THE IMPACT OF OXIDATIVE STRESS ON OXYGEN TRANSPORT AND UTILIZATION IN HEALTH AND DISEASE

by

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ABSTRACT

The overall objective of this dissertation was to examine the impact of oxidative stress on oxygen transport and utilization, and ultimately physiological function, in older individuals and patients with chronic obstructive pulmonary disease (COPD). The goal of the first study was to better understand the age-associated attenuation in leg blood flow (LBF), with a focus on the role of redox balance, at rest and during exercise. Under control conditions, by experimental design, aging was associated with ~15% reduction in LBF. During knee extensor exercise (KE), the old also exhibited greater leg free radical outflow, assessed by electron paramagnetic resonance (EPR) spectroscopy, than the young. At rest, administration of an acute, oral antioxidant cocktail (AOC) increased antioxidant capacity, decreased the EPR signal, and consequently, restored LBF in the old such that it was not different from the young. During exercise, however, the AOC did not alter free radical outflow from the muscle or LBF. Thus, these data document exaggerated free radical production during exercise in older individuals exhibiting attenuated LBF, and identify a favorable effect of decreasing oxidative stress on resting hemodynamics in these individuals. However, the inability of the oral AOC to alter free radical outflow or LBF during exercise suggests that the formidable, pro-oxidant state elicited by exercise in the old likely necessitates a more potent antioxidant strategy to alter free radical outflow and potentially improve LBF in this population. The second study sought to determine the impact of acute, oral AOC administration on oxygen

transport and utilization in a population recognized to have elevated oxidative stress, patients with chronic obstructive pulmonary disease (COPD). AOC administration led to an improvement in LBF during submaximal KE exercise, which was accompanied by an increase in muscle oxygen consumption, in the patients with COPD, but minimal effects in healthy subjects. Additionally, arterial oxygen saturation was improved in the patients with COPD, but unaltered in the healthy subjects. These results reveal detrimental consequences of elevated oxidative stress in patients with COPD in terms of vascular control, and oxygen transport and utilization during exercise. The third study examined the functional consequences of reducing oxidative stress in patients with COPD in terms of skeletal muscle fatigue development. Following intravenous ascorbate administration, an overall attenuation in the ventilatory and metabolic responses to high-intensity KE performed for the same duration and at the same intensity as the placebo condition was observed. Additionally, following the exercise matched for time, the patients exhibited less peripheral quadriceps fatigue. These results suggest a beneficial role for antioxidant administration in COPD, and further implicate oxidative stress in the systemic, pathophysiological consequences of the condition. Collectively, this research has identified novel, biological mechanisms by which oxidative stress may adversely impact oxygen transport and utilization in health and disease.

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CHAPTER 1

INTRODUCTION

Oxidative stress can be described as an imbalance between pro- and antioxidant forces in favor of the former (40, 43, 44). Although acute oxidant production is likely necessary for cell signaling (17) and inflammatory processes (14), as well as cellular adaptation (38), there are also deleterious consequences of chronic oxidative stress in many tissues (3, 12, 14). Indeed, oxidative stress has been implicated in the etiology and pathophysiological processes of diseases such as heart failure (53) and chronic obstructive pulmonary disease (COPD) (43). In addition, normal healthy aging is associated with a pro-inflammatory, pro-oxidant phenotype (12, 48), which likely plays a role in the detrimental consequences of the aging process for many organ systems (18). Consequently, research examining the impact of oxidative stress on physiological function, and how the role of oxidative stress may change in pathophysiological conditions, is of utmost importance.

There are many sources of oxidant, or free radical production that may be dysregulated and contribute to oxidative stress (12, 14, 18, 24, 41, 43). Perhaps most notably, free radicals are produced by electron leak from complexes within the mitochondrial electron transport chain. Here, the leaked electrons reduce molecular oxygen, producing the free radical superoxide in several physiological and pathophysiological states (51). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase have also been documented to contribute, via superoxide production, to oxidative stress, to varying degrees depending on the tissue examined (12, 21, 40). Numerous nonenzymatic and enzymatic antioxidants, including superoxide dismutase and catalase, exist within the mitochondria and cytosol to neutralize radical species and protect the cell. However, excessive oxidant formation can overwhelm these

antioxidant defenses and lead to a chain of oxidation-reduction reactions, whereby additional radical species are generated, and cellular constituents, such as lipids, proteins, and DNA, are damaged (14).

Interestingly, acute exercise is associated with a transient increase in free radical production (2, 3, 44), and this pro-oxidant state is likely necessary for optimal contractile function of skeletal muscle (40), and is thought to confer beneficial adaptations following exercise (38). In health, therefore, upsetting the normal oxidant/antioxidant balance can attenuate exercise-training induced adaptations and may actually be detrimental to physiological function (13, 20, 56). Excessive oxidant production during exercise, however, has been linked to skeletal muscle dysfunction (9), increased fatigability (25), and may adversely impact exercise-induced hyperemia (10). Thus, the normally favorable pro-oxidant potential of exercise in health, may have deleterious consequences in populations predisposed to oxidative stress, such as patients with COPD and aged individuals. Therefore, the overall purpose of this dissertation was to study the interaction between oxidative stress, exercise, aging, and disease, and their collective impact on physiological function. The first study examined the influence of age and oxidative stress on peripheral hemodynamics. The second study further assessed the impact of oxidative stress on oxygen transport and utilization in a population with a greater predisposition to oxidative stress, patients with COPD. The third study of this dissertation investigated the functional consequences of oxidative stress, in terms of skeletal muscle fatigue development, in patients with COPD.

Healthy aging is associated with a decline arterial function, primarily manifested as vascular endothelial dysfunction (12, 48, 54). Oxidative stress has been documented to

contribute to this process, and as such, circulating markers of oxidative stress are inversely related to brachial artery flow-mediated dilation (FMD) (16), an assessment of endothelial cell mediated vascular function. As a result, antioxidant administration has been documented to improve FMD in older individuals (50, 54). Within the vasculature, mitochondria-derived free radicals as well as the upregulation of NADPH oxidase contribute to elevated superoxide production (12, 19). Superoxide, in turn, reacts with endothelial cell-derived, vasodilatory nitric oxide (NO), producing peroxynitrite; the resulting decrease in NO bioavailability impairs endothelially-mediated vasodilation (50). Furthermore, peroxynitrite may oxidize tetrahydrobyopterin, an essential cofactor for endothelial nitric oxide synthase (eNOS), causing eNOS uncoupling and additional superoxide production (26). Collectively, these processes, among others, are responsible for the observed impairment in vascular responsiveness with age to physiological and pharmacological stimuli that target the NO pathway (48).

In addition to diminished vascular function, aging is also commonly associated with reduced skeletal muscle blood flow at rest and during exercise (11, 24, 27, 28, 34, 37, 39). Although the role of NO in exercise hyperemia is equivocal (34, 42), previous research has suggested that reduced NO bioavailability may be, at least partially, responsible for attenuated exercise hyperemia with age (10, 55). Specifically, antioxidant administration has been documented to improve skeletal muscle blood flow and end exercise skeletal muscle perfusion in older individuals (10, 55). However, these previous studies did not simultaneously investigate the impact of antioxidant administration on exercise-induced oxidative stress. In fact, the preponderance of evidence examining the impact of exercise-induced oxidative stress with age in humans is derived from changes

in antioxidant enzyme status (35) or from tissue samples taken immediately following exercise (4). Previously, our group has utilized *ex vivo* spin-trapping and electron paramagnetic resonance (EPR) spectroscopy of femoral arterial and venous blood, in combination with femoral artery blood flow measurements, during dynamic knee extensor exercise (KE) to elucidate the impact of exercise on directly measured free radical outflow from an isolated muscle bed in young individuals (3). While this previous work documented an increase in free radical outflow that was proportional to the increase in muscular work, the impact of age, as well as the potential impact of antioxidant administration was not evaluated.

Thus, the purpose of the first study of this dissertation was to examine the impact of administering an acute, readily available, oral antioxidant cocktail (AOC, Vitamins C, E and alpha-lipoic acid) with previously documented efficacy (13, 22, 44, 54), on redox balance in the femoral artery and vein and peripheral hemodynamics in young and old subjects. It was hypothesized that old subjects, characterized by attenuated leg blood flow (LBF), in comparison to young subjects would exhibit evidence of a greater pro-oxidant status at rest, and this would be translated into greater leg free radical outflow, assessed by EPR spectroscopy, during KE exercise. Additionally, it was hypothesized that the administration of an acute, oral AOC would increase antioxidant capacity, decreasing the augmented oxidative stress in the old, and correct the age-associated impairment in LBF.

Chronic obstructive pulmonary disease, comprised of chronic bronchitis and emphysema, is a chronic inflammatory condition primarily impacting the lungs, resulting in an impaired ability of oxygen to diffuse from ambient air to the blood. Several systemic abnormalities have also been associated with COPD, including skeletal muscle

(31) and mitochondrial dysfunction (6), increased fatigability (1), and peripheral vascular dysfunction (5, 15, 22). Indeed, our group recently observed reduced vascular function, as measured by FMD, in patients with COPD, and documented that AOC administration improved FMD in these patients such that vascular function was similar to healthy controls (22). These data implicate oxidative stress, and subsequently reduced NO bioavailability, in mediating the reduced vascular function observed in patients with COPD. As the vasculature plays a critical role in regulating skeletal muscle blood flow and oxygen delivery, oxidative stress and reduced NO bioavailability may adversely impact oxygen transport and utilization in patients with COPD during exercise.

Therefore, the purpose of the second study of this dissertation was to examine the impact of the oral AOC on muscle blood flow and oxygen transport in the exercising skeletal muscle of patients with COPD and healthy subjects utilizing an exercise modality with minimal ventilatory demand, KE exercise (46). We tested two hypotheses: 1) that the redox balance would be abnormal in patients with COPD relative to healthy subjects, and 2) administration of the AOC would remedy the redox imbalance in patients with COPD and improve exercising skeletal oxygen transport and utilization, with minimal effects in healthy subjects.

While compromised pulmonary function, intrinsic to lung disease, certainly contributes to limited exercise capacity in COPD (1, 36), peripheral muscle abnormalities have also been implicated (29, 31, 45). Specifically, structural abnormalities of skeletal muscle in COPD patients, such as a higher percentage of type II skeletal muscle fibers (45), may hasten the production of metabolic byproducts known to influence the development of peripheral muscle fatigue (52). Indeed, augmented peripheral locomotor

muscle fatigue occurs in patients with COPD following cycle exercise (1, 30) and the perception of leg fatigue is frequently recognized as the primary symptom contributing to exercise intolerance (23). Interestingly, elevated exercise-induced oxidative stress has also been documented to be in patients with COPD, and the magnitude of lipid peroxidation inversely related to endurance time (7, 8). In addition, transgenic mice overexpressing TNF-alpha in the lungs, resulting in systemic inflammation and a phenotype similar to patients with COPD, demonstrate elevated superoxide production from contracting skeletal muscle and decreased fatigue resistance (57). Oxidative stress, therefore, appears to contribute to skeletal muscle dysfunction in patients with COPD and may impact the development of peripheral muscle fatigue.

Antioxidant treatment has been documented to reduce markers of exercise-induced oxidant damage in patients with COPD (21, 25), and the potent pharmacological antioxidant n-acetylcysteine (NAC) appears to improve exercise capacity (25). NAC, however, has also been documented to improve cycling time to exhaustion in healthy, young subjects (32, 33), and improve pulmonary function in patients with COPD (49), confounding interpretation of antioxidant administration on skeletal muscle function in patients with COPD. Accordingly, our group previously examined the impact of administering an AOC to further elucidate the influence of oxidative stress on skeletal muscle function in patients with COPD (47). Interestingly, the AOC decreased the EPR spectroscopy free radical signal, but did not impact the magnitude of skeletal muscle fatigue developed during KE exercise. The individual responses to the AOC, however, were mixed, with only half of the patients exhibiting a substantially reduced EPR

spectroscopy signal. Therefore, in this prior study (47), the role of free radicals on skeletal muscle fatigue in patients with COPD was not fully elucidated.

Thus, the purpose of the third study of this dissertation was to utilize a more potent antioxidant intervention, intravenous ascorbate (AO) administration, to examine the impact of oxidative stress and skeletal muscle fatigue development during dynamic KE exercise in patients with COPD. We tested the hypotheses that in patients with COPD intravenous AO administration would: 1) improve antioxidant capacity and decrease oxidative stress and, 2) decrease the magnitude of peripheral quadriceps fatigue induced by KE exercise matched for intensity and duration (isotime).

Free radicals play an important role in normal physiological function; chronic, excessive free radical formation, however, can lead to oxidative stress, which has been implicated in the etiology of many of the pathophysiological processes of aging and disease. Understanding the impact of oxidative stress may prove useful in developing novel therapeutic strategies to mollify the sequelae of aging and disease. Therefore, the overall purpose of this dissertation was to study the role of oxidative stress in health and disease by: 1) examining the effect of antioxidant administration on free radical outflow and peripheral hemodynamics in young and old individuals, 2) investigating the influence of antioxidant administration and oxidative stress on oxygen transport and utilization in patients with COPD and healthy controls, and 3) determining the functional consequences of reducing oxidative stress, in terms of skeletal muscle fatigue development, in patients with COPD.

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CHAPTER 2

LEG BLOOD FLOW, AGING, AND OXIDATIVE STRESS: INSIGHT FROM ACUTE ORAL ANTIOXIDANT ADMINISTRATION

Abstract

This study sought to better understand the age-associated attenuation in leg blood flow (LBF), with a focus on the role of redox balance in the vasculature. LBF and pro- and antioxidant status in the femoral artery and vein were documented in control conditions at rest and during knee-extensor exercise (KE) in 10 old (68±2 yrs) subjects characterized by attenuated LBF and 10 young (25±1 yrs) subjects. The rest and exercise assessments were then repeated with an oral antioxidant cocktail (AOC), employed to shift the redox balance from control conditions. By experimental design, under control conditions, LBF was ~15% lower in the old compared to the young at rest and during KE. Interestingly, under control conditions, during KE the old also exhibited greater leg free radical outflow, assessed by electron paramagnetic resonance (EPR) spectroscopy, than the young at each work rate (3W: 14±3 vs 26±4; 6W: 15±4 vs 26±6; 9W: 18±3 vs 27±9 AU L/min, respectively). The AOC improved LBF in the old at rest, abolishing the age-related decrement, but did not alter LBF or free radical outflow in either group during exercise. These data document greater free radical outflow during exercise in old subjects exhibiting attenuated LBF at rest and during KE. Additionally, as the AOC ameliorated the attenuated LBF in the old at rest, but failed to alter free-radical outflow or LBF during exercise, this suggests that the formidable, pro-oxidant state elicited by exercise in the old likely necessitates a stronger antioxidant strategy to restore LBF in this population.

Introduction

Oxidative stress, which can be defined as an imbalance between pro- and antioxidant forces in favor of the former (29), has been documented to contribute to the age-related reduction in peripheral vascular function (23, 31, 32). Indeed, previous

research has documented increased markers of oxidative stress with advancing age, and an accompanying reduction in the vasodilatory response to pharmacological (32) and physiological (23, 35) stimuli targeting the nitric oxide (NO) pathway. Furthermore, vascular function in the old has been documented to improve to a level that it is similar to the young with the administration of free radical scavengers such as ascorbate, and this restoration of vascular function is abrogated by NO synthase (NOS) inhibition (17, 32). Thus, it is likely that the age-associated reduction in vascular function is a consequence of elevated free radicals, which by reacting with NO, decreases NO bioavailability in the vasculature, impairing vasodilation (31).

Potentially as a result of impaired vascular function, reduced LBF both at rest and during exercise is commonly observed in the elderly compared to the young (11, 20-22, 24, 26, 27). Indeed, although numerous factors likely play a role in impairing peripheral hemodynamics with age, limited NO bioavailability as a consequence of elevated oxidative stress may be an important factor (17, 20). Specifically, similar to the effects on vascular function, intra-arterial ascorbate has been documented to ameliorate the age-associated attenuation in limb blood flow and vascular conductance at rest (17) and during exercise (20), while, during exercise, NOS inhibition abolished this improvement (10). Likewise, Wray et al. (36) observed an increase in end-exercise muscle perfusion, as assessed by nuclear magnetic resonance spectroscopy, during plantar flexion exercise, following administration of an AOC in older subjects. Collectively, these studies implicate oxidative stress as a causative factor in the impaired peripheral hemodynamics with age, suggest that reduced NO bioavailability is involved, and indicate a potential remedial role for antioxidants.

Likely due to the difficulty in studying free radicals, owing to their very short half-life (18), few studies have comprehensively evaluated the impact of age on free radical production by exercising skeletal muscle in humans (3, 25). Typically, such studies have relied on changes in antioxidant status (25) or the analysis of tissue/blood samples taken immediately following an exercise bout (3, 13). Aiming to utilize a more comprehensive and real-time approach, our group has utilized *ex vivo* spin-trapping and the EPR spectroscopic detection of α-phenyl-*tert*-butylnitrone (PBN) adducts in femoral arterial and venous blood, in combination with blood flow measurements, to directly document an increase in free radical outflow from skeletal muscle during exercise in young subjects (2). In addition, our group has documented the capability of an acutely administered, readily available, oral AOC to ameliorate the free radical signal in the vasculature at rest and as a consequence of exercise (13, 29). However, to date, there has not been a comprehensive examination of the age-associated attenuation in LBF in terms of pro- and antioxidant status and the impact of an AOC on peripheral hemodynamics.

Thus, this study sought to better understand the age-associated attenuation in LBF, with a focus on the role of redox balance in the vasculature. Specifically, it was hypothesized that old subjects, characterized by attenuated LBF, in comparison to young subjects would exhibit evidence of a greater pro-oxidant status at rest and this would be parlayed into a greater leg free radical outflow during KE exercise. Additionally, it was hypothesized that the administration of an acute, oral AOC would increase antioxidant capacity, decreasing the augmented oxidative stress in the old and correct the age-associated impairment in LBF.

Methods

Subjects

With the intent to study old subjects characterized by attenuated LBF, during preliminary screening, old subjects were selected based on an a priori criteria (14) of a >15% attenuation in LBF relative to 10 young (~25 yrs) subjects. According to these criteria, 10 old (~70 yrs) subjects were identified and a total of 20 subjects participated in the study. General subject characteristics and peak KE work rate were determined during preliminary visits to the laboratory. Physical activity of the subjects was assessed using an accelerometer (GT1M; Actigraph, Pensacola, FL), worn for seven consecutive days, and expressed as time spent at differing levels of activity and average steps per day. Written, informed consent was obtained from all participants prior to their inclusion in this study, and the Institutional Review Boards of the University of Utah and the Salt Lake City VA Medical Center approved the protocols.

Exercise Protocols and General Procedures:

On the main experimental day, subjects reported to the laboratory following a 12 hour fast, and rested for ~30 minutes prior to all procedures. Subsequently, catheters were placed in the femoral artery and vein using sterile technique, as previously described (1). After catheter placement, subjects rested for an additional 30 minutes before the resting measurements, which were followed by KE. Three absolute workloads (3, 6, and 9 Watts), with 1 minute of rest between each exercise stage, were performed at 60 rpm on a cycle erogmeter (Monark, Sweden) modified to allow KE exercise (30). Briefly, KE recruits the quadriceps muscle group resulting in leg extension from 90 to ~170 degrees after which a lever arm attached to the cycle ergometer flywheel passively returns the leg

to 90 degrees. Due to the potentially long-lasting effects of the AOC, the AOC trial was always performed after the control condition (~2.5 hrs). However, of note, our group has previously documented the reproducibility of hemodynamic measurements employing this serial exercise testing experimental design, across a range of exercise intensities (5). Leg blood flow, mean arterial pressure (MAP), leg oxygen (O_2) consumption (VO_2), and heart rate (HR), were assessed during the last minute of baseline and each exercise stage. Blood samples were also taken in the same time frame to quantify antioxidant status, oxidative stress, inflammation, as well as for blood gas analysis and co-oximetry.

Antioxidant Supplementation

All subjects were instructed to refrain from vitamin supplements for at least five days prior to data collection. On the experimental day, the AOC was administered in a split dose, consumed 2 and 1.5 hours prior to the second exercise bout, to improve absorption and maximize the time of antioxidant efficacy. The first dose consisted of 300 mg α-lipoic acid, 500 mg vitamin C and 200 IU of vitamin E, and the second dose consisted of the same amounts of α-lipoic acid and vitamin C and 400 IU of vitamin E. This AOC, and the dosing strategy employed, has been previously documented to reduce O_2-centered free radical levels, as measured by EPR spectroscopy, and improve vascular function in older individuals (13, 35, 37).

Mean Arterial Pressure and Heart Rate

Arterial blood pressure measurements were collected continuously from an indwelling catheter placed in the common femoral artery, with the pressure transducer placed at the level of the catheter (Transpac IV, Abbott Laboratories). Mean arterial

pressure (MAP, in mmHg) was calculated as: MAP = diastolic arterial pressure + (arterial pulse pressure × 0.33). Heart rate was monitored continuously from a standard three-lead ECG, a component of the data-acquisition system (Biopac, Goleta, CA).

Leg Blood Flow and Vascular Conductance

The measurement of femoral artery blood velocity and vessel diameter in the leg being studied was performed at rest and during the last minute of each exercise stage, using a Logic 7 ultrasound system (General Electric Medical Systems, WI, USA) as previously described (6). The Logic 7 was equipped with a linear array transducer, operating at an imaging frequency of 9 MHz. The blood velocity profile was obtained with the same transducer with a Doppler frequency of 5 MHz operated in the high-pulsed repetition frequency mode. Blood flow in the femoral artery was calculated as: LBF = (mean velocity)π(vessel diameter/2)^2 × 60. Leg vascular conductance (LVC) was calculated as LBF/catheter-derived MAP.

Blood Analysis

At rest, and in the last 15 s of each exercise stage, femoral arterial and venous blood samples (1-2 ml) were collected. One ml of each sample was presented to a GEM 4000 blood gas analyzer and co-oximeter (Instrumentation Laboratories, Bedford, MA) to determine arterial and venous blood hemoglobin concentration and O_2 saturation, and the partial pressure of oxygen. Arterial and venous blood O_2 content (in ml/dl) was calculated as: blood O_2 content = 1.39 hemoglobin x (O_2 saturation) + 0.003 x partial pressure of O_2. Leg VO_2 was calculated as: VO_2 = (arterial blood O_2 content – venous blood O_2 content) x LBF. A lipid panel was performed on blood attained in a rested and fasted state from all

subjects using standard clinical techniques.

Antioxidant Status, Oxidative Stress, and Inflammation

Femoral arterial and venous blood samples taken at rest and during exercise were centrifuged to facilitate the collection of plasma, and the plasma samples were stored at -80°C until analysis. Total antioxidant capacity was evaluated by determining the ferric reducing ability of plasma (FRAP), using the method described by Benzie and Strain (7). The efficacy of the AOC specific to plasma ascorbate levels was assayed as previously described (8) (CosmoBio, Carlsbad, CA). Concentrations of the pro-inflammatory cytokines C-reactive protein and Interleukin (IL)-6, and the anti-inflammatory cytokine, IL-10 were determined by ELISA (R & D Systems, Minneapolis, MN). Lipid peroxidation was assessed by plasma malondialdehyde (MDA) levels (Bioxytech LPO-586, Foster City, CA) and protein oxidation was assessed by plasma protein carbonyl (PCs) levels (R and D Systems, Minneapolis, MN).

EPR spectroscopy was performed on whole blood samples obtained at rest and during exercise, as previously described (29). Briefly, 1.5 ml of arterial and venous blood was collected into a vacutainer containing 0.5 ml of the spin trap PBN (0.0140 mol/l). After centrifugation, the PBN adduct (200 μl) was pipetted into a precision-bore quartz EPR sample tube (Wilmad, Vineland, NJ). EPR spectroscopy was then performed at 21°C using an EMX X-band spectrometer (Bruker, MA) and commercially available software (version 2.11, Bruker Win EPR System) was used to calculate the area under the curve of the EPR spectroscopy signal by double integration. For the indices of inflammation (CRP, IL6, and IL10) and oxidative stress (EPR signal, MDA, and protein

carbonyls), outflow from the muscle was calculated as: (venous – arterial difference) x LBF (4).

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to determine the impact of age on the physiological variables measured as well as indices of antioxidant status, inflammation and oxidative stress. Two-way repeated-measures ANOVA was used to identify significant changes in measured variables due to AOC administration within the young and old groups. A Tukey post hoc test was used if a significant main or interaction effect was found. Statistical significance was set at $\alpha = 0.05$ for all tests. All group data are expressed as mean ± standard error of the mean.

Results

Subject Characteristics

Initial prescreening of 16 old subjects yielded 10 old subjects who exhibited a >15% attenuation in LBF during rest and exercise relative to the young, and these old subjects were selected to complete the study. The subject characteristics of those who participated in the whole study are documented in Table 2.1. Of note, the old subjects exhibited slightly elevated blood lipids and peak KE work rate was higher in the young, despite exhibiting similar levels of physical activity as the old (Table 2.1).

Aging and Peripheral Hemodynamics

By experimental design**,** LBF was attenuated in the old subjects compared to the young at rest and during KE at 3, 6, and 9 Watts in control conditions (Figure 2.1). Additionally, in control conditions, although mean arterial pressure was not different

between young and old at rest, but was elevated in the old compared to the young during KE, LVC was also consistently attenuated in the old subjects compared to the young at rest and during KE at 3, 6, and 9 Watts (Figure 2.1). The attenuation in LBF in the old corresponded to a reduction in leg O_2 delivery, however, leg VO_2 was maintained in the old by a compensatory increase in leg O_2 extraction (Table 2.2).

Aging and Leg Free Radical Outflow

Directly measured leg free radical outflow, quantified by femoral arterial to venous differences in the EPR signal multiplied by LBF, was unremarkable in both the young and old at rest, but was consistently and significantly elevated in the old during exercise (Figure 2.2). This greater leg free radical outflow during exercise in the old was the combined result of a lower femoral arterial and higher femoral venous EPR signal, with the resulting greater arterial to venous difference offsetting the attenuated LBF, such that free radical outflow was greater in the old relative to the young (Figure 2.2).

Impact of AOC on antioxidant status, LBF, and LVC

Administration of the AOC significantly improved antioxidant status in both young and old individuals, as evidenced by an ~100 % increase in arterial ascorbate concentration, as well as a significant 5-10 % increase in the antioxidant capacity of the arterial blood assessed by the FRAP assay (Figure 2.3). These robust antioxidant increases were present at rest and persisted throughout exercise in both groups (Figure 2.3). AOC administration resulted in an increase in LBF and LVC relative to the control condition in the old at rest (2.4), but there was no impact of the AOC on LBF or LVC during exercise (Figure 2.5).

Oxidative Stress and Inflammation in Control and AOC Conditions

Under control conditions, aging was accompanied by a general increase in inflammation and oxidative stress (Figures 2.6 and 2.7). Specifically, arterial and venous concentrations of the pro-inflammatory cytokines, CRP and IL6, were elevated, and the anti-inflammatory cytokine, IL10, attenuated, at rest and during exercise, in the old compared to the young (Figure 2.6). However, there were no group differences in the outflow of these cytokines from the leg (2.6). In terms of oxidative stress, indices of lipid peroxidation (MDA) and protein oxidation (PCs) were greater in the arterial and venous blood of old subjects at rest and during exercise compared with the young subjects. However, there were no differences in the outflow of these markers of oxidative stress from the muscles of the old and young subjects (Figure 2.7).

Administration of the AOC had no effect on arterial or venous concentrations, or the leg outflow, of CRP or IL10 at rest and during exercise. However, during the AOC trial there was a small, but significant increase in arterial and venous IL6 concentrations and a positive veno-arterial difference in IL6, which translated to greater IL6 outflow from the muscle in both young and old subjects at rest and during exercise (2.6). With regard to oxidative stress, at rest only in the old did the AOC tend to lower the venous EPR signal ($p = 0.09$) and significantly attenuate this signal in the arterial blood (2.7). Other markers of oxidative stress were unaltered by the AOC at rest (Figure 2.7). During exercise, the AOC did not impact arterial or venous concentrations, or outflow from the muscle, of either free radicals or MDA (Figure 2.7). In the young, however, The AOC trial was associated with an increase in the arterial and venous concentration of PCs relative to the control condition, but there was no difference in PC outflow (Figure 2.6).

Discussion

With the goal to better understand the age-associated attenuation in LBF, redox balance in the femoral artery and vein was assessed in both control and oral AOC conditions at rest and during KE in old subjects characterized by attenuated LBF and young subjects. By experimental design, under control conditions, LBF was ~15% lower in the old compared to the young at rest and during KE. In these control conditions, the old exhibited greater leg free radical outflow than the young during KE, assessed by EPR spectroscopy. Interestingly, the AOC improved LBF in the old at rest, abolishing the age-related decrement, but did not alter LBF or free radical outflow in either group during exercise. Therefore, this study documents greater free radical outflow during exercise in old subjects exhibiting attenuated LBF at rest and during exercise. The observation that the AOC ameliorates the attenuated LBF in the old at rest, but fails to alter free-radical outflow or LBF during exercise, suggests that the formidable, pro-oxidant state elicited by exercise in the old likely necessitates a stronger antioxidant strategy to restore LBF in this population.

Aging, LBF, and LVC

The preponderance of evidence suggests that normal, sedentary aging is associated with a reduction in limb blood flow at rest (5, 12, 17, 21, 22, 26, 27, 34), and that this attenuated blood flow persists during exercise (11, 20-22, 24, 27), although this has not been a universal finding (5, 26). Indeed, although LBF and LVC were attenuated in the old at rest in the current study, our group recently reported similar LBF and LVC values in young and old subjects during KE, despite evidence of an enhanced activity of the vasoconstricting peptide, endothelin-1, in the old (5). However, even though some

ambiguity exists, there remains a considerable body of literature identifying an age-associated attenuation in limb blood flow, and as adequate perfusion of the muscle is critical to meet muscle metabolic demand and ensure optimal function at rest and during exercise (28), understanding the mechanisms that contribute to the apparent impairment in vascular control with age remains an important question. Thus, to focus upon this issue, at prescreening, older subjects were identified with a >15% attenuation in LBF both at rest and during exercise relative to the young (Figures 2.1 and 2.2). Of note, this attenuation in LBF during exercise is of a comparable magnitude to that observed previously by our group utilizing the same exercise modality, without preselecting the old subjects for attenuated LBF (14). Additionally, when blood pressure was continuously monitored during the current catheter studies, it became apparent that the attenuated LBF in the old was a result of decreased vasodilation in the leg, as LVC was also attenuated in comparison to the young (Figures 2.1 and 2.4). However, it is interesting to note that despite this attenuation in LBF, leg VO_2 was maintained in the old by a compensatory increase in leg O_2 extraction (Table 2.2).

Aging and Oxidative Stress

Normal, healthy aging has been associated with a pro-inflammatory, pro-oxidant phenotype, which has deleterious effects on many physiological processes (15, 31). Acute exercise in older individuals has been suggested to contribute to the increase in oxidative stress with age (3, 13), although evidence of the impact of aging and exercise on oxidant production in humans is largely based on indirect markers of oxidative stress and/or samples taken following an exercise bout (3, 13, 25). In the vasculature, excessive oxidant production, predominantly in the form of superoxide, has the potential to

adversely impact vascular control by decreasing NO bioavailability, which has been linked to impaired limb blood flow both at rest (17) and during exercise (10, 20). As diminished muscle perfusion may limit physical capacity, and thereby increase cardiovascular disease risk (9), understanding the physiological consequences of age and exercise on oxidative stress and peripheral hemodynamics is of significant importance.

In line with previous findings, the older subjects in the current study demonstrated clear evidence of systemic inflammation and oxidative stress (Figures 2.6 and 2.7). Specifically, relative to the young, the old subjects exhibited higher circulating levels of the pro-inflammatory cytokines CRP and IL6 and lower levels of the anti-inflammatory cytokine, IL10 (Figure 2.6). Oxidative stress in the old was evidenced by greater levels of lipid peroxidation (MDA) and protein oxidation (PCs) in comparison to the young subjects. Furthermore, exercise in the old was associated with amplified oxidant production in comparison to the young, as evidenced by a larger, positive veno-arterial PBN spin adduct concentration difference and greater free radical outflow, assessed directly by EPR spectroscopy (Figure 2.2). As EPR spectroscopy is considered the most sensitive, specific, and direct molecular technique for the detection of free radicals (2, 18, 19), these data provide convincing evidence of the augmented pro-oxidant potential of aging and exercise in the vasculature. Interestingly, it is important to recognize that these data were collected during relatively light, submaximal (<30% of peak work rate) KE, which likely reflects the level of muscular work encountered during many activities of daily living. Therefore, these data demonstrate, for the first time, that exercise in older individuals, who are characterized by impaired peripheral hemodynamics at rest and during exercise, is associated with exaggerated free radical production compared to

young subjects.

Antioxidants and Aging at Rest

Antioxidant administration has been utilized extensively in humans to elucidate the adverse consequences of oxidative stress on vascular function. Specifically, our group has previously documented the capability of the AOC employed in the current study to combat age- and disease-related increases in oxidative stress, revealing improvements in NO-mediated vascular function, as measured by flow-mediated dilation, in such scenarios (16, 33, 35). In addition, Jablonski and colleagues observed a restoration of resting LBF and LVC with intra-arterial ascorbate administration, such that LBF and LVC were no longer different from that of young subjects, and the augmented LBF in the old was suggested to be a result of improved NO bioavailability (17). The findings of the current study confirm and extend the observations of Jablonski et al. (17) in terms of restoring resting LBF and LVC in the old, but with a much lower and more readily available, antioxidant dose, documenting the efficacy of the AOC as a means to negate the impact of age (Figure 2.4). Of note, these significant hemodynamic improvements were coupled with an improvement in arterial antioxidant status (Figure 2.3) and a reduction in the arterial EPR signal (Figure 2.7) in the old. Thus, these data further implicate oxidative stress in the age-associated decline in resting limb blood flow, which may be related to reduced NO bioavailability and deteriorating vascular function with age.

Antioxidants and Exercise Responses

With regard to antioxidant delivery to the old during exercise, Kirby et al. (20) documented an improvement in limb blood flow during intra-arterial ascorbate infusion,

and the increase in hyperemia was abrogated by NOS inhibition (10). In addition, Wray et al. (36) observed an increase in perfusion following plantar flexion exercise assessed by nuclear magnetic resonance spectroscopy in old subjects following AOC administration. In contrast, Nyberg et al. (24) did not observe an impact of arterial N-acetylcysteine administration on LBF or LVC in older individuals, despite detecting an increase in NO metabolites after antioxidant treatment. This study, however, did not evaluate the impact of N-acetylcysteine on oxidative stress. In the current study, the AOC-induced improvement in antioxidant capacity following AOC administration at rest persisted during exercise (Figure 2.3). However, there was no impact of the AOC on free radical outflow from the exercising muscle, or positive effects on other circulating markers of inflammation and oxidative stress (Figures 2.6 and 2.7), and LBF and LVC in the old were unaltered from the control condition during exercise (Figure 2.5). In fact, during the second exercise bout with the AOC there was a small increase in IL6 in both the old and young subjects (Figure 2.6), which may simply have been a consequence of the preceding exercise bout, and PCs were slightly increased in the young (Figure 2.7). The surprising increase in PCs in the young, in whom the EPR data indicate lower free-radical outflow (Figure 2.7), is difficult to interpret, but may indicate differing reaction kinetics for the initial free radical "insult" and downstream protein oxidation between young and old. Regardless, collectively these data suggest that while the AOC may be adequate for decreasing oxidative stress at rest in the old, leading to an improvement in LBF and LVC, the combined pro-oxidant potential of old age and exercise may challenge the efficacy of the AOC such that there is little impact of this intervention during exercise. Further research with different antioxidant strategies may be necessary to fully reconcile

the impact of age, elevated free radical production, and impaired peripheral hemodynamics during exercise.

Conclusions

With a goal of better understanding the age-associated attenuation in LBF, redox balance in the femoral artery and vein was assessed in both control and oral AOC conditions at rest and during KE in old subjects characterized by attenuated LBF, and young subjects. This approach revealed a greater free radical outflow during exercise in old subjects exhibiting attenuated LBF at rest and during KE. While the AOC ameliorated the attenuated LBF in the old at rest, the AOC failed to alter free-radical outflow or LBF during exercise. This suggests that the formidable, pro-oxidant state elicited by exercise in the old likely necessitates a stronger antioxidant strategy to restore LBF in this population.

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Table 2.1 Subject Information

	Young	Old
Male/Female	9/1	8/2
Age (yrs)	25 ± 1	68 ± 2*
Height (cm)	180 ± 2	172 ± 2*
Weight (kg)	77 ± 3	72 ± 5
BMI (kg/m^2)	24 ± 1	24 ± 1
Knee Extensor Maximum (W)	48 ± 3	30 ± 3*
Mean Arterial Blood Pressure (mmHg)	89 ± 2	94 ± 2
Hemoglobin (g/dL)	16 ± 0.4	16 ± 0.3
Cholesterol (mg/dL)	157 ± 11	192 ± 9*
Triglycerides (mg/dL)	78 ± 25	126 ± 18
HDL (mg/dL)	46 ± 4	53 ± 4
LDL (mg/dL)	95 ± 8	125 ± 8*
Physical Activity		
Steps/Day	1039 ± 98	923 ± 117
Sedentary Time (min/day)	1,215 ± 39	1191 ± 19
Low Physical Activity (min/day)	160 ± 34	164 ± 20
Moderate Physical Activity (min/day)	42 ± 6	28 ± 4
High Physical Activity (min/day)	3.8 ± 3	1 ± 0.5

*Significantly different from the young, $p < 0.05$. Values presented as Mean ± Standard Error of the Mean.

Table 2.2 Selected Physiological Variables at Rest and During Exercise

Work Rate		Rest	3 Watts	6 Watts	9 Watts
Young					
Mean arterial pressure, mmHg	CTRL	111±2	114±2	115±2	114±2
	AOC	107±2	111±2	112±3	111±2
Heart rate, beats/min	CTRL	63±3	74±3	75±4	78±4
	AOC	64±3	72±4	75±4	77±4
Arterial O_2 content, ml/dl	CTRL	20.8±0.6	20.8±0.6	20.9±0.6	20.8±0.6
	AOC	20.8±0.6	20.9±0.6	20.8±0.6	20.8±0.6
Leg O_2 delivery, ml/min	CTRL	63±5	343±29	367±17	409±20
	AOC	62±6	325±30	379±18	406±18
Leg a-vO_2 difference, ml/dl	CTRL	8±0.4	11.5±0.3	11.9±0.3	12±0.4
	AOC	7.6±0.6	11.6±0.5	11.6±0.5	12.2±0.4
Leg VO_2 ,ml/min	CTRL	24±2	189±18	209±11	235±8
	AOC	23±2	181±11	210±9	234±11
Old					
Mean arterial pressure, mmHg	CTRL	116±2	125±4*	125±4*	128±2*
	AOC	115±2	124±3*	125±4*	127±4*
Heart rate, beats/min	CTRL	65±3	72±3	74±4	78±4
	AOC	64±3	74±4	76±4	80±5
Arterial O_2 content, ml/dl	CTRL	20.9±0.5	20.7±0.4	20.7±0.5	20.8±0.5
	AOC	20.7±0.5	20.7±0.4	20.9±0.5	20.9±0.5
Leg O_2 delivery, ml/min	CTRL	52±5*	284±16*	322±15*	364±20*
	AOC	61±6#	285±13*	346±14	382±19*
Leg a-vO_2 difference, ml/dl	CTRL	9±0.5	13.2±0.3*	13.5±0.3*	13.4±0.4*
	AOC	9±0.3	13.3±0.3*	13.7±0.4*	13.6±0.4*
Leg VO_2, ml/min	CTRL	22±3	181±11	211±12	234±15
	AOC	26±3	182±12	226±9	248±11

*Significantly different from the young, $p < 0.05$. #Significantly different from control condition (CTRL), $P < 0.05$. AOC, antioxidant cocktail. Values presented as Mean ± Standard Error of the Mean.

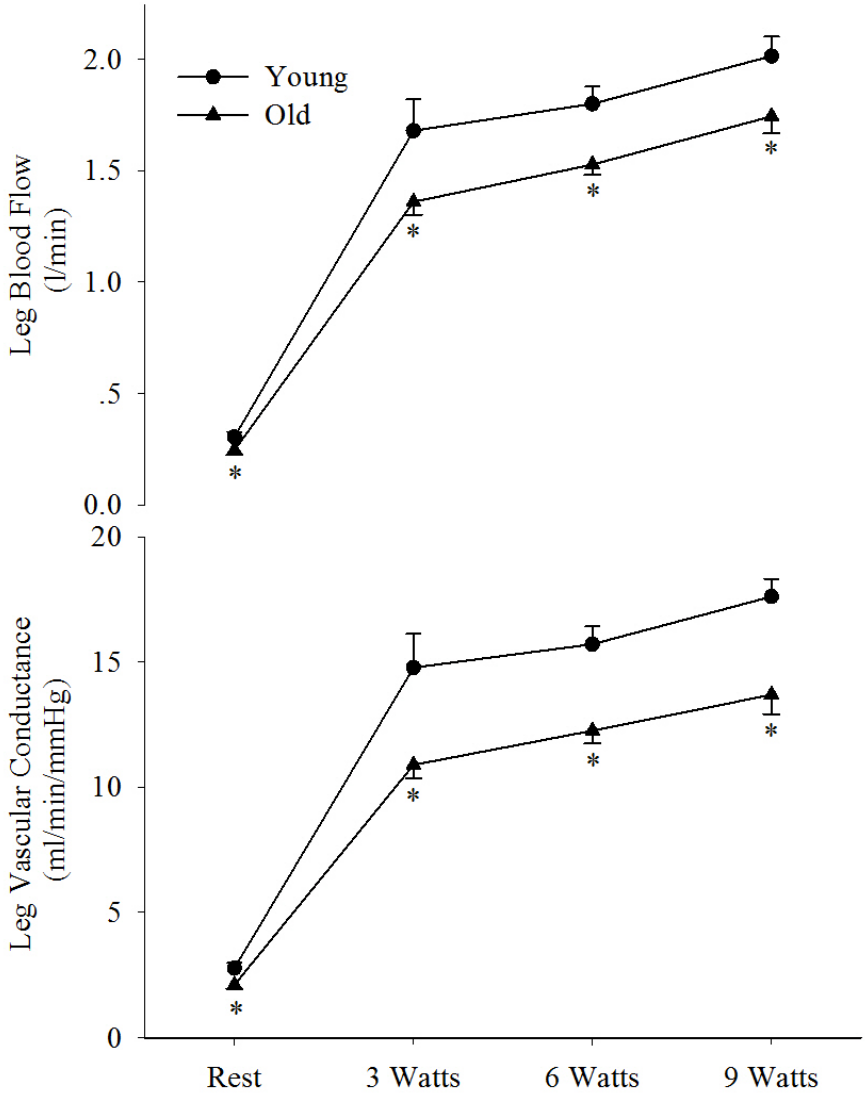

Figure 2.1 Impact of age on leg blood flow and leg vascular conductance at rest and during knee extensor exercise. Values are presented as mean ± S.E.M *Significantly different from the young, $p < 0.05$.

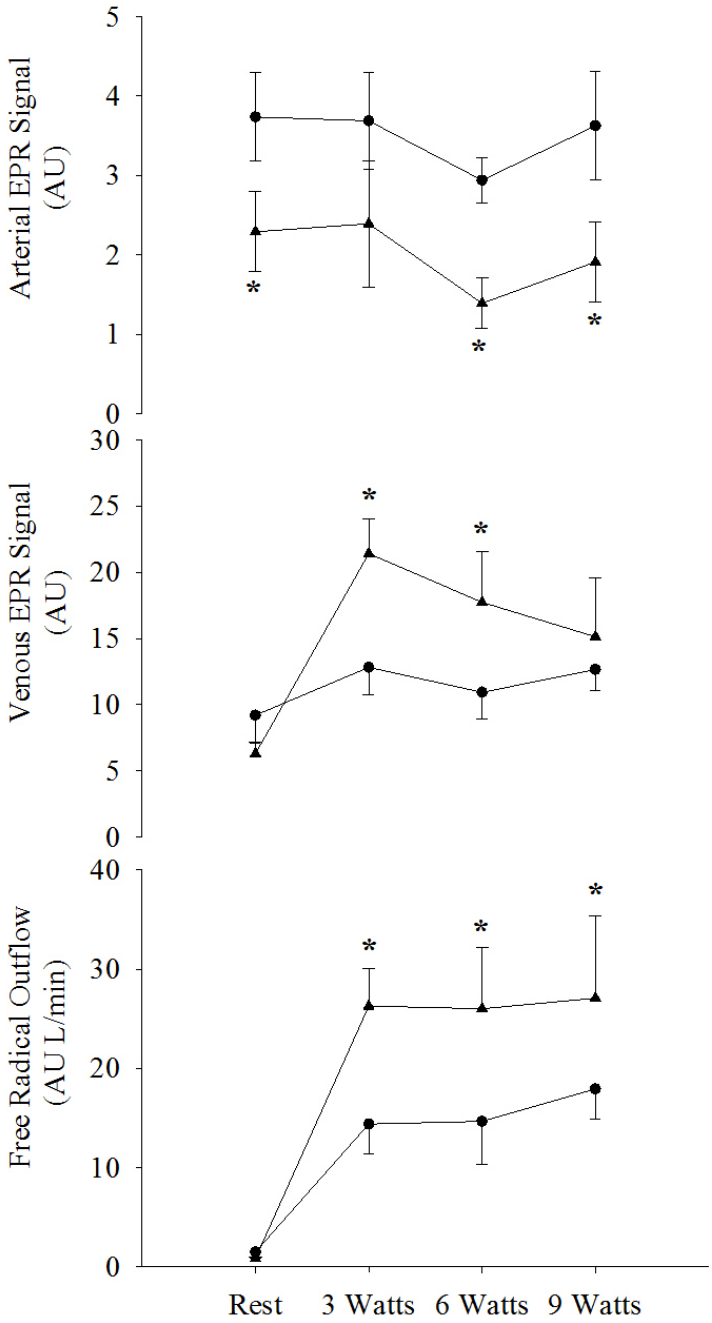

Figure 2.2 Impact of age on the electron paramagnetic resonance (EPR) spectroscopy signal of α-phenyl-tert-butylnitrone (PBN) radical adducts in arterial and venous blood, and free radical outflow at rest and during knee extensor exercise. Free radical outflow was determined by multiplying the arterial to venous PBN adduct difference by leg blood flow. Values are presented as mean ± S.E.M *Significantly different from the young, $p < 0.05$

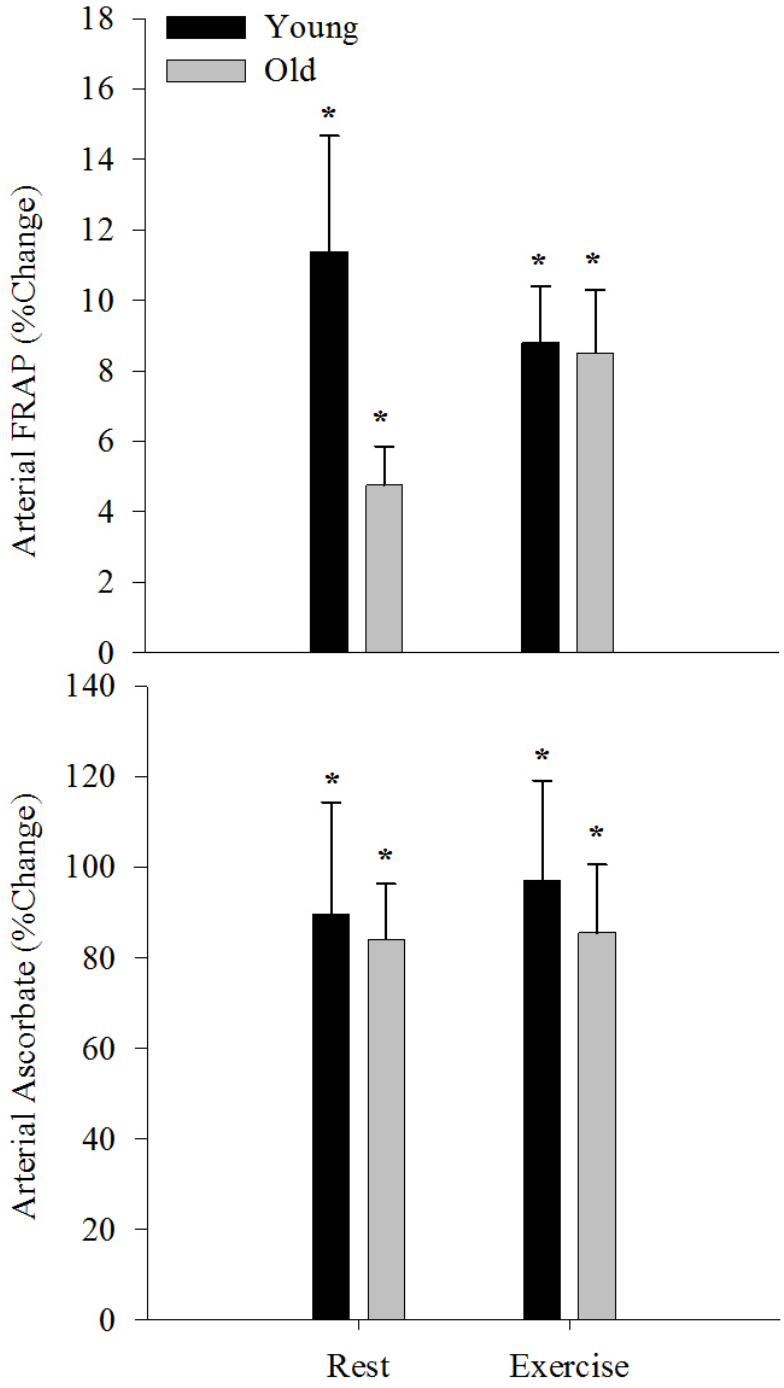

Figure 2.3 Percent change in arterial antioxidant status from control conditions following administration of an oral antioxidant cocktail (AOC) in young and old subjects at rest and during exercise (pooled data from 3, 6, and 9 Watts). FRAP, ferric reducing ability of plasma. Values are presented as mean ± S.E.M. *Significant difference from control condition, $p < 0.05$.

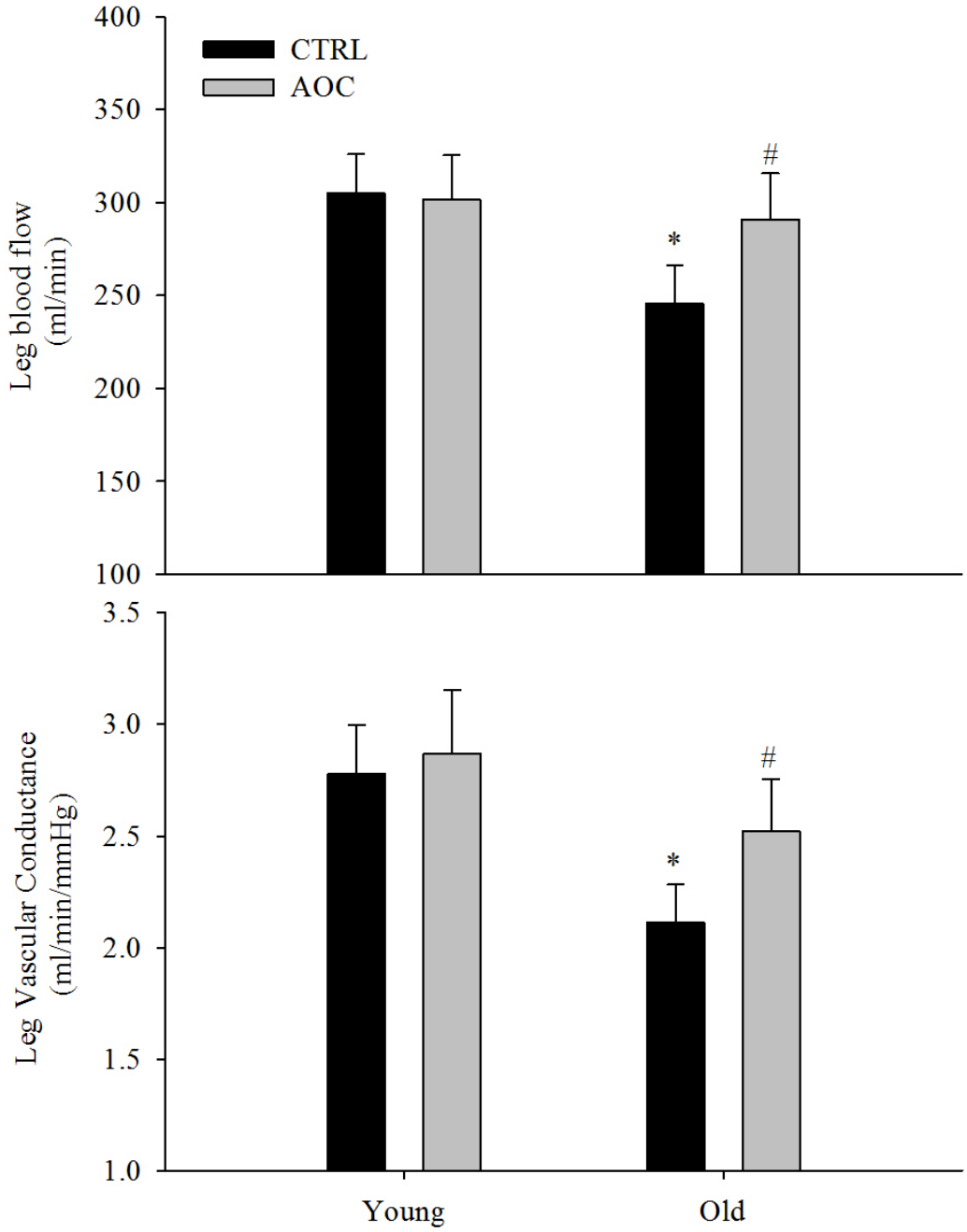

Figure 2.4 Impact of antioxidant cocktail (AOC) administration on leg blood flow and leg vascular conductance in young and old subjects at rest. CTRL, control conditions. Values are presented as mean ± S.E.M. *Significantly different from the young, $p < 0.05$. #Significantly different from the AOC condition.

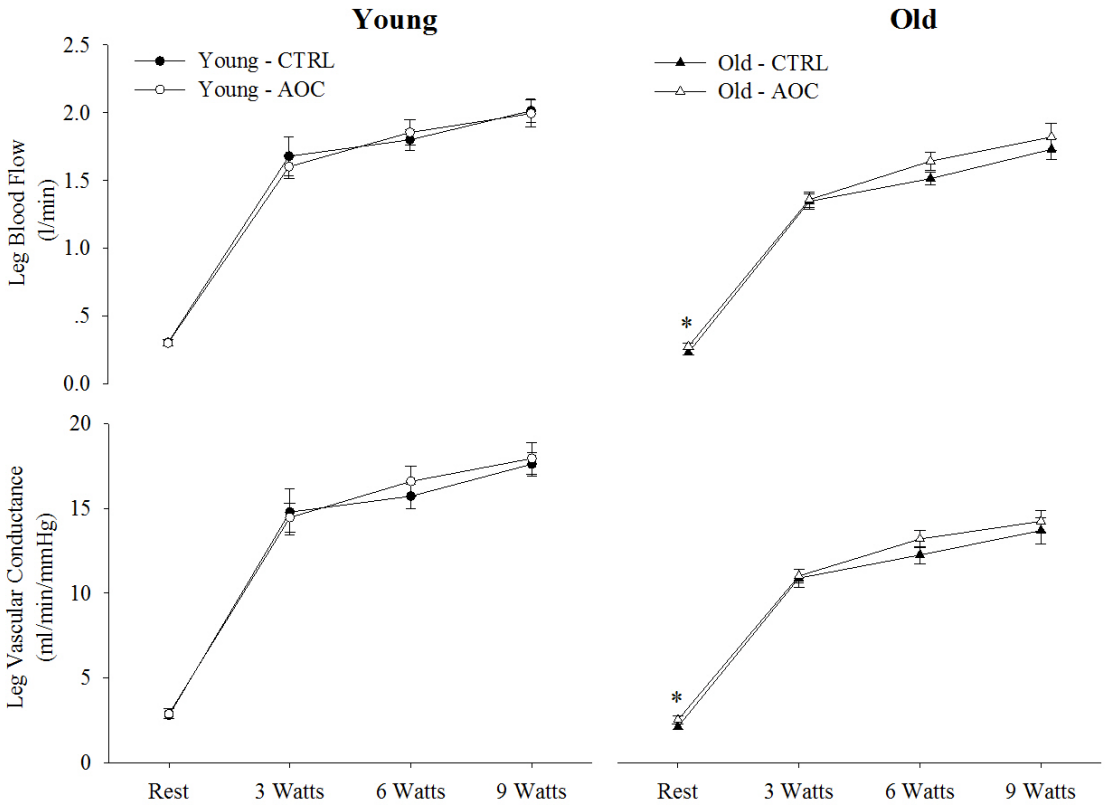

Figure 2.5 Impact of antioxidant cocktail (AOC) administration on leg blood flow and leg vascular conductance in young and old subjects at rest and during knee extensor exercise. CTRL, control conditions. Values are presented as mean ± S.E.M. *Significant difference from CTRL condition, $p < 0.05$.

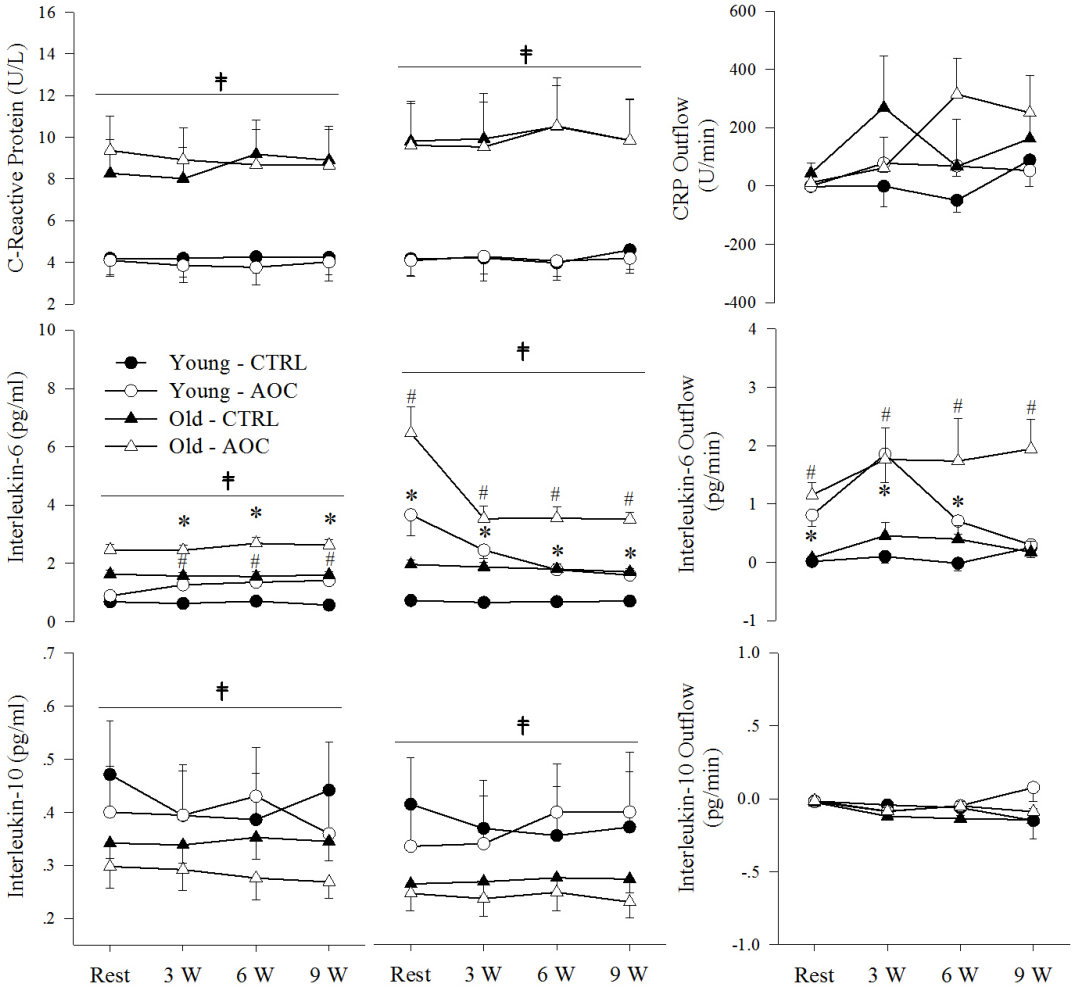

Figure 2.6 Arterial and venous concentrations, and outflow of markers of inflammation. Outflow was determined by multiplying the arterial to venous concentration difference by leg blood flow. CRP, C-reactive protein. Values are presented as mean ± S.E.M. ǂ Main effect of age, $p < 0.05$. *Significant difference from control (CTRL) condition within young, $p < 0.05$. #Significant difference from CTRL condition within old, $p < 0.05$.

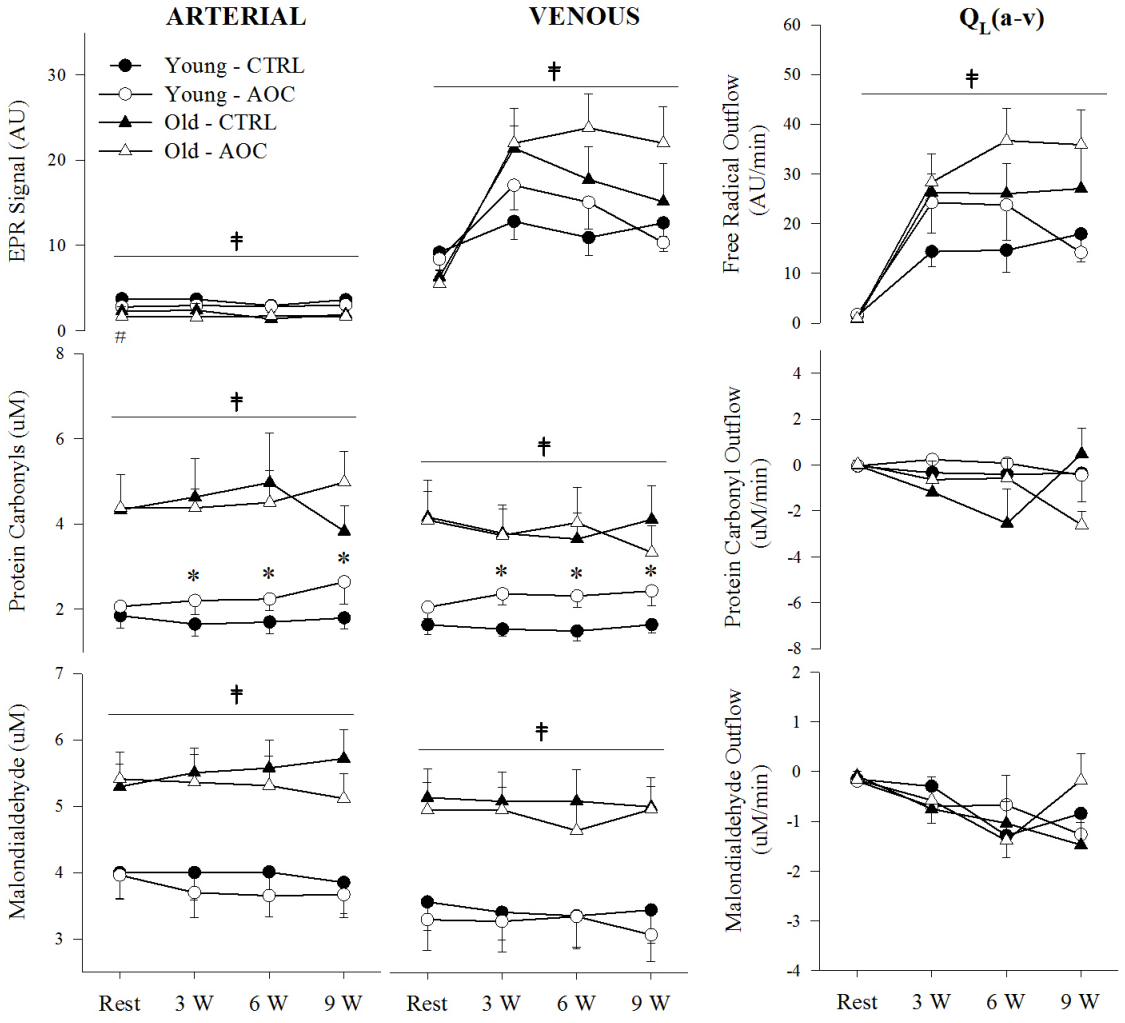

Figure 2.7 Arterial and venous concentrations, and outflow of markers of oxidative stress. Outflow was determined by multiplying the arterial to venous concentration difference by leg blood flow. EPR, electron paramagnetic resonance. Values are presented as mean ± S.E.M. ǂ Main effect of age, $p < 0.05$. *Significant difference from control (CTRL) condition within young, $p < 0.05$. #Significant difference from CTRL condition within old, $p < 0.05$.

CHAPTER 3

LIMB BLOOD FLOW DURING EXERCISE IN PATIENTS WITH COPD: THE IMPACT OF ANTIOXIDANTS

Abstract

The consequence of elevated oxidative stress on exercising skeletal muscle blood flow, and the transport and utilization of oxygen (O_2), in patients with chronic obstructive pulmonary disease (COPD) is not well understood. This study examined the impact of an oral antioxidant cocktail (AOC) on skeletal muscle blood flow and O_2 consumption during dynamic, small muscle mass exercise in 16 patients with COPD and 16 healthy subjects. Subjects performed submaximal (3W, 6W, and 9W) single-leg knee extensor exercise (KE) while leg blood flow (Doppler ultrasound), mean arterial pressure, arterial O_2 saturation, leg arterial-venous O_2 difference, and leg O_2 consumption (direct Fick) were evaluated under control conditions (CTRL) and following AOC administration. AOC administration increased leg blood flow (3W: 1604±100 vs 1798±128; 6W: 1832±109 vs 1992±120; 9W: 2035±114 vs 2187±136 ml/min, $P<0.05$, CTRL vs AOC, respectively), leg vascular conductance, and leg O_2 consumption (3W: 173±12 vs 210±15; 6W: 217±14 vs 237±15; 9W: 244±16 vs 260±18 ml O_2/min, $P<0.05$, CTRL vs AOC, respectively) during exercise in COPD, while no effect was observed in the healthy subjects. In addition, the AOC afforded a small, but significant, improvement in arterial O_2 saturation only in the patients (3W: 92±1 vs 93±1; 6W: 92±1 vs 93±1; 9W: 92±1 vs 93±1%, $P<0.05$, CTRL vs AOC, respectively). Thus, these data demonstrate a novel, beneficial role of AOC administration on exercising skeletal muscle blood flow, O_2 utilization, and oxygenation at the lung in patients with COPD, implicating oxidative stress as a potential therapeutic target for impaired exercise capacity in this population.

Introduction

COPD is a pro-inflammatory condition that primarily impacts the lungs, resulting in diminished pulmonary function (24). Other detrimental sequelae of this condition include mitochondrial (30) and skeletal muscle (26) dysfunction, and, consequently, exercise intolerance and decreased fatigue resistance are recognized hallmarks of patients with COPD (2). Interestingly, peripheral vascular function is also impaired in these patients (19). As the vasculature plays a critical role in regulating skeletal muscle blood flow (32), and therefore the delivery of O_2 and nutrients, poor vascular function also has the potential to influence exercise capacity and fatigability in this population (35). However, the mechanistic link between COPD, vascular dysfunction, and exercising skeletal muscle blood flow remains to be elucidated.

Peripheral vascular dysfunction in COPD has been attributed to numerous factors, including systemic inflammation and oxidative stress (17, 19). Indeed, relative to age-matched healthy subjects, both elevated systemic inflammation and oxidative stress have been well documented, in patients with COPD (5, 19, 27). Specific to oxidative stress, our group has recently demonstrated the beneficial effects of an acutely administered, oral antioxidant cocktail (AOC) on vascular function, as assessed by flow-mediated dilation, in patients with COPD (19). The AOC, and the dosing strategy employed, has previously been documented to reduce O_2 and carbon centered free radicals in patients with COPD (36). Thus, the improvement in vascular function following AOC administration in the prior work was attributed to the free radical scavenging ability of the AOC, restoration of the redox balance in the patients, and subsequent improvement in nitric oxide (NO) bioavailability (19). Based on these findings, targeting oxidative stress

with an acute AOC appears to represent a viable mechanism for improving NO bioavailability and peripheral vascular function in patients with COPD.

The role of NO in regulating exercising skeletal muscle blood flow is equivocal (15, 29, 38). Crecelius et al. (15), however, documented that an intra-arterial infusion of ascorbate improved forearm blood flow in older individuals during rhythmic handgrip exercise, and this effect was abrogated when ascorbate was co-infused with a NO synthase inhibitor. In addition, Wray et al. (38) documented elevated end plantar flexion exercise muscle perfusion, assessed by nuclear magnetic resonance spectroscopy, following administration of the AOC in older individuals. Collectively, these studies implicate antioxidant administration, free radical scavenging, and improved NO bioavailability as potential mechanisms to improve exercise hyperemia. Additional rationale for examining the contribution of NO in the regulation muscle blood flow during exercise in COPD comes from the observation that NO has also been suggested to play a substantial role in hypoxic "compensatory vasodilation," whereby blood flow is increased to maintain O_2 delivery when arterial O_2 content is reduced (11, 12). Therefore, although bulk blood flow is typically not reduced in COPD (34), this pathology is associated with elevated oxidative stress, and can be associated with periodic reductions in arterial O_2 content. Both of these may be exacerbated during exercise (21), with decreased NO bioavailability perhaps playing an especially deleterious vascular role in terms of O_2 transport and utilization in the muscle bed of patients with COPD.

Therefore, the purpose of this study was to examine the impact of an acutely administered oral AOC on muscle blood flow and O_2 transport in the exercising skeletal muscle of patients with COPD and healthy subjects. We tested two hypotheses: first, that

the redox balance, as assessed by antioxidant status relative to markers of oxidative stress, would be abnormal in patients with COPD relative to healthy subjects, and second, administration of the AOC would remedy the redox imbalance in patients with COPD and improve exercising skeletal muscle blood flow, with less of an effect in healthy subjects.

Methods

Subjects

A total of 32 subjects, 16 patients with spirometric evidence of COPD, and 16 age and sex matched healthy subjects, completed this study. Subjects with overt cardiovascular disease, or other confounding conditions, such as diabetes, were excluded from the study. All subjects performed standard pulmonary function tests during an initial visit to the laboratory. General morphometric characteristics, and peak KE work rate, were also determined during this visit. Physical activity was assessed using an accelerometer (GT1M; Actigraph, Pensacola, FL), worn for seven continuous days, in a subset of 10 patients with COPD and 10 healthy subjects. Average total daily physical activity was expressed as both average steps per day, and average total accelerometer counts per minute. The latter assessment was parsed into sedentary, low-, moderate-, high-intensity activity using device-specific software (Actilife, Actigraph, Pensacola, FL). The Institutional Review Boards of the University of Utah and the Salt Lake City VA Medical Center approved all protocol and written, informed consent was obtained from all participants prior to their inclusion in the study.

Exercise Protocols and General Procedures

On the experimental day, subjects reported to the laboratory following a 12 hour fast and rested for ~30 minutes prior to all procedures. Subsequently, catheters were placed in the femoral artery and vein using sterile technique, as previously described (3). After catheter placement, subjects rested for an additional 30 minutes before beginning KE exercise as presented in Figure 3.1. KE exercise was performed at 60 rpm on a cycle erogmeter (Monark, Sweden) modified to allow KE exercise (33). Briefly, this exercise modality recruits the quadriceps muscle group for active leg extension from 90 to ~170 degrees before a lever arm attached to a flywheel passively returns the leg to 90 degrees. Due to the potentially long-lasting effects of the AOC, the AOC trial was always performed after the CTRL condition. However, of note, our group has previously documented the reproducibility of hemodynamic measurements achieved with this serial exercise testing experimental design, without an intervention, across a range of exercise intensities (7). Each workload (3, 6, and 9 Watts) was sustained for 3 minutes. One-minute of rest was allowed between each stage. Leg blood flow, mean arterial pressure (MAP), leg O_2 consumption, and heart rate (HR) were assessed during the last minute of baseline and each exercise stage. Blood samples were taken anaerobically during the last minute of both baseline and each exercise stage.

Antioxidant Supplementation

All subjects were instructed to refrain from vitamin supplementation for at least five days prior to data collection. On the experimental day, the AOC was administered in a split dose, consumed 2 and 1.5 hours prior to the second exercise bout, to improve absorption and maximize the time of antioxidant efficacy. The first dose consisted of 300

mg α-lipoic acid, 500 mg vitamin C and 200 IU of vitamin E, and the second dose consisted of the same amounts of α-lipoic acid and vitamin C and 400 IU of vitamin E. This AOC, and the dosing strategy employed, has been previously documented to lower carbon and O_2-centered free radical levels, as measured by EPR spectroscopy, and improve vascular function in patients with COPD (19, 36).

Central Cardiovascular Responses

Arterial blood pressure measurements were collected continuously from an indwelling catheter placed in the common femoral artery, with the pressure transducer at the level of the catheter (Transpac IV, Abbott Laboratories). MAP (in mmHg) was calculated as follows: MAP = diastolic arterial pressure + (arterial pulse pressure × 0.33). Heart rate was monitored from a standard three-lead ECG, a component of the data-acquisition device (Biopac, Goleta, CA).

Leg Blood Flow and Vascular Conductance

Measurements of femoral artery blood velocity and vessel diameter in the leg being studied were performed at rest and during the last minute of each exercise stage, using a Logic 7 ultrasound system (General Electric Medical Systems, WI, USA) as previously described (8). The Logic 7 was equipped with a linear array transducer, operating at an imaging frequency of 9 MHz. The blood velocity profile was obtained with the same transducer with a Doppler frequency of 5 MHz operated in the high-pulsed repetition frequency mode. Blood flow in the femoral artery was calculated as: leg blood flow = (mean velocity)π(vessel diameter/2)^2 × 60. Leg vascular conductance was calculated as leg blood flow/arterial catheter-derived MAP.

Blood Analysis

A standard lipid panel was obtained for all subjects. At rest, and in the last 15 s of each exercise stage, femoral arterial and venous blood samples (1-2 ml) were collected. One ml of each sample was presented to a GEM 4000 blood gas analyzer and cooximeter (Instrumentation Laboratories, Bedford, MA) to obtain arterial and venous blood hemoglobin (Hb) concentration and O_2 saturation (SO_2), the partial pressure of O_2 (PO_2) and carbon dioxide, lactate and pH. Arterial (C_aO_2) and venous (C_vO_2) blood O_2 content (in ml/dl) were calculated as: blood O_2 content = 1.39 (Hb) x O_2 saturation/100) + 0.003 x PO_2. Leg O_2 consumption (VO_2; in ml/min) was calculated as: $VO_2 = (CaO_2 - C_vO_2)$ x leg blood flow.

Oxidative Stress and Inflammation

Venous blood samples taken at rest were centrifuged to collect plasma, and the plasma samples were stored at -80°C until analysis. Lipid peroxidation, a marker of oxidant damage, was assessed by plasma malondialdehyde (MDA) levels (Bioxytech LPO-586, Foster City, CA). Total antioxidant capacity was evaluated by determining the ferric reducing ability of plasma (FRAP), using the method described by Benzie and Strain (9). The efficacy of the AOC specific to plasma ascorbate levels was assayed as previously described (10) (CosmoBio, Carlsbad, CA). Endogenous antioxidant activity was assessed by catalase activity in the plasma (37) (Cayman Chemical Company, Ann Arbor, MI). Plasma C-reactive protein levels, an index of systemic inflammation, were determined by a high sensitivity ELISA assay (R & D Systems, Minneapolis, MN).

Statistical Analysis

Two-way repeated-measures analysis of variance (ANOVA) was used to identify significant changes in measured variables due to AOC administration within healthy subjects and the patient group. Two-way ANOVA was used to determine the impact of COPD on the measured physiological variables during exercise, and to evaluate the effect of the AOC on indices of antioxidant status, inflammation, and oxidative stress. A Tukey post hoc analysis was used if a significant main effect is found. Statistical significance was set at $\alpha = 0.05$ for all tests. All group data are expressed as mean ± standard error of the mean.

Results

Subject Characteristics

Subject characteristics are documented in Table 3.1. Patients with COPD exhibited reduced pulmonary function relative to healthy subjects, and blood gas characteristics consistent with COPD (Table 3.1). Apart from pulmonary function, arterial blood gases, and pulmonary disease medications, the healthy subjects were well matched with the patients with COPD (Table 3.1). By experimental design, physical activity levels were similar between healthy subjects and the patient group, resulting in similar peak KE work rates between groups (Table 3.1). Two of the patients with COPD were current smokers, who refrained from the use of tobacco products for 12 hours prior to all data collection. Four patients qualified for supplemental O_2; only one of these patients, however, required the use of supplemental O_2 during exercise (the blood gas data for the individual utilizing supplemental O_2 was excluded from the analyses).

Antioxidants, Oxidative Stress and Inflammation

Baseline plasma ascorbate was not different between patients with COPD and healthy subjects (Figure 3.2C). Relative to healthy subjects, patients with COPD exhibited a reduced antioxidant capacity, as assessed by FRAP (Figure 3.2B). In addition, C-reactive protein, a marker of systemic inflammation, was elevated in patients with COPD compared to healthy subjects (Figure 3.2A). Plasma catalase activity and malondialdehyde levels were not different between patients with COPD and healthy subjects (Figure 3.2D and E, respectively). Administration of the AOC increased plasma ascorbate levels similarly in both groups (Figure 3.2C). Interestingly, however, both FRAP (Figure 3.2B) and catalase activity (Figure 3.2D) were only increased in the patients with COPD as a consequence of ingesting the AOC.

Resting Responses

In patients with COPD at rest, administration of the AOC did not impact MAP, leg blood flow, vascular conductance, HR, CaO_2, leg arterial-venous O_2 difference, net lactate release or venous pH (Table 3.2), however, arterial O_2 saturation was elevated slightly (91.7 ± 1.3 vs 92.2 ± 1.1%, CTRL vs AOC, respectively), but significantly. In contrast, in the healthy subjects, leg blood flow and vascular conductance were elevated following the AOC consumption, while the other measured variables were unchanged (Table 3.2).

Exercise Responses

During exercise at 3, 6, and 9 W, leg blood flow was elevated relative to the CTRL condition following AOC consumption in patients with COPD (Figure 3.3A,

Table 3.2). The elevated leg blood flow, in combination with an unaltered arterial-venous O_2 difference, resulted in an increase in leg O_2 consumption in the AOC condition in the patients with COPD (Figure 3.3). In the healthy subjects, leg blood flow, arterial-venous O_2 difference, and leg O_2 consumption, and were unaltered by AOC administration (Figure 3.3A, B, and C, Table 3.2). In addition, leg vascular conductance was elevated with the AOC over the control condition in the patients with COPD (Figure 3.4), but not in the healthy subjects. This was also the case for SaO_2 (Figure 3.5A). PaO_2, however, was somewhat randomly elevated at 6W in the healthy subjects (Figure 3.5B), but not different in patients with COPD. CaO_2 was not different between conditions in either group, because in the patient group the elevated SaO_2 was offset by a small, but significant, decrease in hemoglobin concentration during exercise in the AOC condition (Table 3.2).

Discussion

This study examined the impact of an acutely administered, oral AOC with previously documented efficacy, on exercise-induced skeletal muscle blood flow, vasodilation, O_2 transport and utilization during small muscle mass exercise in patients with COPD and age and sex matched healthy subjects. Patients with COPD exhibited basal evidence of elevated inflammation and reduced antioxidant capacity. AOC consumption improved the abnormal redox balance in the patients, and these alterations were associated with favorable changes in the central and peripheral cardiorespiratory responses to exercise. Specifically, leg blood flow and vascular conductance during single-leg KE was augmented in patients with COPD following AOC consumption, while no changes were observed in the healthy subjects. The elevation in limb blood flow, in

combination with an unaltered arterial-venous O_2 difference from control conditions, led to increased O_2 consumption during exercise in the patients with COPD. In addition, arterial O_2 saturation was improved, at rest and during exercise, in patients with COPD with the AOC, whereas there was no apparent effect in the healthy subjects. These data demonstrate beneficial effects of antioxidant administration on exercise-induced hemodynamics and skeletal muscle metabolism in patients with COPD, and indicate that impaired O_2 transport, as a consequence of elevated oxidative stress, may represent a novel mechanistic link between oxidative stress and exercise intolerance in this population.

Oxidative Stress and Exercise

Oxidative stress has previously been documented to be elevated in patients with COPD relative to age-matched, healthy subjects at rest (5, 19, 27). In addition, indices of oxidative stress, such as protein carbonyls, have been inversely correlated with disease severity (5). Exercise appears to especially augment oxidative stress in patients with COPD, and as such, markers of oxidative damage are typically elevated in patients with COPD in comparison to healthy subjects following exercise (27). Interestingly, this amplified oxidant production during exercise in COPD occurs following exercise which minimally taxes the pulmonary system, such as isolated quadriceps exercise (13, 14), implying that organs beyond the lung may be contributing to the free radical production. Increased oxidative stress in patients with COPD has been attributed to mitochondrial electron transport chain dysfunction (30) and the systemic inflammation (24) accompanying COPD, among other factors (31).

The current data support the concept of elevated oxidative stress and inflammation in patients with COPD. Specifically, decreased plasma antioxidant capacity and increased C-reactive protein levels were documented in the patients with COPD relative to the healthy subjects (Figure 3.2). Administration of the AOC partially corrected the pro/antioxidant imbalance only in the patient group, increasing both FRAP and catalase activity, with minimal effects in healthy subjects (Figure 3.2). Although not examined in the current study, these data are accordance with the previously observed free-radical diminishing effects of the AOC in patients with COPD using *ex vivo* spin-trapping and electron paramagnetic resonance spectroscopy (19, 36). In the current study, the absence of a change in antioxidant or oxidant status in the healthy subjects, despite an equal increase in plasma ascorbate (Figure 3.2A), is likely due to the absence of a substantial redox imbalance in these healthy individuals. Thus, these data further document elevated oxidative stress in patients with COPD, as well as beneficial effects of antioxidant administration on redox balance in patients with COPD, that may be useful in the face of the elevated free radical production associated with exercise in this population.

Exercise Hyperemia and Oxidative Stress

Independent of COPD, aging itself is associated with a pro-inflammatory, pro-oxidant phenotype, which has been implicated as a causative factor in the age-associated decline in vascular function (16). As such, previous research has suggested that the increase in oxidative stress with age may impair resting limb blood flow and exercise hyperemia (11, 12, 15, 38). In this context, antioxidant administration has been documented to augment resting blood flow and exercise-induced hyperemia in older

individuals (15, 20, 38), although this is not a universal observation (29). Likewise, an abnormal pro/antioxidant balance has repeatedly been documented in patients with COPD (5, 19, 25), as observed in the current study (Figure 3.2). In addition, our group has previously observed beneficial effects of AOC administration on vascular function in patients with COPD (19). Collectively, therefore, these observations support the possibility that oxidative stress, and the consequent decrease in NO bioavailability, may negatively impact skeletal muscle blood flow in this population. Similar to previous research in older individuals, the current data, for the first time, demonstrate an augmented exercise-induced hyperemia following AOC administration in patients with COPD (Figure 3.3A). Interestingly, there was a positive relationship between the change in FRAP and the change in limb blood flow from the CTRL condition ($r = 0.45$), further implying an association between improved antioxidant status and improved exercise hyperemia. This increase in blood flow in the patients with COPD was observed across all submaximal workloads (Figure 3.3), and can be attributed to an increase in limb vascular conductance (Figure 3.4), as MAP was unaffected by AOC administration. These data imply an increase in leg vasodilation during exercise in the patients with COPD, providing a novel mechanism to target with the goal of improving O_2 transport in this population.

In the presence of increased limb blood flow following AOC administration, and no change in arterial-venous O_2 difference, exercising skeletal muscle O_2 consumption was elevated in the patients with COPD (Figure 3.3). Although the typical response to augmented O_2 delivery is to decrease O_2 extraction, and thereby maintain O_2 consumption, according to the Fick principle (18), there is growing evidence that skeletal

muscle can adjust VO_2 at a given workload when O_2 availability is altered (3, 4, 6, 23, 28). These changes in VO_2, accompanying alterations in O_2 delivery, do not always result in compensatory changes in glycolytic metabolism, suggestive of intramuscular changes in energy turnover (28), which may have favorable effects on skeletal muscle fatigue development. Indeed, it has previously been demonstrated that augmented leg blood flow during submaximal KE in heart failure patients resulted in an increase in skeletal muscle VO_2, which attenuated exercise-induced skeletal muscle fatigue (1, 4). The data from the current study suggest that augmenting exercise hyperemia, by reducing oxidative stress with the AOC, may enhance aerobic metabolism during exercise in patients with COPD, without altering glycolytic metabolism, as evidenced by a lack of an effect of the AOC on lactate release (Figure 3.3C, Table 3.2). As enhanced aerobic metabolism during exercise has been associated with a greater fatigue resistance during exercise (1, 4), it is tempting to speculate that increased aerobic metabolism during exercise may enhance fatigue resistance in patients with COPD.

Central Responses

Secondary to a significant ventilation-perfusion mismatch in the diseased lungs of patients with COPD (21), depressed arterial O_2 saturation is common in patients with COPD and is typically associated with a reduced arterial PO_2. In addition, the degree of hypoxemia is inversely related to exercise capacity, and hypoxemia exacerbates oxidative stress in patients with COPD (22). As expected, the patients with COPD in the current study exhibited reduced PaO_2 and SaO_2 relative to healthy subjects at rest (Table 3.1) and during exercise (Figure 3.5). Interestingly, following administration of the AOC, a small, but significant, increase in arterial O_2 saturation was observed in patients with COPD,

both at rest (Table 3.2) and during exercise (Figure 3.5A), with no effect in healthy subjects. The changes in SaO_2 were observed without any changes in PaO_2, suggestive of an increase in the O_2 affinity for hemoglobin, which may have been related to a decrease in exercise-induced acidosis as a consequence of the increase in aerobic metabolism afforded by the AOC (Figure 3.3C), although no measurable changes in lactate or pH were observed. Although the practical significance of the ~1% increase in saturation is questionable, as this increase was accomplished with a relatively small antioxidant dose, the impact on saturation of further decreases in oxidative stress with a more potent antioxidant intervention potentially deserves further examination. An increase in SaO_2 has the potential to improve CaO_2 and O_2 delivery, which would likely have beneficial effects for exercising skeletal muscle (1). CaO_2, however, was not altered in the patients in the current study because of the small, but significant, decrease in hemoglobin concentration that offset the increased SaO_2, and maintained CaO_2 (Table 3.2). These data do, however, suggest that reducing oxidative stress in patients with COPD has the potential to attenuate arterial hypoxemia, which may improve exercise tolerance in this population.

Summary and Conclusions

The purpose this study was to examine the impact of an AOC on oxidative stress and antioxidant capacity, and subsequently exercise hemodynamics during small muscle mass exercise in patients with COPD and healthy subjects. Patients with COPD exhibited evidence of reduced antioxidant capacity relative to healthy subjects. Administration of the AOC improved the redox balance in the patients with COPD, with little effect in the healthy subjects. These favorable changes in redox balance were accompanied by

improved exercise hyperemia and leg vascular conductance as well as increased skeletal muscle O_2 consumption during submaximal knee-extensor exercise in patients with COPD, while minimal effects were observed in healthy subjects. In addition, arterial O_2 saturation was improved at rest and during exercise in patients with COPD following AOC administration. Collectively, these data illustrate the role of oxidative stress in the integration of O_2 transport and utilization during exercise in this population, and further implicate oxidative stress in the systemic pathophysiological consequences of COPD.

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Table 3.1 Descriptive characteristics

	Healthy Subjects	COPD
Age, yr	68 ± 2	62 ± 3
Male/Female	13/3	13/3
Height, m	1.73 ± 0.02	1.74 ± 0.02
Weight, kg	75 ± 4	85 ± 4
BMI, kg/m^2	25 ± 1	28 ± 1
Peak knee-extensor work rate, W	31 ± 3	26 ± 3
Glucose, mg/dl	84 ± 4	84 ± 3
Cholesterol, mg/dl	201 ± 14	202 ± 16
HDL, mg/dl	52 ± 3	61 ± 5
LDL, mg/dl	132 ± 10	120 ± 13
Triglycerides, mg/dl	94 ± 16	117 ± 14
Pulmonary function		
Forced vital capacity, l (% predicted)	4.7 ± 0.3 (113 ± 5)	4.1 ± 0.3* (94 ± 5*)
Forced expiratory volume in one s, l/s (% predicted)	3.4 ± 0.2 (115 ± 4)	1.8 ± 0.2* (54 ± 6*)
FEV_1/FVC (%)	75 ± 1	48 ± 4*
Resting arterial blood gases		
Oxyhemoglobin, %	94.4 ± 0.5	91.7 ± 1.2*
Partial pressure of oxygen, mmHg	87 ± 4	71 ± 3*
Partial pressure of carbon dioxide, mmHg	30 ± 1	35 ± 1*
Bicarbonate, mmol/l	19.7 ± 0.5	23.4 ± 0.8*
pH	7.43 ± 0.01	7.43 ± 0.01
Medications (% of Group)		
Long Acting Beta Agonists	0%	26%
Short Acting Beta Agonists	0%	80%
Acetylcholine Antagonists	0%	53%
Inhaled Corticosteroids	15%	33%
Physical Activity, n = 10/group		
Sedentary, min/day	1215 ± 17	1156 ± 75
Light, min/day	153 ± 9	127 ± 20
Moderate, min/day	26 ± 5	15 ± 6
High, min/day	0.4 ± 0.2	0.1 ± 0.01*
Steps, counts/day	5101 ± 671	3798 ± 728

COPD, Chronic Obstructive Pulmonary Disease; FEV_1/FVC, forced expiratory volume in one s relative to forced vital capacity; BMI, body mass index; Values expressed as mean ± S.E.M. *Significantly different from healthy subjects.

Table 3.2 Impact of Antioxidant Cocktail (AOC) Administration on Physiological Variables

Work Rate		Rest	3 Watts	6 Watts	9 Watts
Healthy Subjects					
Mean arterial pressure, mmHg	CTRL	119±2	127±3	125±2	127±3
	AOC	117±2	124±2	125±3	127±2
Leg blood flow, ml/min	CTRL	282±37	1591±77	1856±80	2113±99
	AOC	322±35*	1674±105	1934±107	2126±125
Leg vascular conductance, ml/min/mmHg	CTRL	2.4±0.2	12.6±0.6	14.9±0.6	16.7±0.8
	AOC	2.8±0.3*	13.1±0.8	15.4±0.9	16.7±1.0
HR, beats/min	CTRL	64±2	76±4	78±4	81±5
	AOC	64±2	77±4	79±4	83±5
Leg O_2 Delivery, l/min	CTRL	0.06±.01	0.31±0.03	0.36±0.02	0.41±0.02
	AOC	0.06±0.01	0.32±0.02	0.38±0.02	0.41±0.02
Hemoglobin, g/dl	CTRL	15±0.3	14.8±0.2	14.8±0.2	14.8±0.2
	AOC	14.8±0.4	15.0±0.3	14.8±0.2	14.8±0.2
Arterial O_2 Content, ml/dl	CTRL	19.9±0.3	19.6±0.2	19.6±0.2	19.7±0.3
	AOC	19.6±0.3	19.8±0.3	19.8±0.3	19.7±0.3
Net lactate release, mmol/min	CTRL	8±18	338±82	417±113	975±179
	AOC	34±10	343±99	571±184	799±212
Venous pH	CTRL	7.41±0.01	7.31±0.01	7.30±0.01	7.29±0.01
	AOC	7.38±0.01	7.29±0.02	7.28±0.02	7.27±0.02
COPD					
Mean arterial pressure, mmHg	CTRL	129±4#	137±4#	137±4#	138±4#
	AOC	131±4#	138±4#	136±4#	140±4#
Leg blood flow, ml/min	CTRL	281±37	1604±100	1832±109	2036±114
	AOC	315±38	1798±128*	1992±120*	2187±136*
Leg vascular conductance, ml/min/mmHg	CTRL	2.1±0.3	12.0±0.9	13.8±1.1	15.2±1.3
	AOC	2.3±0.3	13.5±1.2*	15.2±1.3*	16.7±1.5*
HR, beats/min	CTRL	72±4	87±4	90±4	92±4
	AOC	72±4	85±4	87±4	90±4
Leg O_2 Delivery, l/min	CTRL	0.05±0.01	0.3±0.02	0.34±0.02	0.38±0.02
	AOC	0.06±0.01	0.32±0.02	0.36±0.02	0.39±0.03
Hemoglobin, g/dl	CTRL	14.0±0.4	14.3±0.4	14.1±0.4	14.3±0.4
	AOC	13.9±0.4	13.8±0.4*	14.0±0.4*	13.8±0.4*
Arterial O_2 Content, ml/dl	CTRL	18.1±0.4#	18.5±0.4#	18.2±0.4#	18.5±0.5#
	AOC	18.0±0.4#	17.9±0.4#	18.3±0.4#	18.0±0.4#
Net lactate release, mmol/min	CTRL	48±7	1777±373#	1968±369#	2083±442#
	AOC	70±11	1606±346#	1596±298#	2258±461#
Femoral Venous pH	CTRL	7.38±0.01	7.29±0.02	7.28±0.02	7.27±0.02
	AOC	7.38±0.01	7.31±0.02	7.29±0.02	7.28±0.02

Values expressed as mean ± S.E.M. *Significantly different from control (CTRL) conditions. #Significantly different from healthy subjects

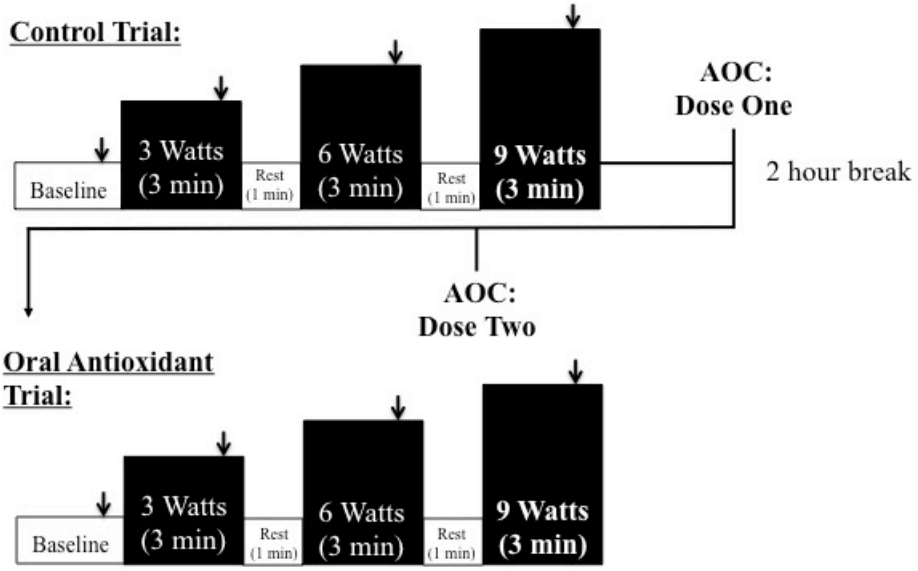

Figure 3.1 Experimental protocol. Arrows indicate points at which leg blood flow was recorded and arterial and venous blood samples were obtained.

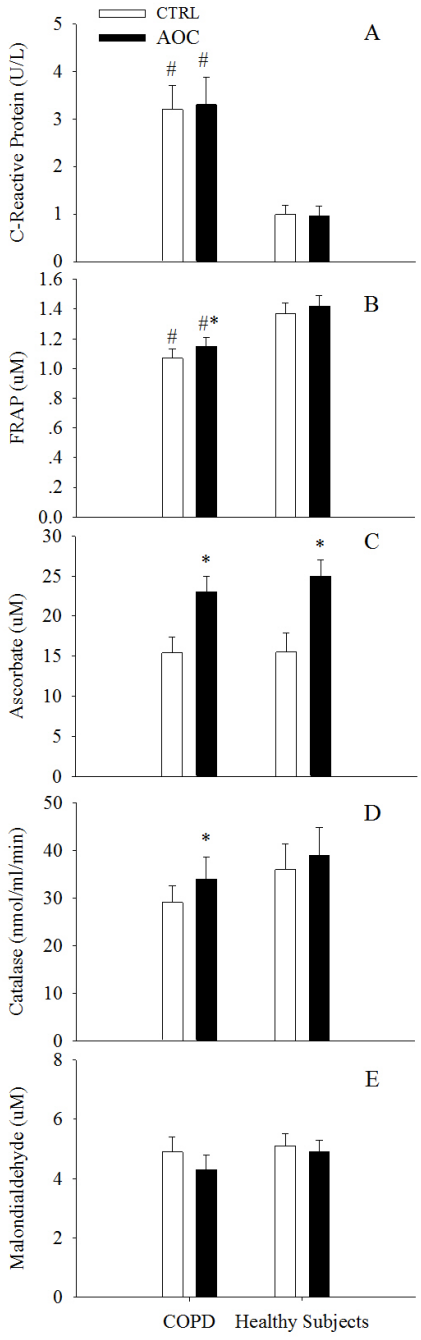

Figure 3.2. Impact of an antioxidant cocktail (AOC) on indices of inflammation, antioxidant capacity and oxidative stress in patients with chronic obstructive pulmonary disease (COPD) and healthy subjects. Values are presented as mean ± S.E.M *Significantly different from the control (CTRL) conditions, $p < 0.05$. #Significantly different from healthy subjects, $p < 0.05$.

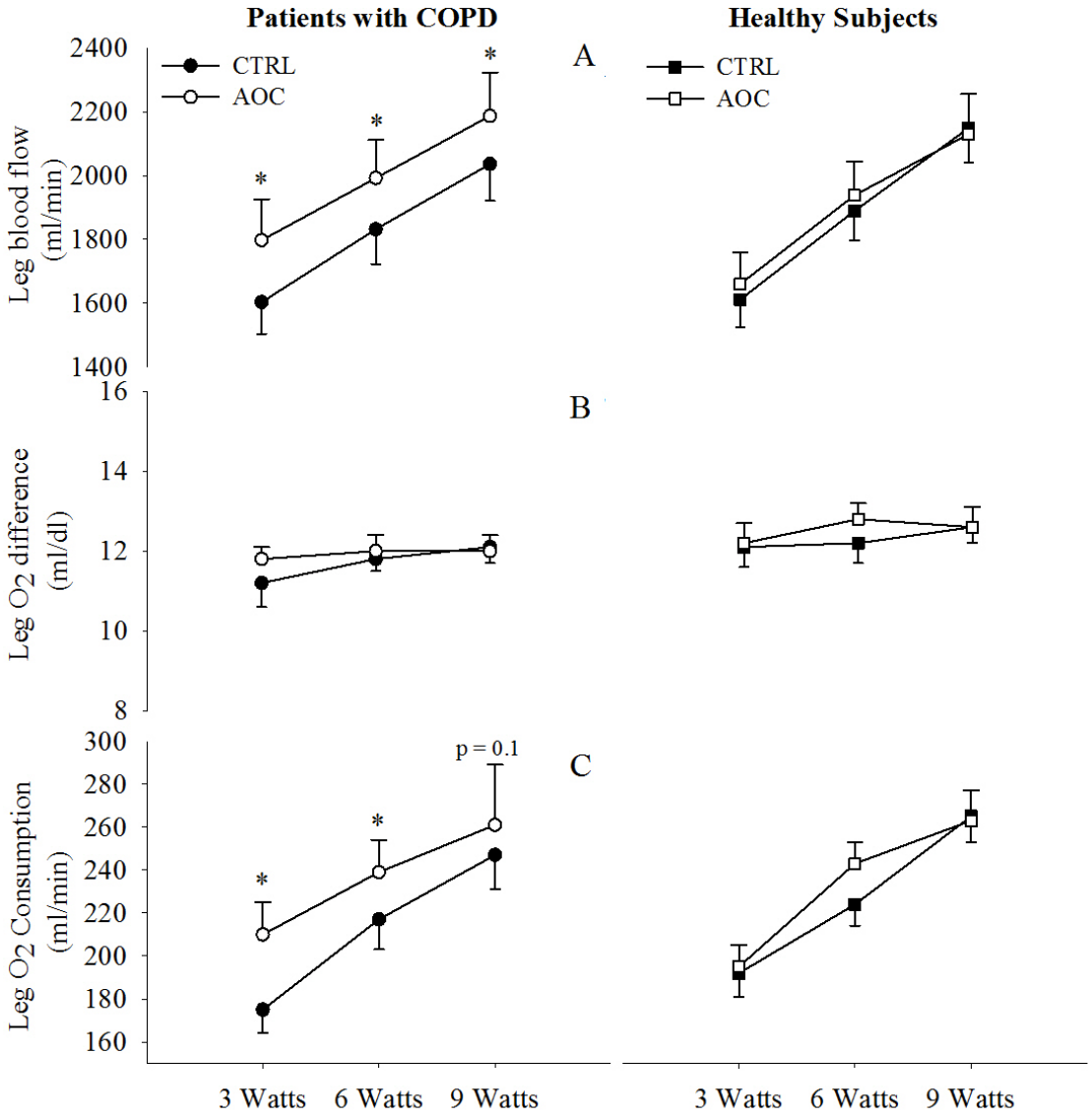

Figure 3.3 Impact of an antioxidant cocktail (AOC) on exercising limb blood flow (A), oxygen extraction (B) and oxygen consumption (C) in patients with chronic obstructive pulmonary disease (COPD) and healthy subjects. Values are presented as mean ± S.E.M. *Main effect of AOC, $p < 0.05$.

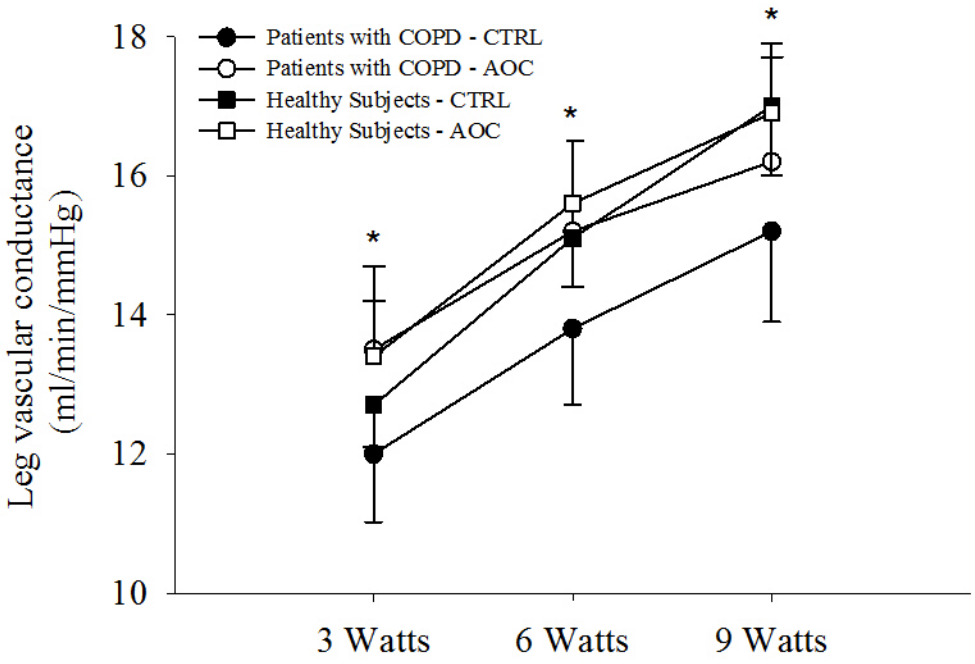

Figure 3.4 Impact of an antioxidant cocktail (AOC) on leg vascular conductance during exercise in patients with chronic obstructive pulmonary disease (COPD) and healthy subjects. Values are presented as mean ± S.E.M. *Significantly different from the control (CTRL) conditions within patients with COPD.

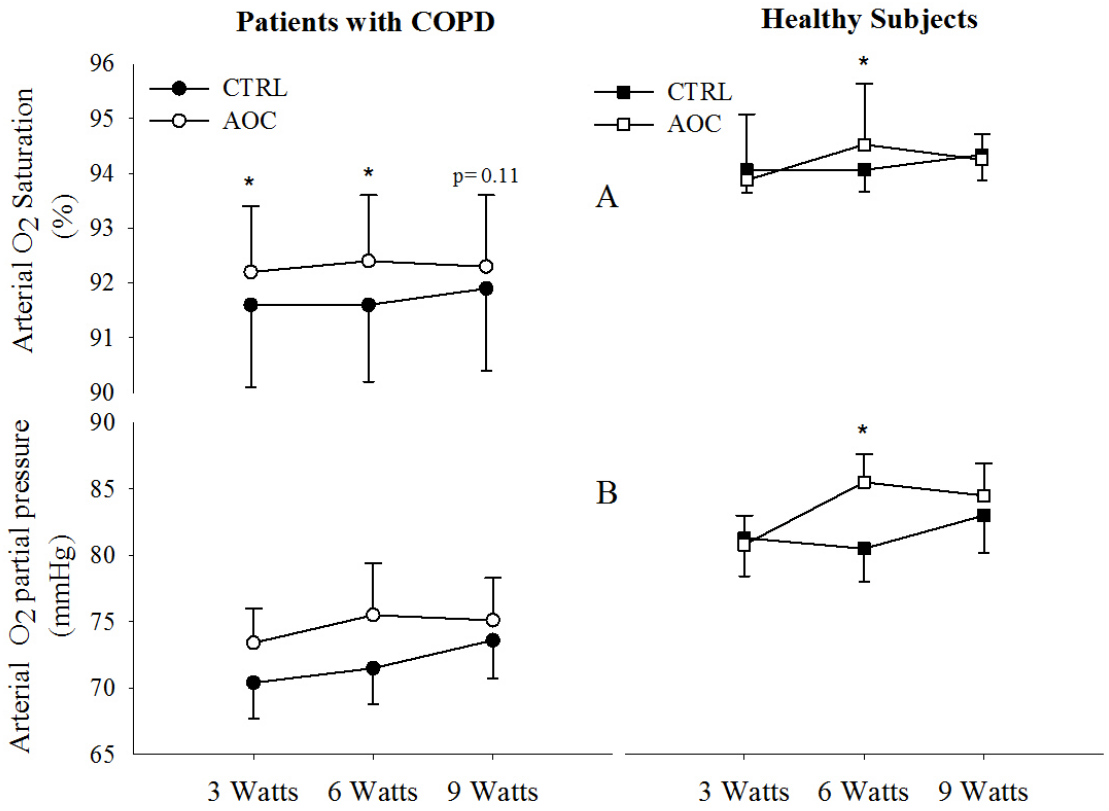

Figure 3.5 Impact of an antioxidant cocktail (AOC) on arterial oxygen saturation (A) and partial pressure (B) during exercise in patients with chronic obstructive pulmonary disease (COPD) and healthy subjects. Values are presented as mean ± S.E.M. *Main effect of AOC, $p < 0.05$.

CHAPTER 4

ASCORBATE INFUSION INCREASES SKELETAL MUSCLE FATIGUE RESISTANCE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Rossman, MJ, Garten, RS, Groot HJ, Reese, VR, Zhao, J, Amann, M, and Richardson, RS. *Am J Physiol Regul Integr Comp Physiol* 305: R1163-R1170, 2013

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Ascorbate infusion increases skeletal muscle fatigue resistance in patients with chronic obstructive pulmonary disease

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Rossman MJ, Garten RS, Groot HJ, Van Reese, Zhao J, Amann M, Richardson RS. Ascorbate infusion increases skeletal muscle fatigue resistance in patients with chronic obstructive pulmonary disease. *Am J Physiol Regul Integr Comp Physiol* 305: R1163–R1170, 2013. First published September 25, 2013; doi:10.1152/ajpregu.00360.2013.—Chronic obstructive pulmonary disease (COPD) is associated with systemic oxidative stress and skeletal muscle dysfunction. The purpose of this study was to examine the impact of intravenous ascorbate administration (AO) on biological markers of antioxidant capacity and oxidative stress, and subsequently skeletal muscle function during dynamic, small muscle mass exercise in patients with COPD. Ten patients with spirometric evidence of COPD performed single-leg knee extensor (KE) trials matched for intensity and time (isotime) following intravenous ascorbate (2 g) or saline infusion (PL). Quadriceps fatigue was quantified by changes in force elicited by maximal voluntary contraction (MVC) and magnetic femoral nerve stimulation ($Q_{tw,pot}$). AO administration significantly increased antioxidant capacity, as measured by the ferric-reducing ability of plasma (PL: 1 ± 0.1 vs. AO: 5 ± 0.2 mM), and significantly reduced malondialdehyde levels (PL: 1.16 ± 0.1 vs. AO: 0.97 ± 0.1 mmol). Additionally, resting blood pressure was significantly reduced (PL: 104 ± 4 vs. AO: 93 ± 6 mmHg) and resting femoral vascular conductance was significantly elevated after AO (PL: 2.4 ± 0.2 vs. AO: 3.6 ± 0.4 $ml \cdot min^{-1} \cdot mmHg^{-1}$). During isotime exercise, the AO significantly attenuated both the ventilatory and metabolic responses, and patients accumulated significantly less peripheral quadriceps fatigue, as illustrated by less of a fall in MVC (PL: −11 ± 2% vs. AO: −5 ± 1%) and $Q_{tw,pot}$ (PL: −37 ± 1% vs. AO: −30 ± 2%). These data demonstrate a beneficial role of AO administration on skeletal muscle fatigue in patients with COPD and further implicate systemic oxidative stress as a causative factor in the skeletal muscle dysfunction observed in this population.

free radicals; peripheral fatigue; ascorbate

SKELETAL MUSCLE DYSFUNCTION plays a prominent role in limiting exercise and activities of daily living in patients with chronic obstructive pulmonary disease (COPD) (21, 22). Numerous factors, including inactivity and skeletal muscle detraining (34), mitochondrial dysfunction (9), and oxidative stress (30) have all been implicated in the skeletal muscle dysfunction associated with COPD. Of these factors, the contribution of oxidative stress to reduced exercise capacity in patients with COPD has been well documented (11, 13, 24). Specifically, previous research has demonstrated an inverse correlation between exercise time to exhaustion and evidence of lipid peroxidation (12), as well as the favorable effects of preexercise antioxidant pretreatment with *N*-acetylcysteine (24) on performance in patients with COPD. Therefore, in patients with COPD, exercising skeletal muscle is a significant source of oxidative stress, the magnitude of oxidative stress likely impairs skeletal muscle function, and the modulation of redox state may enhance exercise capacity in this population.

Accordingly, our group previously utilized an acute, readily available, oral antioxidant cocktail (vitamins C, E, and α-lipoic acid), with documented efficacy (43), to examine the impact of oxidative stress on skeletal muscle function in COPD (32). The antioxidant cocktail decreased the electron paramagnetic resonance (EPR) spectroscopy free radical signal, but did not impact skeletal muscle fatigue measured after isotime knee extensor (KE) exercise in patients with COPD. However, the individual responses to the oral antioxidant cocktail were mixed, with only half of the patients exhibiting a substantially reduced EPR spectroscopy signal. Therefore, in this prior study (32), the role of free radicals on skeletal muscle fatigue in patients with COPD was not fully elucidated.

Likely due to free radical scavenging, the infusion of supraphysiological doses of the antioxidant ascorbate (AO) have previously been documented to restore vascular function in several pathophysiological conditions such as heart failure (19), hypertension (36), diabetes (38), as well in chronic smokers (18). In addition, high-dose AO infusion has been documented to improve resting (20) and exercising (14, 23) skeletal muscle blood flow in healthy older individuals. Improving limb blood flow, and possibly oxygen delivery, has the potential to attenuate the rate of development of peripheral muscle fatigue (3). Interestingly, intravenous AO administration also ameliorated the exaggerated exercise pressor reflex during plantar flexion exercise in patients with peripheral artery disease, which was attributed to a reduction in excessive group III/IV afferent stimulation under basal conditions (27). Decreasing the group III/IV afferent signal from the lower limbs has also been documented to extend exercise time to exhaustion in patients with COPD (17). Therefore, free radical scavenging by AO may confer beneficial vascular effects and dampen group III/IV afferent signaling, potentially translating into fatigue resistance in patients with COPD.

Thus the purpose of this study was to examine the impact of intravenous AO administration, a potent water-soluble antioxidant with no known side effects, on oxidative stress and skeletal muscle fatigue during dynamic KE exercise in patients with COPD. In addition, this study sought to comprehensively evaluate the impact of reducing oxidative stress with AO on the physiological responses to KE exercise in patients with COPD.

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We tested the hypotheses that in patients with COPD intravenous AO administration would *1*) improve antioxidant capacity and decrease oxidative stress and, *2*) decrease the magnitude of peripheral quadriceps fatigue induced by KE exercise matched for intensity and duration (isotime).

METHODS

Subjects. Written, informed consent was obtained from all participants before their inclusion in this study, and the Institutional Review Boards of the University of Utah and the Salt Lake City Veterans Affairs Medical Center approved all protocols. Ten patients with COPD were enrolled based on spirometric evidence of moderate to severe airflow obstruction [$FEV_1/FVC \leq 0.7$ (10)], as assessed by standard pulmonary function tests performed during an initial visit to the laboratory. General anthropometric characteristics, including thigh volume, which was used to estimate quadriceps muscle mass (16), were also determined during this visit. Resting arterial blood analyses, collected in a parallel study in which the current subjects took part, are also presented here to better characterize the patients.

Exercise protocols and general procedures. All subjects were familiarized with single-leg KE exercise, which was performed at a cadence of 60 rpm, during two preliminary visits to the laboratory. Subsequently, peak KE work rate was determined with subject-specific protocols designed to reach exhaustion within 8–12 min, consisting of 2–5 W/min increases. The experimental protocol is depicted in Fig. 1. After the peak work rate tests, subjects performed at least two practice constant-load exercise trials at 80% of maximal workload to the limit of tolerance to determine a target exercise intensity and duration for the subsequent isotime trials. The intensity of the practice trials was adjusted until subjects could maintain the intensity, at a cadence of 60 rpm, for ~6 to 8 min before their cadence dropped below 50 rpm and the trial was terminated. Once these criteria were met, the trial time was adopted as the target time for the subsequent isotime trials. Next, in a repeated-measures design, isotime trials, separated by 48–96 h, were performed following either a bolus infusion of AO (100 mg/ml AO dissolved in normal saline, infused at 1 ml/min for 20 min) or saline (PL: 0.9% NaCl infused at 1 ml/min for 20 min) via an intravenous catheter in the arm (Fig. 1). The patient and all members of the research team, except for the individual administering the AO or PL, were blinded to the experimental condition.

Neuromuscular function tests were performed before infusion, after infusion (but before exercise), and 10 min after the isotime trials. In addition, venous blood samples were taken before and immediately after the isotime trials to determine pro- and antioxidant status, and for spin trapping and EPR spectroscopy to directly assess free radical concentration. Before each exercise bout, 1 min of resting data were collected and subjects performed 1 min of unloaded warmup KE exercise. Ventilation, gas exchange, heart rate (HR), mean arterial pressure (MAP), ratings of perceived exertion and breathlessness, arterial oxygen saturation, femoral blood flow, and quadriceps electromyograms (EMG) were measured during the isotime trials.

Oxidative stress, antioxidant assays, and direct measurement of free radicals. Plasma samples were stored at −80°C until analysis. Lipid peroxidation, a marker of oxidant damage, was assessed by plasma malondialdehyde levels (Bioxytech LPO-586, Foster City, CA). Total antioxidant capacity was evaluated by determining the ferric-reducing ability of plasma (FRAP), using the method described by Benzie and Strain (6). The efficacy of the AO specific to plasma ascorbate levels was assayed as previously described (8) (CosmoBio, Carlsbad, CA). Free radical scavenging, assessed by superoxide dismutase and catalase activity, was also assayed in the plasma (42) (Cayman Chemical, Ann Arbor, MI). EPR spectroscopy was performed on pre- and postexercise blood samples to directly assess the ability of the AO to reduce the concentration of free radicals with an EMX X-band spectrometer (Bruker, MA), as previously described (31, 32).

Pulmonary and cardiovascular responses. Ventilation and pulmonary gas exchange were measured at rest and during exercise using an open-circuit system (ParvoMedics, Sandy, UT). HR, determined from the R-R interval of a three-lead electrocardiogram, and arterial oxygen saturation (Sa_{O_2}), estimated using a pulse oximeter (Nellcor N-595, Pleasanton, CA) with adhesive forehead sensors, were also acquired during these trials at 200 Hz using a data acquisition system (AcqKnowledge; Biopac Systems, Goleta, CA). MAP was determined with a finometer (Finapres Medical Systems, The Netherlands) at heart level. Patients were asked how hard their leg was working (rating of perceived exertion, RPE) and how labored was their breathing

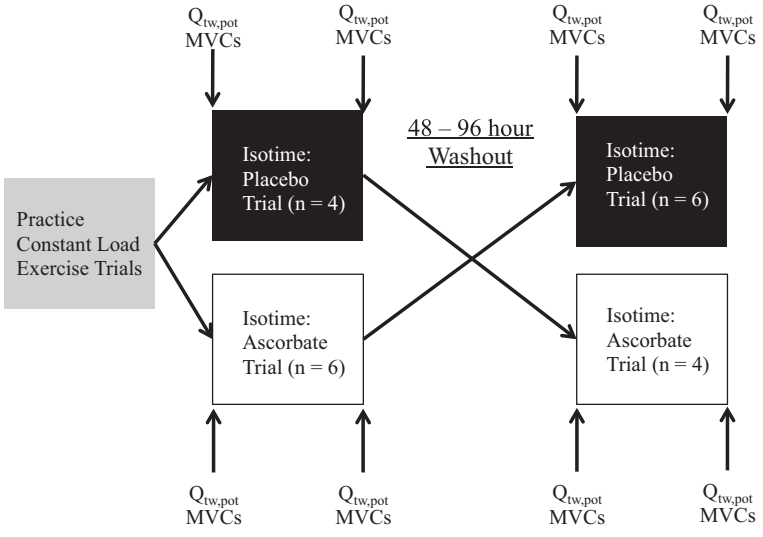

Fig. 1. Study protocol schematic. MVC, maximal voluntary contraction. $Q_{tw,pot}$, potentiated twitch force.

(dyspnea) every minute during the exercise trials using Borg's CR10 scale (7).

Leg blood flow. Measurements of femoral artery blood velocity and vessel diameter in the leg being studied were performed at rest and throughout isotime exercise, using a Logic 7 ultrasound system (General Electric Medical Systems) as previously described (39). Blood flow in the femoral artery was calculated as the following: blood flow = (mean velocity)π(vessel diameter/2)^2 $\times$ 60.

Quadriceps electromyograms. Quadriceps EMGs were recorded from the vastus lateralis muscle during exercise from electrodes placed in a bipolar configuration with an interelectrode distance of 20 mm over the middle of the muscle belly, with the active electrodes placed over the motor point of the muscle and the reference electrode in an electrically neutral site (4). To ensure similar electrode placement between trials, the electrode location was marked with indelible ink. Raw EMG signals were filtered with a bandpass filter (with a low-pass cut-off frequency of 15 Hz and a high-pass cut-off frequency of 650 Hz) and after visual inspection of the filtered signal; a threshold voltage was set to identify the onset of EMG activity (AcqKnowledge; Biopac Systems). For data analysis, the integral of each EMG burst (integrated EMG) was calculated to determine the percent increase in integrated EMG from the first minute of exercise (4), an index of the development of peripheral fatigue during exercise. The EMG electrodes were also used to record magnetically evoked compound action potentials (M-waves, area and peak-to-peak amplitude) to evaluate changes in membrane excitability from pre- to postexercise during potentiated twitch force ($Q_{tw,pot}$) assessments.

Neuromuscular function assessment. The magnitude of peripheral quadriceps fatigue was quantified by pre- to postinfusion, and pre- to postexercise changes in quadriceps maximal voluntary contraction (MVC) and $Q_{tw,pot}$ evoked by supramaximal magnetic stimulation of the femoral nerve (4, 29) with a magnetic stimulator (Magstim 200, The Magstim, Wales, UK) connected to a double 70-mm coil (26). Specifically, while laying semirecumbent with a knee joint angle of 90 degrees, subjects performed a series of six MVCs separated by 30 s, with $Q_{tw,pot}$ assessments interspersed 5 s after each MVC. Patients viewed a computer monitor displaying real-time visual feedback to ensure maximal effort during all MVCs. The neuromuscular function assessment procedure (6 MVCs and 6 $Q_{tw,pot}$ maneuvers) was performed before the AO or PL infusion, after the infusion (but before exercise), and 10 min after exercise. In addition, to quantify voluntary activation of the quadriceps during the MVCs, the additional force generated by a single twitch superimposed on the MVC was compared with the force produced by the potentiated twitch immediately following the MVC to determine the percent voluntary muscle activation (4). Force was obtained from a calibrated load cell (Transducer Techniques, Temecula, CA) connected to a noncompliant strap placed around the subject's ankle and acquired at 200 Hz with a data acquisition system (AcqKnowledge; Biopac Systems). On a separate visit, to ensure supramaximality of stimulation during magnetic stimulation of the femoral nerve, the plateau in evoked force following serial twitch forces, obtained every 30 s, at 70, 80, 85, 90, 95, and 100% of maximal stimulator output, was also evaluated.

Statistical analysis. Two-way repeated measures ANOVA were used to compare the effect of antioxidant treatment on physiological parameters during exercise, with a Tukey post hoc analysis if a significant main effect was found. Student's paired *t*-tests were used to compare the effect of AO in terms of antioxidant efficacy and indices of peripheral fatigue. Statistical significance was set at $\alpha = 0.05$ for all tests. All group data are expressed as means ± SE.

RESULTS

Subject characteristics. Subject characteristics are documented in Table 1. One patient was a current smoker, who refrained from the use of tobacco products for 12 h before all data collection. Two patients qualified for supplemental oxygen; these patients, however, at the time of data collection, only used the supplemental oxygen while sleeping. No patients reported any side effects of AO or PL administration and were therefore successfully blinded to the experimental condition. Supramaximality of magnetic nerve stimulation was demonstrated in all patients by evidence of a plateau in evoked force with increasing stimulus intensity.

Table 1. *Subject characteristics*

Age, yr	62 ± 3
Height, m	1.73 ± 0.03
Weight, kg	84 ± 7
BMI, kg/m^2	28 ± 2
Quadriceps muscle mass, kg	1.7 ± 0.2
Peak knee-extensor work rate, W	28 ± 3
Male/Female	7/3
Pulmonary function	
FVC, l (% predicted)	3.6 ± 0.2 (86 ± 5)
FEV in 1 s, l/s (% predicted)	1.8 ± 0.2 (57 ± 5)
FEV_1/FVC, %	51 ± 5
Resting arterial blood gases	
Hemoglobin concentratio, g/dl	14 ± 1
Oxyhemoglobin, %	92 ± 1
Partial pressure of oxygen, mmHg	70 ± 2
Partial pressure of carbon dioxide, mmHg	32 ± 2
Bicarbonate, mmol/l	22 ± 1
pH	7.45 ± 0.01

Values expressed as means ± SE. FEV_1/FVC, forced expiratory volume in 1 s relative to forced vital capacity; BMI, body mass index.

Antioxidant efficacy. Before exercise, AO caused an ~10-fold elevation in plasma ascorbate levels (Fig. 2*A*). AO infusion also increased endogenous antioxidant capacity, as measured by FRAP, and resulted in greater free radical scavenging, as evidenced by increased superoxide dismutase enzymatic catalase activities (Fig. 2, *B–D*). Consequently, resting malondialdehyde levels, a marker of lipid peroxidation and oxidative stress, were decreased following AO infusion (Fig. 2*E*). In contrast, and somewhat surprisingly, there was no detectable difference in plasma free radical levels, directly measured by EPR spectroscopy, between conditions (AO: 10.9 ± 3.1 AU vs. PL: 11.6 ± 3.7 AU, $P > 0.05$).

After exercise, AO and FRAP remained elevated over PL values in the AO condition (AO: 107.6 ± 8.1 μg/ml vs. 12.9 μg/ml, $P < 0.05$; FRAP: 1.5 ± 0.08 mM vs. PL: 0.97 ± 0.08, $P < 0.05$ exercise, for AO and PL, respectively). In addition, MDA was decreased to a similar extent as before exercise (AO: 0.94 ± 0.1 μM vs. PL: 1.2 ± 0.1 μM, $P < 0.05$). Postexercise, there were no differences between conditions in terms of antioxidant enzyme (superoxide dismutase or catalase) activity or plasma free radical levels, assessed by EPR spectroscopy.

Isotime trials. Acutely, MVC and $Q_{tw,pot}$ were unaffected by AO infusion (MVC: 374 ± 52 N to 374 ± 53 N, $P > 0.05$; $Q_{tw,pot}$: 105 ± 13 N to 103 ± 13 N, $P > 0.05$, for pre- and postinfusion, respectively), and these values were not different from the pre- and post-PL infusion values. At baseline, before exercise, MAP was reduced following the AO infusion (Fig. 3). Despite the decrease in perfusion pressure, femoral artery blood flow was not different between conditions ($P = 0.1$), and thus femoral vascular conductance was significantly elevated in the AO condition (Fig. 3). Because of movement artifact, differences in MAP could not be evaluated throughout the

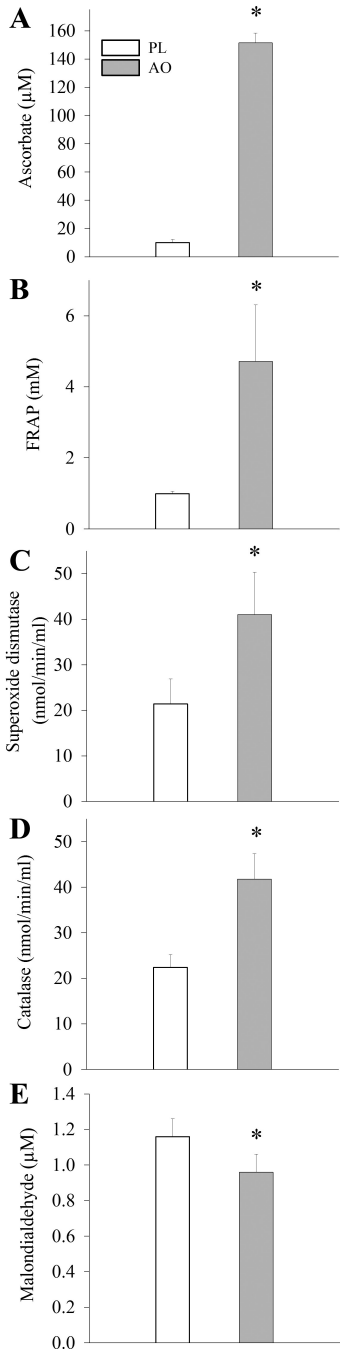

Fig. 2. Quantitative assessment of antioxidants and markers of oxidative stress after intravenous saline (PL) or ascorbate (AO) administration. FRAP, ferric-reducing ability of plasma. Values are presented as means ± SE. *Significantly different from the PL condition.

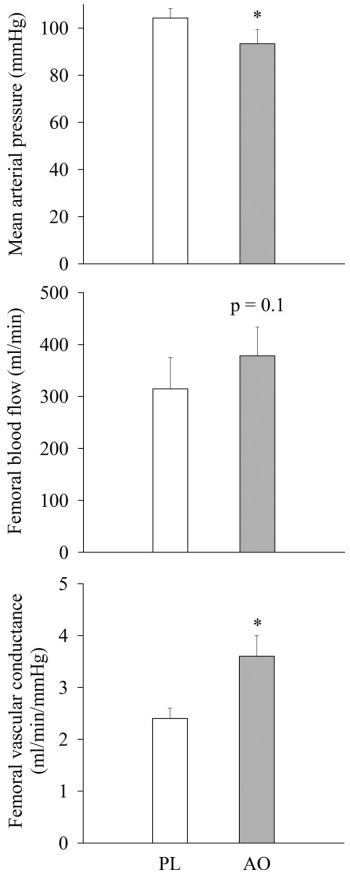

Fig. 3. Resting mean arterial pressure and hemodynamic parameters following intravenous PL or AO administration. Values are presented as means ± SE. *Significantly different from the PL condition.

isotime trials. The cardiorespiratory responses to the isotime trials are depicted in Fig. 4. As illustrated, oxygen consumption (V_{O_2}) and carbon dioxide production (V_{CO_2}) were reduced during exercise following AO infusion at isotime *minute 4*, but reached similar levels at the end of exercise. In addition, ventilation rate (VE) and the ventilation relative to carbon dioxide production (VE/V_{CO_2}) ratio were reduced in the AO condition during exercise and at the end of the isotime trials. Arterial oxygen saturation and femoral artery blood flow were not different between conditions.

With respect to the development of peripheral fatigue during exercise, the percent increase in the integrated EMG signal (Fig. 4) was reduced during exercise in the AO condition and tended to be lower at end exercise ($P = 0.09$). Subjects' ratings of perceived exertion were also lower during exercise following AO infusion, as well as at the end of exercise. In line with these observations, the patients' dyspnea ratings were reduced at the end of the isotime trials in the AO condition (6.3 ± 1 vs. 4.8 ± 1, $P > 0.05$, for PL and AO, respectively). There were no changes in m-wave area (PL: 80 ± 9 mVms vs. 72 ± 10

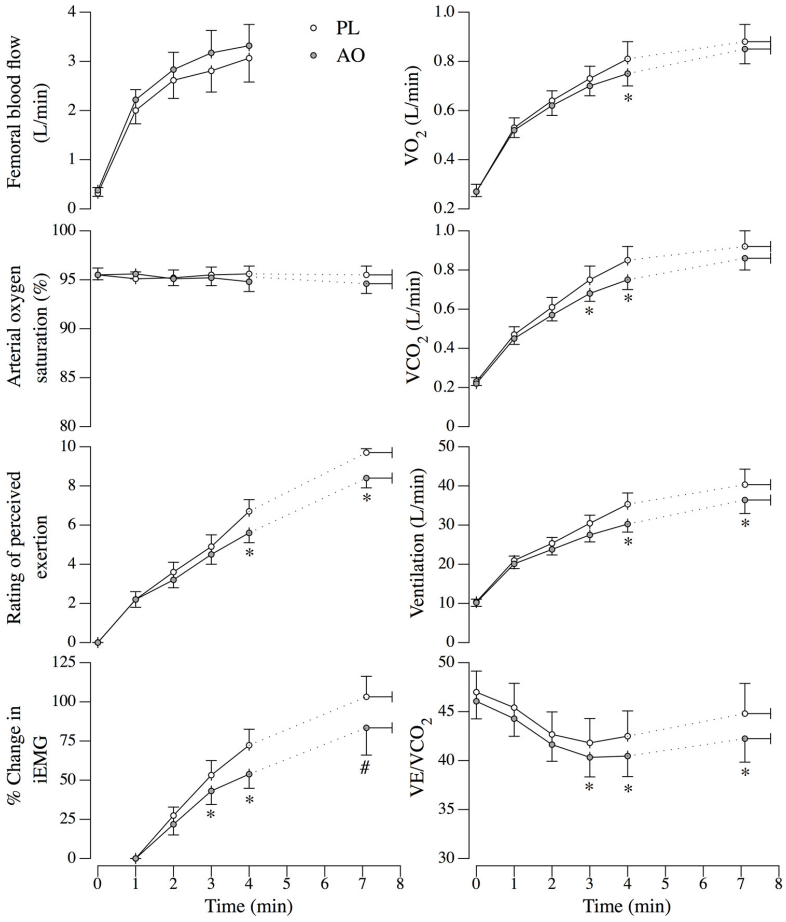

Fig. 4. Physiological responses to constant workload isotime knee extensor exercise matched for intensity and duration following intravenous PL or AO administration. Group mean data (±SE) over the first 4 min of exercise, which were attained by all subjects. The final time point represents end-exercise values, which were not obtained for femoral blood flow due to loss of signal. VE/V_{CO_2}, ventilation relative to carbon dioxide production; V_{CO_2}, carbon dioxide production; V_{O_2}, oxygen consumption; iEMG, integrated electromyogram from the vastus lateralis. *Significantly different from the PL condition. #$P = 0.09$.

mVms, $P > 0.05$; AO 85 ± 11 mVms vs. 82 ± 11 mVms, $P > 0.05$) or peak-to peak-amplitude (PL: 8.7 ± 0.8 mV vs. 8.1 ± 0.9 mV, $P > 0.05$; AO 9.3 ± 1.2 mV vs. 8.8 ± 1.2 mV, $P > 0.05$) in either condition from pre- to postexercise, and these values were not different between PL and AO trials. Voluntary activation was reduced following exercise to a similar extent in both conditions (AO: −3.1 ± 0.7% vs. PL: −3.5 ± 1.6%, $P > 0.05$). Additionally, the pre- to postexercise changes in MVC and $Q_{tw,pot}$ were reduced to a lesser extent in the AO condition, suggestive of less peripheral quadriceps fatigue (Fig. 5).

DISCUSSION

This study sought to evaluate the impact of intravenous AO on systemic antioxidant capacity and oxidative stress in patients with COPD and subsequently determine the effects of

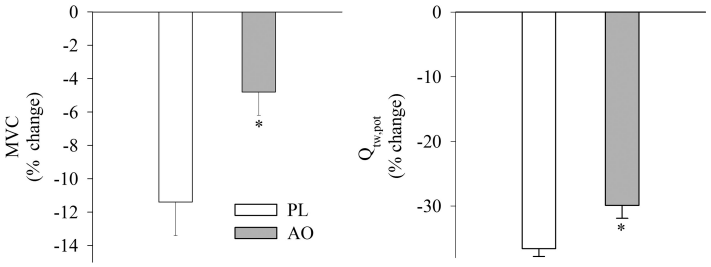

Fig. 5. Changes from preexercise values in quadriceps muscle function following constant-load knee extensor exercise matched for intensity and duration preceded by either intravenous PL or AO administration. Data are presented as means ± SE. *Significantly different from the PL condition.

this intervention on skeletal muscle fatigue following exercise in this population. Before exercise, AO increased antioxidant capacity and reduced oxidative stress, and these changes in the pro- and antioxidant balance were accompanied by a reduction in MAP and an increase in femoral artery vascular conductance. Exercise after AO administration, matched for time with the PL trial, was associated with attenuated ventilatory and metabolic responses to the work and a slowed rate of fatigue development (rise in quadriceps iEMG). Thus the exercise bout ultimately resulted in less of a decrease in quadriceps MVC and evoked twitch force following exercise, revealing improved fatigue resistance during exercise. Collectively, these data demonstrate a beneficial effect of intravenous AO administration on systemic oxidative stress and skeletal muscle function in patients with COPD. Moreover, these data further implicate oxidative stress as a factor contributing to skeletal muscle dysfunction in COPD.

Oxidative stress and fatigue. Previously, dynamic KE exercise has been documented to increase markers of oxidative damage in patients with COPD, but not in healthy control subjects (11, 12). In this prior study, within the patient group, the magnitude of increase in oxidative stress was negatively correlated with exercise time to exhaustion (12). Furthermore, when patients with COPD were pretreated with the pharmacological antioxidant *N*-acetylcysteine before performing KE exercise, markers of oxidative damage were reduced and exercise time to exhaustion was improved (24). Excessive elevations in free radicals, within muscle itself, have been suggested to impair function by decreasing the calcium sensitivity of the myofilaments and attenuating calcium reuptake by the sarcoplasmic reticulum, among other mechanisms (1). Collectively, these studies suggest that oxidative stress contributes to skeletal muscle dysfunction in patients with COPD, and decreasing the oxidant load has the potential to improve the intramuscular redox state and therefore skeletal muscle function in this population.

In the current study, intravenous AO decreased plasma markers of oxidative damage, improved the antioxidant status (Fig. 2), and attenuated exercise-induced fatigue (Fig. 5) in patients with COPD. Consequently, these data reveal that KE exercise performed by patients with COPD for the same duration and at the same intensity with AO infusion is associated with less peripheral quadriceps fatigue than without AO infusion. Specifically, the rate of increase in the integrated EMG signal from the vastus lateralis, an index of peripheral fatigue development during exercise, was attenuated (Fig. 4), and the magnitude of decrease in quadriceps MVC and $Q_{tw,pot}$ were diminished by ~50% and ~20%, respectively (Fig. 5). These data contrast with the lack of effect observed previously by our group following oral antioxidant administration in this population (32). However, the plasma concentration of ascorbate achieved in the current study, and consequently antioxidant capacity as assessed by FRAP, were elevated by approximately fivefold over the values obtained in the previous investigation (32), which may have enhanced the ability of the ascorbate to enter the muscle and exert beneficial effects on the myofilaments. It is therefore reasonable to hypothesize that the altered redox state following AO administration was translated into improved muscle function, potentially due to decreased intramuscular free radical accumulation and greater fatigue resistance during KE exercise. Thus these data suggest that oxidative stress contributes to skeletal muscle dysfunction in patients with COPD, and a reduction in oxidative stress lessens the magnitude of fatigue accumulated during high-intensity, small muscle mass exercise.

Physiological responses to exercise. Feedback from skeletal muscle group III and IV afferent fibers contribute to the cardiovascular and ventilatory response to dynamic exercise (2). In the current study, ascorbate infusion led to a reduction in V_E and V_E/V_{CO_2} ratio (Fig. 4) as well as attenuated sensations of dyspnea at the same exercise time points in the PL condition. The attenuated increase in the integrated EMG signal during exercise in the AO condition suggests less peripheral fatigue development during exercise. This is largely determined by the accumulation of metabolic by-products such as hydrogen ions and inorganic phosphates (41), as well as reactive oxygen species, within the muscle (1). These exercise-induced metabolites, as well as oxidative stress, have also been documented to activate group III and IV afferent fibers (15, 28). Thus the attenuated ventilatory responses to the exercise bouts may have been the result of improved muscle function following antioxidant administration due to an improved intramuscular redox state. This reduced metabolic perturbation during exercise would, in turn, diminish the requisite increase in V_{O_2} and V_{CO_2} during exercise in the AO condition (Fig. 4).

Alternatively, oxidative stress has been documented to directly stimulate group IV afferent fibers (15), and blocking afferent feedback with spinal anesthesia attenuated the ventilatory response to exercise in patients with COPD (17). Therefore, reducing oxidant-driven afferent activity with the AO infusion may have contributed to the reduced ventilatory response in the AO condition in the current study. Collectively, these data reveal that reducing oxidative stress by intravenous AO administration was associated with an overall attenuation in the ventilatory and metabolic responses to exercise. These, likely positive, changes may be attributed to reduced stimulation of lower limb afferent fibers potentially due to improved muscle function during exercise and decreased metabolite accumulation, or less direct stimulation of afferent fibers by oxidative stress.

Blood pressure and vascular conductance. Research regarding the hypotensive effect of antioxidant administration is equivocal. However, in a small, tightly controlled experiment, our group has previously observed a tendency for acute oral antioxidant supplementation to reduce arterial blood pressure in normotensive, older individuals (44), whereas chronic antioxidant treatment has been associated with reduced blood pressure in young healthy males (33). Under basal conditions in the current study, as is not unusual in this population (35), the patients with COPD had an average "high-normal" MAP of ~104 mmHg, and this tendency to exhibit elevated blood pressure is associated with increased cardiovascular disease risk (40). Intriguingly, intravenous AO administration resulted in an ~10- to 15-mmHg reduction in MAP, such that the average resting blood pressure for the patients with COPD in the AO condition returned to a healthy, normal value (Fig. 3). Because COPD is an independent predictor of cardiovascular disease mortality, and cardiovascular disease is a leading cause of hospitalizations in patients with mild to moderate COPD (35), ameliorating cardiovascular disease risk factors is of utmost importance. Thus these data suggest that some form of

antioxidant treatment may be important for cardiovascular disease risk management in patients with COPD.

Despite reduced arterial blood pressure with the AO, femoral artery blood flow at rest was unchanged (Fig. 3). Thus when femoral artery blood flow was normalized for the decrease in perfusion pressure, resting femoral vascular conductance was significantly elevated in the AO condition (Fig. 3). Interestingly, the magnitude of increase in femoral vascular conductance was similar to that demonstrated previously in healthy, older individuals following a similar intravenous AO infusion (20). AO has been documented to activate endothelial nitric oxide synthase (25), and coinfusion of AO and a nitric oxide synthase inhibitor negates the ability of AO to improve blood flow and vascular conductance (14). Thus, in the current study, intravenous infusion of AO may have improved nitric oxide bioavailability, perhaps by both reducing oxidative stress and promoting nitric oxide production by nitric oxide synthase, which resulted in a reduction in total peripheral resistance, leading to reduced MAP and improved femoral vascular conductance. The potential increase in nitric oxide may have also improved oxygen distribution in the working muscle and improved aerobic metabolism (37), increasing fatigue resistance during exercise. Collectively, these data support a favorable role of reducing oxidative stress on resting hemodynamic parameters in patients with COPD.

Perspectives and Significance

This study documents the ability of an intravenous AO infusion to improve antioxidant capacity and decrease oxidative stress in patients with COPD. These changes in redox balance were associated with a reduction in resting blood pressure and elevated femoral vascular conductance. In addition, dynamic KE exercise performed for the same duration and at the same intensity as the placebo condition, was associated with an attenuated rate of development of peripheral quadriceps fatigue, improved metabolic and ventilatory responses, and less of a reduction in quadriceps force production assessed after exercise. These data further implicate oxidative stress in the systemic, pathophysiological consequences of COPD and suggest a beneficial role for reducing oxidative stress in this population. Therefore, targeting oxidative stress with some form of antioxidant therapy in a clinical setting may represent an important therapeutic avenue for patients with COPD.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.J.R., M.A., and R.S.R. conception and design of research; M.J.R., R.S.G., H.J.G., and M.A. performed experiments; M.J.R., V.R., and J.Z. analyzed data; M.J.R., R.S.G., M.A., and R.S.R. interpreted results of experiments; M.J.R. prepared figures; M.J.R., M.A., and R.S.R. drafted manuscript; M.J.R., R.S.G., H.J.G., M.A., and R.S.R. edited and revised manuscript; M.J.R., R.S.G., H.J.G., V.R., J.Z., M.A., and R.S.R. approved final version of manuscript.

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CHAPTER 5

CONCLUSION

The overall purpose of this dissertation was to elucidate the impact of age- and disease-induced oxidative stress on physiological function. Specifically, the consequences of oxidative stress for oxygen transport and utilization, as well as peripheral hemodynamics, was evaluated in young and old individuals. The influence of oxidative stress on these parameters, as well as skeletal muscle fatigue development, was also examined in patients with COPD. Collectively, this research sought to identify novel, mechanistic strategies to improve physiological function, and thereby favorably affect physical capacity, with the overarching goal of ameliorating some of the adverse sequelae of aging and disease.

With the goal to better understand the age-associated attenuation in LBF, The first study of this dissertation examined redox balance in the femoral artery and vein under control conditions and following administration of an AOC in old subjects characterized by attenuated LBF and young subjects, at rest and during KE exercise. By experimental design, under control conditions, LBF was ~15% lower in the old compared to the young at rest and during KE exercise. In these control conditions, the old exhibited greater leg free radical outflow than the young during KE exercise, assessed by EPR spectroscopy. Interestingly, the AOC improved LBF in the old at rest, abolishing the age-related decrement, but did not alter LBF or free radical outflow in either group during exercise. Therefore, in summary, this study documented greater free radical outflow during exercise in old subjects exhibiting attenuated LBF at rest and during exercise under control conditions. The observation that the AOC ameliorates the attenuated LBF in the old at rest, but fails to alter free-radical outflow or LBF during exercise, suggests that the formidable, pro-oxidant state elicited by exercise in the old likely necessitates a stronger

antioxidant strategy to restore LBF in this population.

The second study of this dissertation examined the impact of the AOC on oxygen transport and utilization during submaximal KE exercise in patients with COPD and healthy controls. Patients with COPD exhibited basal evidence of elevated inflammation and reduced antioxidant capacity. AOC consumption improved the abnormal redox balance in the patients, and these alterations were associated with favorable changes in the central and peripheral cardiorespiratory responses to exercise. Specifically, LBF and leg vascular conductance (LVC) during KE exercise were augmented in patients with COPD following AOC consumption, while no changes were observed in the healthy controls. The elevation in LBF, in combination with an unaltered arterial-venous oxygen difference from control conditions, led to increased oxygen consumption during exercise in the patients with COPD. In addition, arterial oxygen saturation was improved, at rest and during exercise, in patients with COPD with the AOC, whereas there was no apparent effect in the control subjects. These data demonstrate beneficial effects of antioxidant administration on exercise-induced hemodynamics and skeletal muscle metabolism in patients with COPD, and indicate that impaired oxygen transport, as a consequence of elevated oxidative stress, may represent a novel mechanistic link between oxidative stress and exercise intolerance in this population.

The third study of this dissertation evaluated the impact of intravenous ascorbate administration on oxidative stress, as well as the effects of this intervention on skeletal muscle fatigue following high-intensity KE exercise in patients with COPD. Prior to exercise, ascorbate increased antioxidant capacity and reduced oxidative stress, and these changes in the pro- and antioxidant balance were accompanied by a reduction in mean

arterial pressure and an increase in LVC. Exercise after ascorbate administration, matched for time with the placebo trial, was associated with attenuated ventilatory and metabolic responses to the work, and a slowed rate of fatigue development. Thus, the exercise bout ultimately resulted in less of a decrease in quadriceps maximal voluntary contraction and evoked twitch force assessed following exercise, revealing improved fatigue resistance during exercise. Collectively, these data demonstrate a beneficial effect of intravenous ascorbate administration on systemic oxidative stress and skeletal muscle function in patients with COPD. Moreover, these data further implicate oxidative stress as a factor contributing to skeletal muscle dysfunction in COPD.

In summary, this research has elucidated biological mechanisms by which elevated oxidative stress may impair physiological function with aging and disease. Accordingly, therapeutically targeting age- and disease-induced increases in oxidative stress, especially in relation to oxygen transport, may enhance exercise tolerance and physical activity. As low levels of physical activity are linked to elevated mortality risk and cardiovascular disease development, the conclusions garnered from this dissertation have broad implications for increasing functional capacity with aging and disease and thereby potentially enhancing quality of life and longevity.