Objective: To examine the interaction of oxidative stress biomarkers with age, and also factors that influence oxidative stress such as body mass index (BMI) and fitness in a population of individuals with established higher risk of cardiovascular disease, African Americans.

Methods: Blood samples were obtained from healthy college-age and middle-age to older African Americans. Participants underwent a graded exercise test. Superoxide dismutase (SOD) activity, total antioxidant capacity and thiobarbituric acid reactive substances (TBARS) levels were measured.

Results: TBARS levels were significantly (P<0.001) lower in young participants relative to middle-age to older participants. SOD activity was significantly (P<0.001) lower in middle-age to older participants with low fitness relative to participants with normal fitness, and lower (P=0.04) in middle-age to older participants that were overweight relative to normal weight participants.

Discussion: In a healthy middle-age to older population of African Americans, BMI and fitness are crucial for maintaining a healthy endothelium. (Ethn Dis. 2010;20:403–408)

Key Words: Aging, Oxidative Stress, African Americans, Fitness, BMI

INTRODUCTION

Many of the factors that underlie age-related changes in the vasculature are also implicated in the development of cardiovascular disease (CVD). Endothelial dysfunction as a pathological state due to chronic oxidative stress has been associated with both aging and CVD, and evidence suggests these two separate processes are highly intertwined. Clinical observations involving the epidemiology of CVD also point toward an unequal ethnic distribution of the disease. In 2010, African Americans had statistically higher levels of total CVD than Caucasians or Mexican Americans. The mechanisms underlying these racial disparities are multifactorial and involve genetic factors, socioeconomic status, psychosocial issues, and other environmental factors. However, all of these factors have the ability to influence oxidative stress.

The ability of cellular antioxidant enzymes to process and remove reactive oxygen species (ROS) is critical to maintaining a healthy endothelium and nitric oxide (NO) bioavailability. If ROS such as superoxide anion (O$_2^-$), hydroxyl radical (OH$^-$), hydrogen peroxide (H$_2$O$_2$), or hydroperoxides (ROOH) overwhelm the antioxidant system, the redox state of the cell will shift and the cell will be placed under oxidative stress. Interestingly, in a basal in vitro experiment, Kalinowski et al observed that NO bioavailability was diminished by higher levels of O$_2^-$ in African American endothelial cells compared to Caucasian endothelial cells. The researchers suggested there are underlying racial differences in the redox state of endothelial cells between African Americans and Caucasians.

The pathophysiology of essential hypertension is still uncertain and there are many factors that contribute to chronically raised blood pressure (BP). Lifestyle modifications such as exercise, healthy diet, and weight management are critical for the prevention of high blood pressure and also the progression of CVD. Exercise has been implicated in the preservation of endothelial function with advancing age in the face of adverse effects of conventional risk factors for CVD. Weight gain into overweight and obese body mass index (BMI) categories is powerfully associated with overall mortality and is a principal causative factor in the development of metabolic syndrome. It is also directly correlated to increased production of ROS and systemic markers of oxidative stress.

The purpose of our study was to examine the interaction of oxidative stress biomarkers with age, and also factors that influence oxidative stress within younger and middle-age to older age groups of normotensive and pre-hypertensive African Americans.
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hypertensive African Americans. To our knowledge, no study to date has investigated oxidative stress biomarkers in pre-hypertensive African Americans as they relate to aging and clinically relevant measures of fitness and body weight.

Materials and Methods

Participants

College-aged African Americans, aged 18–25 yrs, were recruited through advertisements and word of mouth. Middle-age to older African Americans, aged 40–75 yrs, were recruited through advertisements and mailings. All participants were apparently healthy. This was assessed by completion of an extensive health history form for college-aged participants, and by more extensive screening in the middle-age to older African American participants. Demographic and health history information were collected during an initial laboratory visit and qualified participants provided their written, informed consent. The study was approved by the institutional review board of Temple University, Philadelphia, Pa.

Middle-age to older African Americans were non-smoking, not on lipid-lowering medication, and had no history of CVD, diabetes, hypercholesterolemia, liver disease, renal disease, or lung disease. Middle-age to older African Americans were also screened for cholesterol level and fasting blood glucose level. Exclusion criteria included cholesterol level >240 mg/dL and fasting blood glucose >126 mg/dL. In addition, middle-age to older African American participants underwent a physician administered physical examination and echocardiogram bicycle stress test to confirm that participants displayed no evidence of cardiovascular, pulmonary, or other chronic diseases.

Experimental Design

For each participant, the average of three BP values measured in accordance with JNC-7 guidelines was considered the study BP. Fasting blood samples were obtained via venipuncture at an antecubital vein. Participants were asked to refrain from vitamins, caffeine, alcohol, or exercise training for 24 hrs prior to the acute exercise test. A modified Bruce sub-max treadmill (TM) exercise test was performed. The TM test was terminated when the participant reached 75–80% of their estimated heart rate reserve. Regression analysis using data collected by indirect calorimetry was used to predict VO_{2max} levels (pVO_{2max}).

Assessment of Oxidative Stress Biomarkers

Superoxide dismutase (SOD) is the primary enzyme responsible for removing O_2^- and total antioxidant capacity (TAC) is a biomarker indicative of activity of all antioxidants found in peripheral blood plasma. Superoxide dismutase activity and TAC levels were determined using commercially available kits (Cayman Chemical, Ann Arbor, MI); SOD inter-assay and intra-assay coefficients of variation were 5.9% and 12.4%, respectively; TAC inter-assay and intra-assay coefficients of variation were 6.7% and 9.2%, respectively.

A commonly used indicator of oxidative stress via lipid peroxidation is thiobarbituric acid reactive substances (TBARS). This indicator was colorimetrically quantified following the reaction of malondialdehyde (MDA), contained in the sample, with thiobarbituric acid (TBA). All reagents were obtained from Cayman Chemical (Ann Arbor, MI). Inter-assay and intra-assay coefficients of variation for TBARS were 12.9% and 15.1%, respectively. Blood samples were collected in Sodium-Heparin and EDTA tubes, centrifuged at 2000g for 20 minutes at 4°C, and plasma was frozen at −80°C until assayed. Absorbencies were read using a SpectraMax Microplate Reader (Molecular Devices, Sunnyvale, CA).

Statistics

Data are presented as means ± SEM and significance was set at P<.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 age groups, young groups divided by BMI, young groups divided by fitness level, middle-age to older groups divided by BMI, and middle-age to older groups divided by fitness level. Statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL).

Results

Whole Population

Nine college-aged males (n=6) and females (n=3) aged 21.3 ± 0.5 years participated in this study along with 15 middle-age to older males (n=3) and females (n=12), aged 52.7 ± 1.4 years. Demographic information is presented in Table 1. There was no significant difference in systolic BP (SBP) or diastolic BP (DBP). Participants were matched for BMI; therefore there was no significant difference between the young and middle-age to older groups. It was observed that the young group had a significantly higher (P=.001) average predicted VO_{2max} (44.9 ± 3.41 ml/kg/min) compared to the middle-age to older group (26.2 ± 1.42 ml/kg/min).

Subgroup Analysis

For analysis, young and middle-age to older participants were assigned within their age groups to normal weight or overweight categories, and normal fitness or low fitness categories (Table 2). Both young and middle-age to older groups were composed of normotensive (BP<120/80 mm Hg) and pre-hypertensive (BP 120–139/80–89 mm Hg) participants. Normal
weight and overweight were defined using BMI criteria (normal weight = BMI<25, overweight = BMI≥25). Fitness was categorized by age and sex specific VO\textsubscript{2max} ranges for healthy non-athletes.\(^{13}\)

In order to elucidate any potential differences in oxidative stress variables due to weight or fitness that may be age-specific, these categories were analyzed within age groups (young and middle-age to older). Superoxide dismutase activity levels in the younger and middle-age to older groups stratified by weight and fitness levels are illustrated in Figure 1. While BMI or fitness did not significantly affect SOD activity in the young group, there were significant differences in the middle-age to older group. Middle-age to older participants with normal fitness (2.94 ± 0.78 mM) or normal weight (3.14 ± 0.99 mM) compared to middle-age to older participants with low fitness (1.63 ± 0.41 mM) or overweight (2.17 ± 0.52 mM). However, these values did not reach statistical significance (Figure 2).

Levels of TBARS were significantly lower in young participants compared to middle-age to older participants (P=0.02). Though non-significant, TBARS levels were observed to be higher in overweight middle-age to older participants (8.24 ± 1.32 μmol/L) relative to middle-age to older normal weight participants (6.51 ± 2.00 μmol/L) (Figure 3).

**DISCUSSION**

The incidence of CVD increases exponentially with age and may share a common mechanism of progression such as oxidative stress. In order to understand how clinical observations such as BMI and fitness are related to molecular biomarkers of endothelial health, we assessed these relationships in a population of young and middle-age to older healthy normotensives and pre-hypertensives. In addition, these relationships were assessed in African Americans. African Americans have an established higher incidence of hypertension, and a greater age-adjusted heart disease death rate compared to Caucasians.\(^{14,15}\)

In our study, TBARS levels were observed to be the only significantly different oxidative stress biomarker between age groups in normotensive and pre-hypertensive African Americans. This finding is in agreement with previous studies which have reported elevated TBARS in healthy older populations.\(^{16,17}\)

Markers of antioxidant capacity, SOD activity and TAC levels, were lower in middle-age to older African Americans with compromised fitness relative to middle-age to older African Americans with normal fitness. This relationship was also true for SOD activity and TAC levels in middle-age to older overweight African Americans relative to middle-age to older normal weight African Americans. The significantly lower SOD activity may indicate a compromised ability to handle O\textsuperscript{2−}, or that SOD capacity is saturated. In either situation, an excess of O\textsuperscript{2−} may be present which may cause increased lipid peroxidation as seen in the middle-age to older population.

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**Table 1. Demographic characteristics of young and middle-age to older African Americans**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yrs)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>BMI (kg/m(^2))</th>
<th>pVO\textsubscript{2max} (ml/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (n=9)</td>
<td>*21.3 ± 0.50</td>
<td>122.6 ± 4.31</td>
<td>79.0 ± 3.82</td>
<td>25.8 ± 1.15</td>
<td>*44.9 ± 3.41</td>
</tr>
<tr>
<td>Middle-age to old (n=15)</td>
<td>52.7 ± 1.40</td>
<td>123.9 ± 3.86</td>
<td>77.4 ± 2.19</td>
<td>26.6 ± 0.63</td>
<td>26.2 ± 1.42</td>
</tr>
</tbody>
</table>

* P<.01 Young vs middle-age to older. Systolic blood pressure, SBP; diastolic blood pressure, DBP; body mass index, BMI; predicted VO\textsubscript{2max}; pVO\textsubscript{2max}.

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**Table 2. Weight and fitness categories for young and middle-age to older African Americans**

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal Weight</th>
<th>Overweight</th>
<th>Normal</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>*23.0 ± 0.78 (n=4)</td>
<td>28.0 ± 1.31 (n=5)</td>
<td>*49.8 ± 8.59 (n=6)</td>
<td>35.1 ± 2.34 (n=3)</td>
</tr>
<tr>
<td>Middle-age to old</td>
<td>*23.6 ± 0.51 (n=5)</td>
<td>28.0 ± 0.40 (n=10)</td>
<td>27.8 ± 1.73 (n=7)</td>
<td>24.5 ± 2.21 (n=7)</td>
</tr>
</tbody>
</table>

* P<.01 between BMI group or fitness for each age group. Body mass index, BMI; predicted VO\textsubscript{2max}; pVO\textsubscript{2max}.
Epidemiological studies have demonstrated that chronic diseases and all-cause mortality are increased in sedentary adults relative to physically active adults.\textsuperscript{18–22} Taddei et al speculated based on their findings in younger and older participants that aerobic exercise training increases protection of the vascular endothelium from cardiovascular risk during aging.\textsuperscript{23} Decreased SOD activity has been closely linked to impaired endothelial-dependent vasodilation, and may contribute to endothelial dysfunction.\textsuperscript{24} In addition, Durrant et al recently demonstrated that SOD activity in the aorta of old mice that performed voluntary wheel running was significantly greater than SOD activity in old sedentary mice.\textsuperscript{25} Taken together, these studies lend support to the hypothesis proposed by Taddei et al that aerobic exercise training may confer protective effects against the age-induced decline in vascular function via the maintenance of redox status.

Franzoni et al found less oxidative stress in older athletes compared to older sedentary participants.\textsuperscript{26} They observed that total oxyradical scavenging capacity was significantly lower in older sedentary participants relative to older athletes while MDA was significantly higher in older sedentary participants relative to older athletes. In the current study, middle-age to older participants were overall sedentary. Yet, it was observed that normal fitness was significantly related to SOD activity. Mejer et al found that higher physical activity levels, as measured by average daily metabolic rate, were related to reduced exercise-induced oxidative stress.\textsuperscript{27} Perhaps differences in fitness generated by physical activities of daily living may be important in the middle-age to older healthy population for prevention of lipid peroxidation and oxidative stress via maintenance of the antioxidant system.

Body mass index is another essential clinical tool for assessing overall health. In the current study, it was observed that body weight deviation above a BMI of 25 kg/m\textsuperscript{2} into the overweight category was significantly associated with reduced SOD activity. This is in opposition to the results of Brown et al as they did not observe significant differences in SOD activity between normal weight, overweight, and obese participants.\textsuperscript{28} However, participants in the Brown et al study ranged from 18–50 years of age, and a possible age-dependent difference in SOD activity was not analyzed.

In a recent study by Collins et al, young and old mice were fed high fat diets. The researchers observed a decline
in SOD gene expression along with other antioxidant enzymes only in older mice. Thus, aging is a factor in the ability of the vasculature to respond to oxidative stress in the face of pro-atherosclerotic conditions. While the current study did not detect an age-dependent difference in SOD activity, we did observe significant differences in SOD activity based on BMI in a clinically healthy middle-age to older population. Therefore, the complex juxtaposition of aging, elevated weight, and physical inactivity that is clinically central to many chronic diseases may also influence age-dependent SOD activity.

Our study used BMI matched young and middle-age to older African Americans; therefore a limitation of the study was a restricted sample size. However, the significant associations that were found in this study were well powered variables that ranged in power from .66 to .95.

Middle-age to older participants were selected carefully and were disease-free, non-smoking, and not on any medication. This homogenous population of middle-age to older participants was clinically healthy. However, our study suggests a compromised endothelium in overweight, sedentary, CVD-free African Americans. While the young group also demonstrated individuals that were pre-hypertensive, overweight, and of low fitness, none of these criteria were significantly associated with differences in oxidative stress biomarkers relative to young normotensive, normal weight, and normal fitness individuals.

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**REFERENCES**


**Author Contributions**

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- **Data analysis and interpretation:** Sturgeon
- **Manuscript draft:** Sturgeon, Diaz, Veerabhadrappa
- **Statistical expertise:** Sturgeon, Brown
- **Acquisition of funding:** Sturgeon, Brown
- **Administrative:** Feairheller, Williamson, Brown
- **Supervision:** Sturgeon, Brown