Subjects

Young college-aged African American and Caucasian students 18 to 25 years of age were recruited through advertisements and word of mouth. Following the completion of an extensive health history form during the initial laboratory visit, all subjects were apparently healthy and free of cardiovascular risk factors. This study was approved by the Institutional Review Board of Temple University, Philadelphia, PA., was conducted under HIPPA guidelines, and all qualified students provided their written, informed consent.

Experimental Design

The subjects were asked to refrain from vitamins for 2 weeks prior to the study, from caffeine, alcohol, and exercise training for 24 hours prior to testing, and they were asked to fast for at least 12 hours the night before the study. Research has suggested that the hormone fluctuations during the menstrual cycle can influence oxidative stress responses to exercise (16). Therefore, all females

were tested during days 1 through 5 of their menstrual cycle because hormone levels tend to be lowest early in the follicular phase.

On the morning of the study, height and weight were measured, and a pre-exercise blood sample was collected. Blood samples were collected in EDTA and Sodium-Heparin tubes, centrifuged at 2000g for 20 minutes at 4°C, and then the plasma was frozen at -80°C until assay. Then a modified Bruce submaximal treadmill (TM) exercise test was performed. The TM test was terminated when the subjects reached 75 to 80% of their heart rate reserve, then regression analysis using data collected by indirect calorimetry was used to predict VO_2 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. All subjects remained in the lab for 2 hours following the exercise period in order to control for food and water intake. During this time, they were instructed to sit and read, or work on the computer. They were allowed to drink up to 1L of water. At the completion of the test, juice and snacks were provided to replenish glucose levels. Subject data were only included if 80% of the blood samples were collected.

Assays (All assays were done in duplicate)

Plasma Superoxide Dismutase (SOD). Plasma samples were diluted 1:5 in sample buffer (50 mM 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 dethylenetriaminepentaacetic 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). All reagents were obtained from Cayman Chemical (Ann Arbor, MI). The detection limit was 0.025 U/ml. Inter-assay and intra-assay coefficients of variation were 5.9% and 12.4%, respectively.

Total Antioxidant Capacity (TAC). Plasma samples were diluted 1:20 in Assay buffer (5 mM 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[®] (2,2'-Azino-di- to ABTS^{®+•} by metmyoglobin). The capacity of the antioxidants in plasma to prevent ABTS[®] 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. All reagents were obtained from Cayman Chemical (Ann Arbor, MI). The detection limit was 0.044 mM. 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[™] Protein Carbonyl ELISA Kit (Cell Biolabs, Inc., San Diego, CA). The manufacturer's instructions were followed as described previously (9). Absorbance was read at 450 nm using a SpectraMax Microplate Reader (Molecular Devices, Sunnyvale, CA). The detection limit was 0.375 nmol/mg. 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. 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). Inter-assay and intra-assay coefficients of variation were 12.9% and 15.1%, respectively.

Statistical Analyses

The data are presented as means \pm SE and significance was set at $p < 0.05$. The distribution of all variables was examined using the Shapiro-Wilk test of normality, and homogeneity of variances was determined using Levene's test. All data were normal. Independent t-tests were used to determine if there were significant differences between ethnic groups. Two-way repeated measures ANOVA, with Huynh-Feldt or Greenhouse-Geisser correction when necessary, were conducted to assess whether significant effects of race or time existed. This was followed by post-hoc paired t-test analyses. Area under the curve (AUC) was calculated by polynomial integration and independent t-test analyses were conducted to examine for differences between ethnic groups. The statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL).

RESULTS

For analysis by ethnic groups, the participants were divided by race into the African American group and the Caucasian group. Table 1 shows the subject characteristics in each group. There were no significant differences between the two groups for any of the variables.

Table 1. Subject Characteristics by Ethnic Group (N=18)

	African Americans (N=9)	Caucasians (N=9)	P-Value
Age, yrs	21.3 \pm 0.5	20.6 \pm 0.7	0.40
BMI, kg/m ²	25.8 \pm 1.1	25.2 \pm 2.2	0.83
VO ₂ max, ml·kg ⁻¹ ·min ⁻¹	44.9 \pm 3.4	44.6 \pm 2.6	0.96
Systolic BP, mmHg	122.6 \pm 4.4	126.2 \pm 3.9	0.55
Diastolic BP, mmHg	79.0 \pm 3.8	78.0 \pm 3.2	0.85

Data are presented as mean \pm SE. N, sample size; BMI, body mass index; VO₂ max, "predicted" maximum oxygen consumption; BP, blood pressure.

SOD Responses by Race. Figure 1 shows SOD activity by race over time. There was a significant time and race effect for SOD. Statistical analysis showed a time effect from pre-exercise to post-exercise ($p = 0.03$), and from the 30-minute to 60-minute ($p = 0.02$) time points in the Caucasian group. In the African American group, the analysis showed a time effect from post-exercise to the 30-minute point ($p = 0.01$). Separate pair-wise analyses showed significant differences between African Americans and Caucasians at pre-exercise ($p = 0.04$), at the 60-minute ($p = 0.00$), and at the 120-minute ($p = 0.01$) time point.

PC Responses by Race. Figure 2 shows PC values by race over time. There was only a significant race effect in PC levels. The African American group had higher PC values at all time points, with the difference between African Americans and Caucasians reaching statistical significance at pre-exercise ($p = 0.01$), post-exercise ($p = 0.00$), at the 30-minute point ($p = 0.01$), and at the 60-minute ($p = 0.01$) time point.

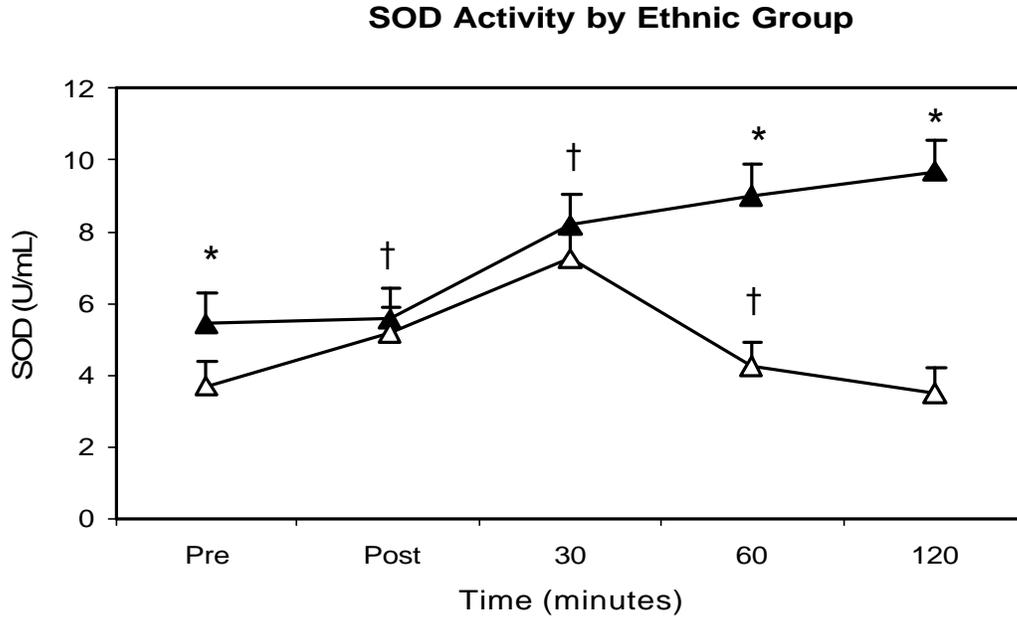


Figure 1: Comparison of SOD levels between African American (closed triangles) and Caucasian (open triangles) adults. Values are means \pm SE. * Significant difference between Ethnic groups. †Significant difference from previous sample time. Significance level set at $p = 0.05$.

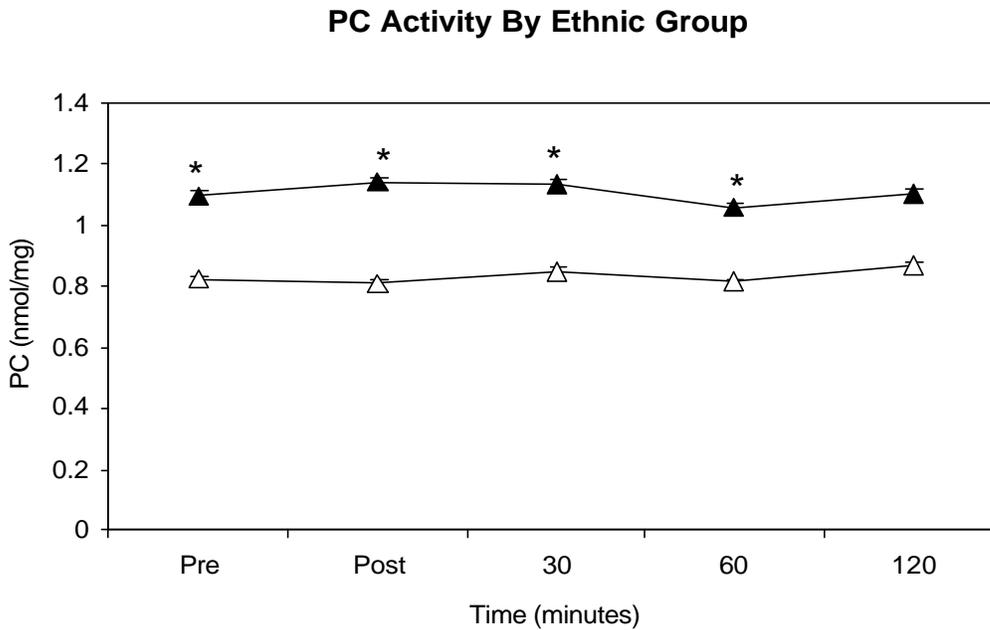


Figure 2: Comparison of PC levels between African American (closed triangles) and Caucasian (open triangles) adults. Values are means \pm SE. * Significant difference between Ethnic groups. Significance level set at $p = 0.05$.

TAC Responses by Race. Figure 3 shows TAC values by race over time. Only a significant race effect was found in TAC responses. While the African American group had higher TAC values at all time points, analysis showed significant difference between African Americans and Caucasians at pre-exercise ($p = 0.01$), post-exercise ($p = 0.00$), and at the 30-minute ($p = 0.00$) time point.

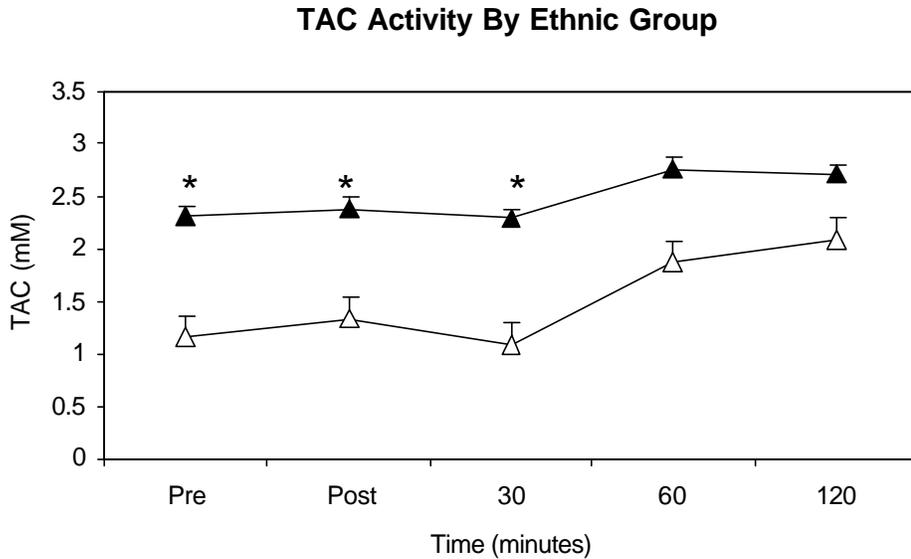


Figure 3: Comparison of TAC levels between African American (closed triangles) and Caucasian (open triangles) adults. Values are means \pm SE. * Significant difference between Ethnic groups. Significance level set at $p = 0.05$.

TBARS Responses by Race. Figure 4 shows TBARS values by race over time. No significant time or race effect was found.

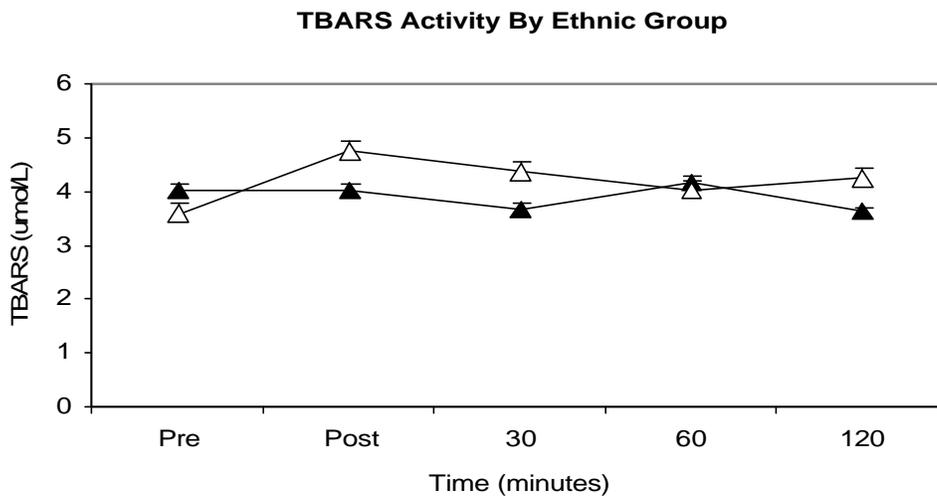


Figure 4: Comparison of TBARS levels between African American (closed triangles) and Caucasian (open triangles) adults. Values are means \pm SE.

Total Area under the Oxidative Stress Response Curves (AUC). Table 2 reports the AUC differences by race. Integration was completed to analyze AUC for each oxidative stress variable. Independent t-test analysis showed significant race differences in SOD, TAC, and PC, but not in TBARS. The data indicate that the African American group had a higher antioxidant and oxidative stress load over the entire sampling time.

Table 2. Total Area Under Oxidative Stress Response Curves, by Ethnic Group

	African Americans (N=9)	Caucasians (N=9)	P-Value
AUC SOD, U/ml per 120 min	986.0 ± 86.3	517.9 ± 51.8	0.00
AUC TAC, mM per 120 min	293.2 ± 31.9	180.7 ± 23.4	0.01
AUC PC, nmol/mg per 120 min	126.1 ± 7.7	96.9 ± 8.7	0.02
AUC TBARS, umol/L per 120 min	405.2 ± 74.9	478.8 ± 54.9	0.44

Data are presented as mean ± SE. N, sample size; AUC, total area under the response curve; SOD, superoxide dismutase activity; TAC, total antioxidant capacity; PC, protein carbonyls; TBARS, thiobarbituric acid reactive substances.

DISCUSSION

The main finding from the present study is that significant racial differences exist in oxidative stress markers, but the submaximal exercise intensity was only enough to elicit a response to the exercise bout in SOD activity. Research has established that chronic aerobic exercise can effectively improve oxidative stress levels, but the results on the oxidative stress responses to acute exercise, especially submaximal levels, remain inconsistent. Reports from previous studies vary by response and by oxidative stress marker, and this discrepancy may be largely influenced by exercise intensity, by vast differences in training status, age, gender, race, and exercise modality. High intensity acute exercise has been shown to increase oxidative stress (21,23), while other studies report differential responses depending on exercise intensity (6,11). For example, Dayan et al. (13) used a modified Balke test to examine lipid peroxidation in healthy males and found no change in lipid oxidation. The submaximal intensity of a Balke protocol is similar to that used in our study and we also found no change in lipid peroxidation. Considering this disparity in the literature, it appears that further research is still needed to elucidate the oxidative stress responses to acute exercise.

In young Caucasian adults, we found that an acute submaximal exercise bout caused a significant increase in SOD activity from pre-exercise to post-exercise compared to no change observed in the African American group. Some studies have shown no change in SOD activity in animal models after acute exhaustive exercise (1,18), while other studies have shown that gender and training status influenced SOD responses (10,19,24). Except for the present study, there are no studies that have examined the racial differences in SOD in response to acute aerobic exercise. As mentioned, we found a racial difference in SOD activity over the time-course sampling period. The Caucasian adults showed a significant increase in SOD activity in response to the acute exercise bout. These levels continued to rise until they peaked at 30 minutes, and by 60 minutes had returned to basal levels.

Conversely, the African American adults had a delayed response in SOD activity to the submaximal exercise stimulus. The levels of SOD activity in African Americans did not begin to rise until the post-exercise time point and continued to trend upward for the following 2 hours. To the best of our knowledge, only one other study has reported racial differences in SOD responses, and this was in diabetic patients. Zitouni et al. (30) reported that the African American patients had significantly higher SOD activity compared to the Caucasian patients, which is similar to what we found in our healthy young adults. In addition, other unpublished data from our lab in healthy adults confirms a

higher SOD activity in African Americans compared to Caucasians, before and after a maximal the Bruce treadmill test. The SOD enzyme is the main antioxidant enzyme that catalyzes the dismutation of superoxide anion into hydrogen peroxide, so a higher SOD activity in African Americans suggests an existing higher level of superoxide production, and thus elevated oxidative stress.

Elevations in oxidative stress interact with proteins, lipids, and DNA causing protein degradation, lipid peroxidation, and DNA damage. However, the effect of damage from exercise depends on duration and degree of exercise as well as training status of the subjects (25). ROS-induced protein modifications lead to altered protein structure or unfolding of proteins, and the most common products of protein oxidation are the protein carbonyl (PC) derivatives of Pro, Arg, Lys, and Thr. Carbonylation is an irreversible and non-enzymatic protein conversion and the byproduct derivatives are chemically stable and easily measurable markers of oxidative stress (12). Recent research has reported PC elevations associated with many endogenous and exogenous factors not related to exercise. Elevated PC levels have been found in patients who are critically ill (28), who have acute infection (27), who have neurodegeneration (15), and in the pathogenesis of aging (4,8). Recently, Yeh et al. (29) found that PC levels were significantly higher in African American compared to Caucasian adults (29). Similarly, in the present study, the African Americans had significantly higher PC levels compared to the Caucasians across all time points, thus indicating elevated oxidative stress.

We also found that acute submaximal exercise did not elicit a change in PC levels. Similar to these findings, Liu et al. (20) compared the oxidative stress responses in rats to acute and chronic exercise. Their results showed that acute exercise did not cause significant changes in PC levels; whereas, chronic exercise induced a small decrease. Studies (2,20) also report that the magnitude of oxidative damage is associated with intensity of the acute exercise bout. High-intensity and high-volumes of aerobic exercise elicit an increase in protein and DNA damage as measured by higher PC levels (5,26). In short, it appears that the intensity of a submaximal treadmill test is not sufficient to elicit a change in PC levels, which was confirmed by the present study.

The submaximal exercise test was also not a sufficient intensity-stimulus to elicit a response in TAC. The same was found by Ashmaig et al. (3) in which no change in TAC was observed in patients with ischaemic heart disease who underwent a standard Bruce treadmill test. On the other hand, Demerbag et al. (14) measured TAC responses to a submaximum modified Bruce treadmill protocol and reported a decrease in TAC. Participants in their study were patients with angina or angina-like symptoms. The variation in study populations may have contributed to the different responses in TAC. While we found no response to exercise in TAC, we did find significant differences between race in TAC levels pre-exercise, post-exercise, and at the 30-minute time points. The African American group was higher at all points, suggesting elevated oxidative stress exists and, therefore, the heightened antioxidant levels.

It must be noted that there are some limitations in the present study. First, the sample size is small but this was due to the exclusion of college athletes, diabetics, smokers, women on birth control, and all other ethnic groups. 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 that showed significant racial differences; SOD, TAC, and PC the statistical power ranged from 65 to 75% with an alpha level of 0.05. The power for TBARS was lower and this may have contributed to the lack of significant difference between the groups. However, the TBARS values for the African American group and the Caucasian group were very similar at all time-points. 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 to those in the present study, the day to day differences in macronutrient ingestion are very minimal (2,7). Finally, most exercise studies use

more aggressive exercise intensities, and the use of a submaximal exercise test in this study may not replicate the oxidative stress responses to other maximal or exhaustive exercises. One of the main purposes of this study was to examine whether racial differences existed in the oxidative stress responses, and we found significant racial differences between African American and Caucasian adults in the oxidative stress biomarkers SOD, TAC, and PC.

CONCLUSION

In conclusion, race had a greater effect on oxidative stress levels than time or exercise did. African Americans had significantly higher TAC, SOD, and PC levels when compared to Caucasians, while SOD was the only biomarker to demonstrate different responses to the submaximal exercise. These data confirm what has been previously reported that African Americans have a higher level of oxidative stress compared to Caucasians. The data also add to existing acute exercise literature by providing evidence that a single submaximal exercise bout will not elicit substantial oxidative stress responses, since the only marker to show response was SOD. Considering that exhaustive exercise to volitional fatigue is not commonplace for most exercise sessions among the general public, these findings suggest that a moderate submaximal exercise intensity may be an appropriate exercise prescription as a non-pharmacologic treatment for chronic diseases like HT and cardiovascular diseases because it does not elicit excessive oxidative stress.

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