SKELETAL MUSCLE FATIGUE IN HEALTH AND DISEASE: THE ROLE OF ACTIVE MUSCLE MASS, AFFERENT FEEDBACK AND OXIDATIVE STRESS

by

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ABSTRACT

The overall objective of this thesis was to examine skeletal muscle function and the development of peripheral quadriceps fatigue in health and in patients with chronic obstructive pulmonary disease (COPD). The aim of the first study was to further elucidate the role of afferent feedback in the regulation of locomotor muscle fatigue during dynamic exercise by varying the amount of active muscle mass. Utilizing cycling (BIKE) and single-leg knee extensor (KE) exercise, far greater quadriceps fatigue at exhaustion was observed following KE exercise. These data imply that when the source of skeletal muscle afferent feedback is confined to a small muscle mass, the central nervous system tolerates a greater magnitude of peripheral fatigue, and likely a greater intramuscular metabolic disturbance; a finding that has important implications for the adoption of small muscle mass exercise in rehabilitative medicine. The second study sought to determine the impact of an acute oral antioxidant cocktail (AOC), with previously documented efficacy, on free radical concentration and KE exercise performance in patients with COPD. In this population, recognized to have elevated oxidative stress, administration of the AOC significantly attenuated resting free radical levels, which were negatively correlated with the degree of airflow limitation and baseline MVC force. Upon secondary analysis, however, a dichotomous response to the AOC was recognized, whereby the AOC appeared to be most efficacious in those patients with high initial free radical levels, with minimal effects when the initial free radical

load was low. Despite these antioxidant effects, no differences in KE exercise performance or the magnitude of peripheral quadriceps fatigue were evident following consumption of the AOC. These findings revealed that acutely reducing free radicals with an oral AOC does not translate to improved exercise capacity and fatigue resistance in patients with COPD. Collectively, this research has provided novel insight into the role of active muscle mass and the regulation of peripheral fatigue, and has better elucidated the link between free radicals, antioxidants, and fatigue in patients with COPD.

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CHAPTER 1

INTRODUCTION

Defined as an acute impairment in performance, including an increase in the perceived effort to exert a desired force, and an eventual inability to produce this force (15), skeletal muscle fatigue directly limits exercise in both healthy and diseased populations (6, 17). Therefore, understanding the physiological and pathophysiological events governing the development of fatigue is important both scientifically and in the context of rehabilitative medicine. The processes contributing to a fatigue-induced decrease in muscle performance can be partitioned into central processes (proximal to the neuromuscular junction) and those arising at or distal to the neuromuscular junction (within the muscle cell) termed peripheral fatigue (35). As the relative contributions from primarily central or peripheral processes vary depending on, among other factors, the exercise task, length of the activity (15, 18), and environmental conditions (9, 27), the etiology of fatigue is often both complex and multifaceted.

During short duration, high-intensity dynamic exercise, peripheral processes contribute significantly to muscular fatigue. Indeed, the accumulation of metabolic byproducts from skeletal muscle contraction (such as hydrogen ions and inorganic phosphates) depress the function of the cellular constituents of excitation-contraction coupling (ECC) (33). In addition, the production of reactive oxygen species (ROS) increases during fatiguing contractions, and these free radicals are similarly detrimental to ECC events (11, 34). The degree of intramuscular perturbation therefore plays a role in dictating the magnitude of peripheral muscle fatigue. In exercising humans, this can be assessed by a pre- to postexercise decline in the force evoked by supramaximal stimulation of the motor nerve innervating the active muscle group, reflective of changes that occurred within the muscle itself during exercise (3).

Interestingly, work by Amann et al. (5), experimentally altering the rate of development of peripheral fatigue by varying arterial oxygenation, has documented that despite improvements in fixed workload exercise time with increased arterial oxygenation (or reductions in performance time with arterial hypoxemia), the magnitude of peripheral quadriceps fatigue following dynamic, large muscle mass exercise was the same at task failure (~34% reduction in potentiated twitch force - $\Delta Q_{pot,tw}$). In addition, following pre-fatiguing cycling bouts to induce a set level of quadriceps fatigue, central motor drive was modulated in subsequent 5 km cycle time trials, in a dose-dependent manner, such that participants again did not accumulate peripheral fatigue beyond the critical level (the ~34% $\Delta Q_{pot,tw}$) (4). Accordingly, the authors postulated that central motor drive to the muscle is regulated by afferent feedback from the working skeletal muscle such that exercise cessation occurs at a sensory tolerance limit and a critical level of ensemble afferent input to the CNS to facilitate homeostasis (2).

The interplay between the magnitude of peripheral locomotor muscle fatigue and afferent feedback from skeletal muscle has been demonstrated by experimentally blocking group III and IV thin-fiber afferents during dynamic exercise (6, 7). These skeletal muscle afferents, sensitive to mechanical deformation and the metabolic milieu of the muscle, are active during dynamic exercise (1) and contribute to the fine-tuning of the cardiovascular response (10). In regard to peripheral fatigue, blocking this source of afferent input to the CNS resulted in drastically elevated central motor drive to the muscle such that at the end of a 5 km cycle time trial the ~34% $\Delta Q_{pot,tw}$ was surpassed (~45% $\Delta Q_{pot,tw}$) and serious atypical ambulatory difficulties were observed at task failure (7). These studies highlight the role of group III/IV afferents in the regulation of

peripheral fatigue; however, the link between the volume of exercise-induced ensemble afferent feedback and the degree of peripheral muscle fatigue during dynamic exercise has not been elucidated.

Utilizing the postexercise circulatory occlusion technique to selectively stimulate metabolically sensitive afferents, Freund et al. (16) varied the mass of occluded muscle following dynamic cycle exercise. This study documented greater post-exercise mean arterial pressure (MAP) when two legs were occluded compared to just one, suggesting augmented afferent input to the CNS in proportion to the occluded muscle mass. As the sensory tolerance limit associated with exercise cessation appears to be substantially determined by the ensemble afferent signal from the working skeletal muscle (2), reducing the mass of active muscle mass could potentially lessen the signal during exercise. Thus, the sensory tolerance limit at task failure with dynamic, small muscle mass exercise would eventually be reached by a focused, but very strong, local afferent signal as opposed to the more diffuse signal (but of equal ensemble magnitude) achieved during whole body exercise. It is likely that the increased metabolic disturbance affecting less group III/IV afferents will take longer to reach the sensory tolerance limit, translating into a greater degree of contractile dysfunction at task failure. Accordingly, the first objective of this thesis was to determine the role of active muscle mass during dynamic exercise in the development of peripheral fatigue.

Although the compromised pulmonary function intrinsic to lung disease certainly contributes to limited exercise capacity in chronic obstructive pulmonary disease (COPD) (8, 28), peripheral muscle abnormalities have also been implicated (23, 30). Specifically, structural abnormalities of skeletal muscle in COPD patients, such as a predominantly

type II skeletal muscle fiber phenotype (30), may hasten the production of metabolic byproducts intrinsic to the development of peripheral muscle fatigue (33). Indeed, significant peripheral locomotor muscle fatigue occurs in patients with COPD following cycle exercise (8, 24) and the perception of leg fatigue is frequently recognized as the primary symptom contributing to exercise intolerance in this population (20). In addition, the inflammatory pathology of lung disease increases the susceptibility of this group to oxidative stress (defined as an imbalance between pro- and antioxidant forces in favor of the former) (12, 13, 29, 32). Currently, the practical application of reducing the level of oxidative stress, and the impact on exercise tolerance, in patients with COPD has not been elucidated.

Although free radicals directly impact the contractile apparatus (11, 34), their production has also been linked to an altered afferent signal. Specifically, ROS have been implicated in the exaggerated exercise pressor response typically observed in some disease states such as heart failure (21), and documented to increase the spontaneous firing of group IV afferents in resting and exercising mouse skeletal muscle (14). As the magnitude of afferent feedback plays an important role in determining exercise performance (2), oxidative stress may pathologically alter this signal. In COPD, antioxidant treatment has been documented to reduce markers of exercise-induced oxidant damage (19, 22), and the potent pharmacological antioxidant n-acetylcysteine (NAC) appears to improve exercise capacity (22). NAC, however, has also been documented to improve cycling time to exhaustion in healthy, young subjects (25, 26), and improve pulmonary function in patients with COPD (31), confounding interpretation of antioxidant administration on COPD patient skeletal muscle function. Thus, the second

aim of this thesis will be to examine the effect of a readily available, oral antioxidant cocktail with documented efficacy (29, 36, 37) on free radical concentration and the development of peripheral muscle fatigue in a population with a heightened susceptibility to oxidative stress and limited exercise capacity, patients with COPD.

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CHAPTER 2

MUSCLE MASS AND PERIPHERAL FATIGUE: A POTENTIAL ROLE FOR AFFERENT FEEDBACK?

Abstract

The voluntary termination of exercise has been hypothesized to occur at a sensory tolerance limit that is significantly determined by feedback from group III and IV muscle afferents, and is associated with a specific level of peripheral quadriceps fatigue during whole body cycling. Therefore, the purpose of this study was to reduce the amount of muscle mass engaged during dynamic leg exercise to constrain the source of muscle afferent feedback to the central nervous system (CNS), and examine the effect on peripheral quadriceps fatigue. Eight young males performed exhaustive large (cycling – BIKE) and small (knee extensor – KE) muscle mass dynamic exercise at 85% of the modality-specific maximal workload. Pre- vs. postexercise maximal voluntary contractions (MVC) and supramaximal magnetic femoral nerve stimulation ($Q_{tw,pot}$) were used to quantify peripheral quadriceps fatigue. Significant quadriceps fatigue was evident following both exercise trials; however, the exercise-induced changes in MVC (-28 ± 1% vs. -16 ± 2%) and $Q_{tw,pot}$ (-53 ± 2% vs. -34 ± 2%) were far greater following KE compared to BIKE exercise, respectively. The greater degree of quadriceps fatigue following KE exercise was in proportion to the greater exercise time (9.1 ± 0.4 vs. 6.3 ± 0.5 minutes, $p < 0.05$), suggestive of a similar rate of peripheral fatigue development. These data suggest that when the source of skeletal muscle afferent feedback is confined to a small muscle mass, the CNS tolerates a greater magnitude of peripheral fatigue, and likely a greater intramuscular metabolic disturbance. An important implication of this finding is that the adoption of small muscle mass exercise may facilitate greater exercise-induced muscular adaptation.

Introduction

During high-intensity cycle exercise (BIKE), a centrally mediated sensory tolerance limit (25) associated with a set level of peripheral locomotor muscle fatigue has been documented (5, 9, 10, 13). This level of peripheral fatigue appears to be consistent across exercise bouts in which variations in arterial oxygenation alter the rate of development of peripheral fatigue and exercise time (13), but at the point of task failure, end-exercise locomotor muscle fatigue appears to be quite similar (10, 13, 14). In line with this observation, utilizing plantar flexion (28) or single leg knee-extension (52), which recruits a larger muscle mass, exercise and nuclear magnetic resonance spectroscopy, the metabolic disturbance at exhaustion has been documented to be invariant despite variations in arterial oxygenation, or work rate, altering exercise time to exhaustion. Therefore, it seems that it is possible to alter the rate of peripheral fatigue development and endurance time, but at exhaustion following short duration, high intensity exercise a similar intramuscular metabolic disturbance and level of peripheral locomotor muscle fatigue is achieved (9, 10, 13, 28, 52).

Group III and IV muscle afferents are active during dynamic exercise (2, 6, 15) and provide input to the central nervous system (CNS) regarding mechanical deformation and the metabolic milieu within the working skeletal muscle (39, 48). Pharmacological blockade of these thin fiber afferents from the lower limbs during high intensity cycling exercise has highlighted the role of afferent feedback in limiting the development of peripheral fatigue (7, 12). Indeed, in the absence of group III/IV muscle afferents, subjects accumulated a significantly greater amount of peripheral fatigue such that atypical ambulatory difficulties were observed at exercise cessation (7, 12). The authors

of this work postulated that group III/IV afferent feedback from the working muscles modifies central motor drive to the locomotor muscle to ensure that the overall homeostasis of the organism is not threatened (5). Thus, the voluntary termination of exercise during high-intensity constant load endurance exercise occurs once a sensory tolerance limit (25) is reached that is substantially dependent, amongst other factors, on the ensemble afferent input from the active locomotor muscles (5, 9, 10, 12, 13). The corresponding level of peripheral fatigue presumably depends on the exercise task, and may vary with the amount of muscle mass recruited.

A relationship between the magnitude of afferent feedback and muscle mass has long been established (22, 29). Freund et al. (22) demonstrated that post-bicycle exercise occlusion of blood flow to both legs maintained mean arterial pressure at a higher level than occlusion of one leg alone. In addition, time to task failure has been documented to be shorter, with less end-exercise quadriceps fatigue, for bilateral compared with unilateral sustained maximal voluntary contractions (MVC) of the knee extensor muscle group (37). In combination, these observations reveal that increasing active muscle mass augments the ensemble feedback to the CNS from the periphery and these changes in ensemble feedback may alter the level of end exercise fatigue and endurance time. However, the link between exercising muscle mass, the associated changes in afferent feedback and the degree of peripheral muscle fatigue following dynamic exercise has not been elucidated.

Therefore, the purpose of this study was to reduce the amount of active muscle mass during dynamic exercise to confine group III/IV afferent feedback to one muscle group. Thus, the sensory tolerance limit associated with task failure and a critical amount

of ensemble afferent input, would eventually be reached by a strong local afferent signal from the isolated muscle group. This would contrast with the sum of the more diffuse weaker signals, with an equal ensemble magnitude, associated with whole body exercise (Figure 1). Specifically, we tested the hypothesis that exhaustive high intensity constant-load dynamic knee extensor (KE) exercise, utilizing ~2.5 kg of muscle (17, 40), would result in a greater degree of end-exercise quadriceps fatigue compared to the equivalent challenge utilizing a far larger muscle mass (BIKE, ~15 kg of muscle (40)).

Methods

Subjects

Eight young, healthy males (24 ± 1 years, 83 ± 6 kg, 178 ± 4 cm) volunteered to participate in this study. Written, informed consent was obtained from participants prior to their inclusion and all protocols were approved by the Institutional Review Boards of the University of Utah and the Salt Lake City VA Medical Center. All testing was performed in a thermoneutral environment (22°C).

Protocol

Prior to data collection, subjects were familiarized with BIKE and KE exercise as well as the neuromuscular function assessment procedures during preliminary visits to the laboratory. On subsequent visits, separated by at least 48 hours, time to exhaustion (T_{lim}) during constant load exercise trials at 85% of maximal workload, for each exercise modality were used to induce quadriceps fatigue. This workload (85%) was selected because, during pilot testing, this intensity of effort elicited task failure in all subjects in ~5 to ~12 minutes in both exercise modalities. Throughout each trial, ventilation, gas

exchange, heart rate (HR), and rating of perceived exertion (RPE) were assessed. Prior to each exercise bout, 2 minutes of resting data were collected and subjects were allowed a 3-minute warm-up period (unloaded KE exercise and BIKE exercise at ~85 W). To quantify peripheral fatigue before exercise and 2 minutes following task failure, neuromuscular function tests were performed on the same leg used for both modalities. Task failure was defined as a drop of 10 rpm for both the KE and BIKE. The experimental limb was randomized and balanced between dominant and non-dominant legs (42).

Bike (BIKE) and Knee-extensor (KE) Exercise

Dynamic, small muscle mass exercise was performed on a cycle erogmeter (Monark, Sweden) modified to allow KE exercise (44). 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. Subjects were instructed to maintain a rate of 60 contractions per minute during KE exercise. For BIKE exercise, a cycle ergometer was employed (Excalibur, Lode, The Netherlands) and a constant pedaling rate was self-selected by all subjects (~75 rpm). For both exercise modalities, subjects performed one-minute stage, incremental exercise tests to exhaustion to determine peak workload (10 W + 5 W min^{-1} for KE and 20 W + 25 W min^{-1} for BIKE (16)).

Ventilation, Gas Exchange and Heart Rate

Ventilation and pulmonary gas exchange were measured at rest and during exercise with a metabolic cart (ParvoMedics, Sandy, UT). HR was determined from the

R-R interval of a three-lead electrocardiogram (ECG) acquired at 200 Hz using a data acquisition system (AcqKnowledge; Biopac Systems, Goleta, CA). RPE was taken every minute during the T_{Lim} trials using Borg's modified CR10 scale (19).

Neuromuscular Function

The magnitude of peripheral quadriceps fatigue was quantified by supramaximal magnetic stimulation of the femoral nerve (9, 31, 43): the exercise induced reduction in potentiated quadriceps twitch force ($Q_{tw,pot}$) assessed before exercise and again 2 minutes after both T_{lim} trials. This time delay was necessary to transfer the subjects from either exercise ergometer (BIKE or KE) to the neuromuscular function assessment apparatus, and was thus standardized for both exercise modalities. For the neuromuscular function test procedure, while subjects were semi-recumbent in a separate KE chair, with a knee joint angle of 90 degrees, a magnetic stimulator (Magstim 200, The Magstim Company Ltd, Wales) connected to a double 70 mm coil was used to stimulate the femoral nerve. The evoked twitch force was obtained from a calibrated load cell (Transducer Techniques, Temecula, CA) connected to a non-compliant strap placed around the subject's ankle. To record magnetically evoked compound action potentials (M-waves) and evaluate changes in membrane excitability, quadriceps EMG was recorded from the vastus lateralis (VL) muscle (9). Electrodes were placed in a bipolar configuration over the middle of the muscle belly, with the active electrodes placed over the motor point of the muscle and reference electrode placed in an electrically neutral site. During a separate visit, supramaximality of stimulation was determined by serial, single unpotentiated twitch (Q_{tw}) forces obtained every 30 seconds at 50, 60, 70, 80, 85, 90, 95, and 100% of maximal stimulator output.

As potentiated, compared to unpotentiated, twitch force has been documented to be a more sensitive indicator of fatigue (31), $Q_{tw,pot}$ was assessed following a 5 second MVC of the quadriceps muscle. A series of 6 MVCs and $Q_{tw,pot}$ maneuvers were performed with 30 seconds between each MVC, such that the entire procedure lasted 2.5 minutes. In addition, to quantify activation of the quadriceps during the MVCs, a superimposed twitch technique was employed (9, 38). Briefly, 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 (%VMA). Peak force, maximal rate of force development (MRFD), and maximal relaxation rate (MRR) were analyzed for all $Q_{tw,pot}$ values (32).

Statistical Analyses

Two-way repeated measures analysis of variance was used to compare the effect of exercise modality by time on the physiological parameters during exercise, with the Tukey's honestly significant difference test used for posthoc analysis if a significant main or interaction effect was found. Student's paired *t* tests were used to compare the effect of exercise modality on end-exercise physiological parameters and the magnitude of peripheral fatigue. Statistical significance was set at $\alpha = 0.05$. Results are expressed as means ± S.E.M.

Results

Exercise Responses

The maximal workload for the incremental exercise test was 280 ± 9 W for BIKE and 60 ± 4 W for KE exercise. Peak oxygen uptake was significantly higher for BIKE compared to KE exercise (3.2 ± 0.1 vs 1.6 ± 0.2 L/min). T_{lim} trial data are documented in Table 1. Cardiovascular and respiratory responses to the BIKE and KE T_{Lim} differed, with ventilation (V_E), oxygen consumption (VO_2), carbon dioxide production (VCO_2), and HR all being higher during BIKE exercise ($p < 0.05$). T_{lim} time was longer, by 31 ± 5% (range 46 seconds to 283 seconds), for KE compared to BIKE exercise ($p < 0.05$). RPE was lower for KE exercise at minutes 4 and 5 ($p < 0.05$), but was not different at exhaustion (Table 1).

Neuromuscular Function

A plateau in Q_{tw} and M-wave amplitudes with increasing stimulus intensity documented maximal depolarization of the femoral nerve in all subjects. Membrane excitability was maintained from pre- to post-exercise in all trials, as indicated by unchanged M-wave characteristics, indicating that the observed changes in $Q_{tw,pot}$ were predominantly due to changes within the quadriceps. $Q_{tw,pot}$ measured after exercise was significantly reduced from pre-exercise values for both exercise modalities, with a fall of 52 ± 2% for KE and 34 ± 2% for BIKE exercise, with no difference in the %VMA from pre- to post-exercise or between modalities. The fall in $Q_{tw,pot}$ was significantly greater for KE compared to BIKE exercise, by 36 ± 4%, and in proportion to the greater exercise time. Other indices of fatigue (MVC, MRFD and MRR) were significantly reduced from

preexercise values, and also revealed greater peripheral fatigue following KE exercise (Figure 2).

Discussion

The magnitude of group III/IV mediated afferent feedback from the active limbs substantially influences the voluntary termination of exercise, which has been suggested to occur once a sensory tolerance limit is reached (5, 25). By reducing the amount of active muscle mass during dynamic exercise, we sought to confine the source of afferent feedback to one muscle group in contrast to the ensemble feedback from many muscles during whole body exercise. Thus, a greater local metabolic disturbance at task failure with small muscle mass exercise would be required to elicit an afferent signal of equal magnitude to that achieved during whole body exercise (Figure 1). As the degree of metabolic disturbance significantly influences the magnitude of peripheral fatigue assessed immediately after exercise, it was hypothesized that small muscle mass exercise to exhaustion would result in a greater amount of peripheral fatigue than large muscle mass exercise. This was, indeed, the case, with KE exercise inducing significantly greater peripheral fatigue than BIKE exercise. These findings suggest that the CNS tolerates a greater degree of peripheral fatigue when the amount of active muscle mass is reduced. As exercise-induced peripheral adaptation responds to the degree of local perturbation, this study has implications for optimizing exercise training-induced muscle adaptations.

Peripheral Fatigue and Large Muscle Mass (BIKE) Exercise

Previous work quantifying exercise-induced locomotor muscle fatigue following constant load, large muscle mass cycle exercise has identified a consistent level of

peripheral fatigue in a variety of populations (5). In a group of young, endurance trained individuals exercising at a high-intensity constant workload, Amann et al. (10) identified a ~34% decline in $Q_{tw,pot}$ from preexercise at exhaustion; altering arterial oxygenation affected endurance time, but not the observed threshold level of peripheral fatigue (10). In older, sedentary individuals, work by Mador et al. (33) documented a similar ~36% fall in $Q_{tw,pot}$ following exhaustive constant-load cycling. Interestingly, even moderate to severe chronic obstructive pulmonary disease patients have been documented to exhibit a ~35% decline in potentiated twitch force following cycle exercise (34, 49).

In the current study, BIKE exercise to exhaustion elicited quadriceps fatigue in all subjects, as exemplified by a consistent pre- to postexercise decline in MVC force (16%) as well as a 34% decline in $Q_{tw,pot}$ (Figure 3). Our intra-twitch indices, MRFD and MRR, which reflect reductions in the rate of calcium reuptake by the sarcoplasmic reticulum as well as cross-bridge dissociation (50), were similarly attenuated (~30% and ~33%, respectively). Of note, the mean decline in $Q_{tw,pot}$ was in agreement with values observed in the aforementioned studies. These similarities suggest that although factors such as oxygen availability, fitness level, age, and disease state influence the rate of development of peripheral fatigue, an apparently similar level of peripheral fatigue coincides with task failure during large muscle mass exercise.

Peripheral Fatigue and Small Muscle Mass (KE) Exercise

The knee extensor ergometer model provides a paradigm in which dynamic exercise can be limited to the quadriceps muscles (44). During KE exercise, Vanhatalo et al. (52) has documented a similar increase in inorganic phosphates and ADP, and fall in phosphocreatine and intramuscular pH assessed by nuclear magnetic resonance

spectroscopy, at task failure across work rates in both normoxia and hyperoxia. In addition, Fulco et al. (23) documented an equivalent fall in MVC force following exhaustive KE exercise in both hypobaria and normoxia. Utilizing evoked, potentiated twitch forces, Burnley et al. (20) documented a ~52% decline in $Q_{tw,pot}$ with electrical femoral nerve stimulation following exhaustive intermittent isometric contractions of the quadriceps, and Polkey et al. (43) found an equivalent reduction (~55%), following a similar exercise bout, with magnetic stimulation of the femoral nerve.

In the current study, the quantification of exercise-induced quadriceps fatigue revealed a 53% reduction in $Q_{tw,pot}$ and ~28% fall in MVC force following KE exercise, whereas MRFD and MRR were reduced by ~53 and 46%, respectively (Figure 3). Interestingly, the magnitude of the exercise-induced decrease in the evoked twitch force in this study (~53%) was very similar to the values observed in previous work (~52% and 55%) utilizing the $Q_{tw,pot}$ maneuver following quadriceps exercise to task failure (20, 43). Taken together, these studies, and our results, are suggestive of a similar level of end exercise peripheral quadriceps fatigue following small muscle mass exercise to task failure. Interestingly, the magnitude of peripheral fatigue when the exercise is confined to a small muscle mass may be greater then that obtained at exhaustion with whole body exercise.

Muscle Mass, Afferent Feedback and Fatigue

This study sought to compare the magnitude of peripheral fatigue following the voluntary termination of KE and BIKE exercise. Despite the matching of exercise intensity (85% of peak workload), KE exercise time was longer, by ~31%, and the magnitude of peripheral fatigue assessed following task failure was ~36% greater in

comparison to BIKE exercise. The proportionality between the increased time to fatigue and magnitude of fatigue, although speculative, suggests a similar rate of peripheral fatigue development, with the ability to achieve a greater degree of end-exercise peripheral fatigue potentially contributing to the greater exercise time. These results imply that at any given time point, achieved in both BIKE and KE trials, the magnitude of peripheral quadriceps fatigue was equivalent for both exercise modalities. During KE exercise with a reduced active muscle mass limiting the source of muscle afferent signal to the quadriceps, a similar magnitude of peripheral fatigue was likely associated with decreased ensemble input to the CNS. Indeed, during BIKE exercise, peripheral fatigue in both legs substantially augmented afferent feedback. Thus, subjects continued to drive the quadriceps muscle and delve deeper into its functional capacity during KE exercise, eventually reaching a greater level of quadriceps fatigue and presumably end-exercise metabolic disturbance, ultimately eliciting a muscle afferent signal of equal ensemble magnitude to that obtained with whole body BIKE exercise at task failure.

A greater magnitude of peripheral fatigue was evident in all measured indices following KE exercise (Figure 2). With this reduction in active muscle mass, subjects surpassed the ~34% decline in $Q_{tw,pot}$ observed following BIKE exercise, and continued until quadriceps contractile function was reduced by ~53%. This difference was also reflected in all other indicators of peripheral fatigue with an approximate two-fold greater decrease in MVC force, despite equal %VMA and therefore similar levels of central fatigue, and attenuated intra-twitch indices (Figure 2) following KE exercise. It is likely that when the source of afferent feedback to the CNS is constrained to the ~2.5 kg of muscle engaged during KE exercise, in contrast to the sum of multiple, more diffuse,

inputs from the ~15 kg of muscle utilized during BIKE exercise, the CNS tolerates a greater local accumulation of metabolic byproducts in the quadriceps, which impact peripheral fatigue (3, 4, 54). Thus, the sensory tolerance limit associated with exercise cessation and significantly influenced by, amongst other factors, the magnitude of ensemble afferent feedback, was likely reached with a greater degree of local skeletal muscle homeostatic disturbance during small muscle mass exercise (Figure 1).

Active Muscle Mass and the Potential for Adaptation

The utility of reducing active muscle mass during endurance training bouts to enhance peripheral muscle adaptation has been previously documented (1, 35, 46). Magnussen et al. (35) trained one set of quadriceps at a time with dynamic KE exercise and elicited large peripheral skeletal muscle adaptations (increased oxidative enzyme activity and capillarity) in chronic heart failure patients. Richardson et al. (46) documented a ~35% increase in quadriceps VO_2peak in young, healthy subjects following an 8 week KE training program, which outstrips the typical 6-20% gains following whole body training. In addition, Abbiss et al. (1) documented increased cytochrome *c* oxidase subunits II and IV following single leg, compared with double leg, cycle training. As exercise training induces an adaptive response to a homeostatic disturbance, a greater degree of peripheral fatigue during small muscle mass exercise may provide a greater impetus for skeletal muscle adaptation. Thus, when the active muscle mass is kept small during dynamic exercise, an enhanced adaptation in response to a greater homeostatic disturbance may therefore be possible. In essence, the adoption of such a small muscle mass approach to exercise training could mean that the sum of the

parts will be greater than the whole, which has significant implications for exercise training in both healthy and diseased populations.

Experimental Considerations

It is important to acknowledge that dissimilarities in the cardiovascular and respiratory responses to KE and BIKE exercise were evident, which could raise concerns about the role of oxygen supply and utilization (Table 1). However, experimental manipulations of oxygen availability achieved by altering arterial oxygen content (8) during exercise performance tests have been proposed to act via oxygen delivery to the working muscle and not the end-exercise level of peripheral fatigue (8). In addition, although fatiguing respiratory muscle work may increase afferent activity (27), the alleviation of diaphragm fatigue with proportional assist ventilation does not alter the end-exercise level of peripheral fatigue during BIKE exercise (11). Therefore, in the current study, as in previous work (5, 24), peripheral fatigue likely played a significant, autonomous role in curtailing exercise performance.

Additionally, although our conceptual schematic (Figure 1) paints a uniform picture of increased afferent feedback with increasing muscle recruitment, we acknowledge that the characteristics of group III/IV afferents are more complicated. Certainly, some pathophysiological conditions are associated with alterations in group III/IV afferent sensitivity or their elicited reflexes (41). In addition, the role of these fibers can vary across skeletal muscle fiber type and muscle group (29, 36), and thus the picture is not actually so simple. Indeed, the increase in afferent feedback achieved by the increase in active muscle mass in our study from KE to BIKE was likely not directly proportional to the change in muscle mass. However, BIKE exercise included the

addition of the contralateral quadriceps performing the same action (knee extension from ~90 to ~180 degrees), with added afferent feedback from other active muscles, which, although of unknown magnitude, would probably have greatly exaggerated the difference in ensemble afferent feedback.

Alternative explanations for the differences in peripheral fatigue following BIKE and KE exercise are also possible. Task specificity is an important facet in the etiology of fatigue (18) and although BIKE and KE exercise are similar, differences in neural activation strategies could have affected our results. Of importance, the relative contributions of peripheral and central fatigue could have varied between the two modalities. Although we attempted to measure central fatigue by the superimposed twitch technique and %VMA, and even though we did not observe any differences from pre- to post-exercise, or between BIKE and KE exercise, the time delay (2 minutes) between task failure and our fatigue measurements was probably too great to validly detect central fatigue. In addition, this time delay potentially could have led to an underestimation of the degree of peripheral fatigue at the moment of task failure, due to the extremely fast kinetics of phosphocreatine recovery. However, this time delay was logistically unavoidable to standardize the fatigue assessment protocol for both KE and BIKE exercise, so these effects, if present, were presumably constant between both trials.

The ability of the magnetic stimulation technique to achieve supramaximility of stimulation following exercise is another potential concern. Specifically, varying degrees of activity-dependent hyperpolarization (51) of the quadriceps motor neurons following exercise may have led to a change in motor unit recruitment elicited by the supramaximal stimulation intensity prior to exercise. However, a recent comparison by Verges et al.

(53) of the magnetic stimulation technique with electrical stimulation revealed no difference between techniques in the magnitude of measured fatigue following exercise. Thus, these data contend that the twitch data does, in fact, represent changes within the quadriceps muscle, but a change in axon excitability, and thus axon recruitment following exercise can not be completely ruled out.

Finally, the conclusions drawn from the current study are largely based on the assumption that afferent feedback was different between exercise modalities and in proportion to the metabolic disturbance in the quadriceps, neither of which were directly measured. Indeed, this study would have benefited greatly from a valid and feasible technique for the measurement of group III/IV afferent activity during dynamic exercise in humans, but currently this does not exist. Our group has previously utilized a partial afferent block to demonstrate the obligatory role of these afferents in the cardiovascular and respiratory response to both BIKE (6) and KE (15) exercise, but in the current study, it was not deemed that this approach would actually have better elucidated the role of afferent feedback. In regard to differences in the end exercise metabolic disturbance, we have previously documented intramuscular pH values following KE exercise of ~6.5 (45), a value considerably lower than that typically reported following maximal BIKE exercise (30). Therefore, in the context of previous research, and not without limitations, it can be contended that afferent feedback was varied, which enabled subjects to accumulate a greater degree of peripheral fatigue during small muscle mass exercise.

Conclusion

Confining group III/IV afferent feedback to a small muscle mass during dynamic KE exercise, in contrast to the multiple sources during whole body cycling, resulted in a

greater degree of peripheral fatigue following constant load exercise to exhaustion. This finding further highlights the role of afferent feedback in limiting the development of peripheral fatigue. Additionally, this study reveals that much greater local skeletal muscle fatigue can be achieved by utilizing small muscle mass exercise and thus may promote enhanced exercise-induced adaptation, with implications for the application of exercise training in both health and disease.

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Disclosures

There are no conflicts of interest to report.

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Table 1. Physiological responses to constant workload trials at exhaustion during large (BIKE) and small (KE) muscle mass leg exercise.

	BIKE (85% of peak workload)	KE (85% of peak workload)
Time to Exhaustion (s)	378 ± 30	547 ± 22.7*
Workload (W)	238 ± 8	52 ± 4*
Oxygen Consumption (L/min)	3.1 ± 0.1	1.8 ± 0.2*
Carbon Dioxide Production (L/min)	3.4 ± 0.2	2 ± 0.2*
Ventilation (L/min)	116 ± 9	84 ± 6*
VE/VCO_2	34.9 ± 1.9	42.6 ± 0.9*
Heart Rate (bpm)	166 ± 4	130 ± 5*
RPE	10 ± 0	10 ± 0

Values expressed as mean ± S.E.M. VE/VCO_2, ventilation relative to carbon dioxide production. RPE, rating of perceived exertion. *Significant difference between KE and BIKE exercise.

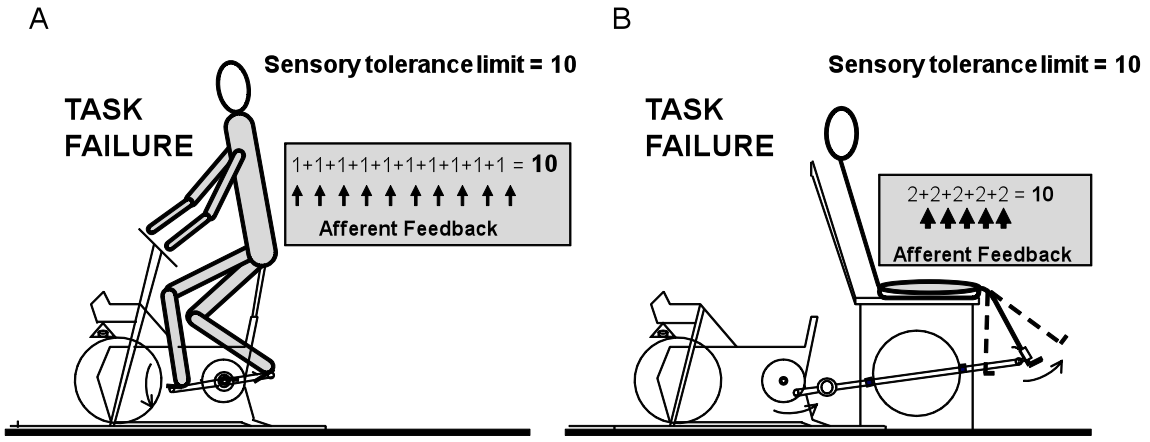

Figure 1. Conceptual schematic illustrating the equal magnitude of ensemble group III/IV skeletal muscle afferent feedback at task failure in both large (cycling - BIKE) and small (knee extensor - KE) muscle mass exercise**.** Group III/IV afferent feedback from active skeletal muscle is represented by the grey area. Accordingly, the sensory tolerance limit (10) influenced by the magnitude of ensemble afferent feedback and obtained by the sum of many diffuse signals during BIKE exercise (thin arrows; 1X10 = 10) (A) is reached with a focused, but very strong, local signal elicited by a greater intramuscular metabolic disturbance in the quadriceps (thick arrows; 5X2 = 10) at task failure during knee extensor exercise (B).

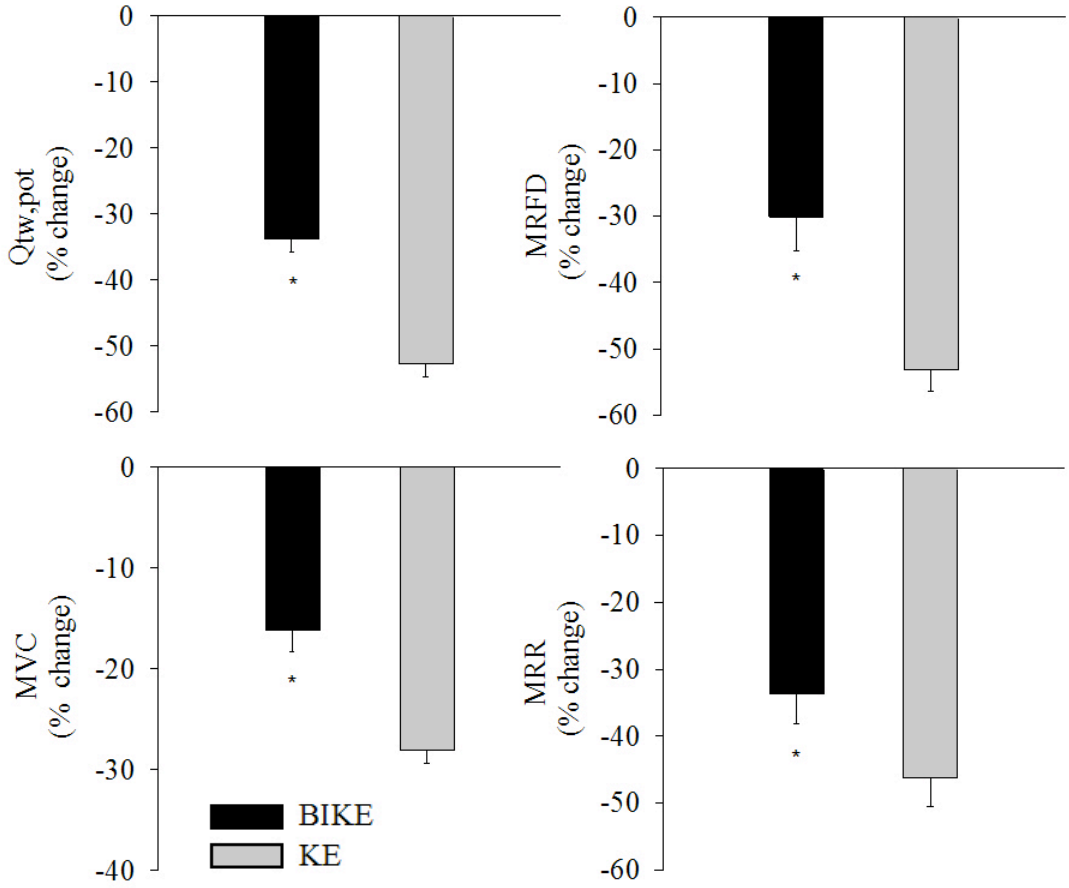

Figure 2. Change in quadriceps muscle function following constant load large (cycle - BIKE) and small (knee extensor - KE) exercise to exhaustion. Data are represented as mean ± S.E.M. and values represent the percent change from pre- to post-exercise. MVC, maximal voluntary contraction; $Q_{tw,pot}$, potentiated twitch force; MRFD, maximal rate of force development; and, MRR, maximal rate of relaxation. *Significant difference between KE and BIKE exercise.

CHAPTER 3

OXIDATIVE STRESS AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE: THE IMPACT OF ORAL ANTIOXIDANTS ON SKELETAL MUSCLE FATIGUE

Abstract

Oxidative stress may contribute to reduced exercise tolerance in patients with chronic obstructive pulmonary disease (COPD). This study sought to determine the effect of an oral antioxidant cocktail (AOC: vitamins C, E, and alpha-lipoic acid), with documented efficacy, on skeletal muscle function in COPD patients during dynamic quadriceps exercise. Ten patients with COPD ($FEV_1/FVC < 0.7$, $FEV_1 \leq 80\%$ predicted) performed knee extensor (KE) exercise to exhaustion and trials matched for time (isotime) following consumption of either the AOC or placebo (PL). Maximal voluntary contractions (MVCs) and supramaximal magnetic femoral nerve stimulation ($Q_{tw,pot}$) quantified the degree of peripheral quadriceps fatigue. Despite increasing plasma ascorbic acid levels (10.1 ± 2.2 to 24.1 ± 3.8 ug/ml, $p < 0.05$) and reducing the electron paramagnetic resonance (EPR) spectroscopy signal (AUC: 11.6 ± 3.7 to 4.8 ± 2.2 AU, $p < 0.05$) prior to exercise, AOC consumption did not alter endurance time or the magnitude of quadriceps fatigue. Closer examination of the EPR spectroscopy data revealed a tendency for the AOC to be most efficacious in patients with high resting free radical levels (n = 5, AUC: 19.7 ± 5.8 to 5.8 ± 4.5 AU, $p < 0.05$) compared to those with lower values (n = 4, AUC: 1.6 ± 0.5 to 3.4 ± 1.1 AU). Interestingly, this baseline index of free radicals was inversely correlated with FEV_1 ($r = -0.54$, $p = 0.08$) and baseline MVC force ($r = -0.56$, $p = 0.09$). Together, although it appears that the heterogeneity of free radical load in patients with COPD might have played a role, these findings reveal that acutely reducing free radicals with an oral AOC does not translate to improved exercise capacity and fatigue resistance in this population.

Introduction

Oxidative stress, defined as an imbalance between pro- and antioxidant forces in favor of the former (45), is prevalent in patients with chronic obstructive pulmonary disease (COPD) (15, 17). Although numerous factors contribute to the elevated oxidant burden documented in these patients, the most accepted source of free radicals is the inflammatory pathology of the lung disease itself (50). However, following exhaustive knee extensor (KE) exercise, which minimally taxes the lungs (47), elevations in markers of oxidative damage, such as plasma lipid peroxidation products (LPP) and stimulated phagocyte superoxide (O_2^-) production, have been recognized to increase in patients with COPD, but not healthy controls (15). Additionally, inhibiting the O_2^- generator xanthine oxidase in these patients ameliorated the exercise induced increase in LPPs and prevented an increase in the oxidized to reduced glutathione ratio (GSSG:GSH) (22). Collectively, these studies implicate exercising skeletal muscle as a significant source of free radicals, contributing to the oxidant burden in this population.

Although the impaired pulmonary function that is intrinsic to lung disease certainly contributes to limited exercise capacity in COPD (6, 39), peripheral muscle abnormalities have also been implicated (29, 46). Indeed, significant peripheral locomotor muscle fatigue occurs in patients with COPD following cycle exercise (6, 31) and the perception of leg fatigue is frequently recognized as the primary symptom contributing to exercise intolerance (23). A failure to improve pulmonary function with treatment is a hallmark of the disease, thus enhancing quality of life and exercise capacity primarily through peripheral muscle therapy is regarded as an important rehabilitative tool (20, 32). As free radicals both depress muscle force production (7) and exaggerate

the discharge from thin-fiber muscle afferents (19, 26), which are inherent in the regulation of peripheral muscle fatigue (3-5), targeting oxidative stress in COPD to directly attenuate muscle fatigue and improve exercise tolerance appears to be a real possibility.

Pretreatment with the potent pharmacological antioxidant N-acetylcysteine (NAC), a GSH precursor, has been documented to improve quadriceps exercise endurance and lessen markers of oxidative damage at exhaustion in patients with COPD (27). However, NAC also appears to improve both small muscle mass (44) and whole body (33, 34) exercise tolerance in young, healthy individuals, suggesting its benefits may not be unique to a cohort with a heightened susceptibility to oxidative stress. In addition, NAC has been documented to improve pulmonary function during exercise in patients with COPD (48), further confounding the interpretation of the apparent benefit to peripheral muscle function in this population. Adverse side effects of NAC, such as lightheadedness and nausea (44), also potentially limit the efficacy of this antioxidant as a therapeutic intervention. There is currently little known about the potential of other antioxidant interventions to improve exercise tolerance in patients with COPD.

Therefore, we sought to examine the effects of an orally administered, readily available, antioxidant cocktail (AOC; vitamin C, vitamin E, and α-lipoic acid) with demonstrated efficacy (45, 53, 54), on free radical concentration and skeletal muscle fatigue following dynamic KE exercise in patients with COPD. Specifically, we tested the hypotheses that in patients with COPD, the AOC would: 1) raise antioxidant levels and decrease free radicals, as assessed by plasma ascorbate concentration and electron paramagnetic resonance (EPR) spectroscopy, 2) improve exercise capacity, as measured

by constant load KE exercise time to exhaustion, and 3) attenuate the development of peripheral quadriceps fatigue, quantified by magnetic stimulation of the femoral nerve, such that the degree of contractile dysfunction at isotime will be diminished.

Methods

Subjects

Eleven patients with COPD were recruited for this study based on spirometric evidence of moderate to severe airflow obstruction (FEV_1 < 80% predicted, FEV_1 / FVC ≤ 0.7 (14)), as assessed by standard pulmonary function tests (36). Written, informed consent was obtained from participants prior to their inclusion and the Institutional Review Boards of the University of Utah and the Salt Lake City VA Medical Center approved all protocols.

Exercise Protocol

Prior to data collection, all subjects were thoroughly familiarized with KE exercise, constant load exercise trials to exhaustion (T_{Lim}), as well as the neuromuscular function assessment. Peak KE work rate was determined with subject specific protocols designed to elicit maximum effort within 8-12 minutes, consisting of a 2-5 Watt/min increases at a cadence of 60 rpm. On subsequent visits, two T_{lim} trials separated by at least 48 hours, at 80% of maximal workload were performed following ingestion of either the AOC or PL in a counterbalanced order. The shorter of the two T_{Lim} times was then matched with the opposite condition (AOC or PL) on a successive visit to allow isotime comparisons. Throughout all trials, ventilation, gas exchange, heart rate, ratings of perceived exertion and breathlessness, arterial oxygen saturation, femoral blood velocity

and quadriceps electromyograms (EMG) were measured. Prior to each exercise bout, one minute of resting data were collected and subjects performed one minute of unloaded warm-up KE exercise. To quantify peripheral fatigue, neuromuscular function tests were performed before exercise and 10 minutes after task failure (<50 rpm). Venous blood samples were taken prior to and 1 hour after ingestion of the second dose of PL or AOC and immediately after KE exercise, to determine pro- and antioxidant status, and for spin trapping and EPR spectroscopy to directly assess free radical concentration.

Oxidative Stress, Antioxidant Assays, and Free Radicals

Plasma samples were stored at -80°C until analysis. Lipid peroxidation, a marker of oxidant damage, was assessed by thiobarbituric acid reactive substances (TBARS) and malondialdehyde levels (55) (Bioassays Systems, Hayward, CA). Total antioxidant capacity was assessed by determining the ferric reducing ability of plasma (FRAP), using the method described by Benzie and Strain (11). Although the efficacy of the AOC specific to plasma ascorbate levels was also assayed, as previously described (13) (CosmoBio, Carlsbad, CA). Endogenous antioxidant activity, assessed by superoxide dismutase (SOD) and catalase (CAT) activity, was also assayed in the plasma (51) (Cayman Chemical Company, Ann Arbor, MI).

To directly assess the ability of the AOC to reduce the concentration of free radicals, EPR spectroscopy was performed on pre- and postexercise blood samples, as previously described (45). Briefly, 3 ml of venous blood was collected into a vacutainer containing 1 ml of the spin trap α-phenyl-tert-butylnitrone (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), which was also used to calculate the AUC of the EPR spectroscopy signal by double integration.

Ventilation, Gas Exchange, Heart Rate, and Perceived Exertion

Ventilation and pulmonary gas exchange were measured at rest and during exercise with a metabolic cart (ParvoMedics, Sandy, UT). Heart rate, determined from the R-R interval of a three-lead electrocardiogram (ECG), and arterial oxygen saturation (SaO2), estimated using a pulse oximeter (Nellcor N-595, Pleasanton, CA, USA) with adhesive forehead sensors, were acquired at 200 Hz using a data acquisition system (AcqKnowledge; Biopac Systems, Goleta, CA). Ratings of perceived exertion (RPE) were taken every minute during the exercise trials using Borg's modified CR10 scale (12).

Leg Blood Flow Assessment

Measurements of femoral artery blood velocity and vessel diameter in the leg being studied were performed at rest and throughout exercise, using a Logic 7 ultrasound system (General Electric Medical Systems, Milwaukee, WI, USA) as previously described (49). Briefly, arterial diameter was measured, and mean velocity (V_{mean}) values (angle-corrected, and intensity weighted area-under-the-curve) were calculated using commercially available software (Logic 7). Using arterial diameter and V_{mean}, blood flow in the femoral artery was calculated as: Blood flow = $V_{mean}\pi(\text{vessel diameter}/2)^2 \times 60$.

Neuromuscular Function

Quadriceps electromyograms (EMG) were recorded from the vastus lateralis (VL) muscle (2). Electrodes were placed in a bipolar configuration 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 over the tibial tuberosity. These electrodes were used to record magnetically evoked compound action potentials (M-waves) to evaluate changes in membrane excitability, as well as EMG from the VL throughout exercise to provide an index of central motor drive. 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, Goleta, CA). For data analysis, the integral of each EMG burst (integrated EMG, iEMG) was calculated to determine a percent increase in iEMG from the first minute of exercise (2).

The magnitude of peripheral quadriceps fatigue was quantified by supramaximal magnetic stimulation of the femoral nerve (2, 28, 42). Specifically, $Q_{tw,pot}$ was assessed before and after each exercise trial. For this procedure, while subjects lay semi recumbent in a KE chair, with a knee joint angle of 90 degrees, a magnetic stimulator (Magstim 200, The Magstim Company Ltd, Wales UK) connected to a double 70 mm coil was used to stimulate the femoral nerve. The evoked twitch force was obtained from a calibrated load cell (Transducer Techniques, Temecula, CA) connected to a non-compliant strap placed around the subject's ankle and acquired at 200 Hz with a data acquisition system (AcqKnowledge; Biopac Systems, Goleta, CA). Supramaximality of stimulation was

determined by serial, single unpotentiated twitch (Q_{tw}) forces obtained every 30 seconds at 70, 80, 85, 90, 95, and 100% of maximal stimulator output on a separate visit to the laboratory.

A series of six MVCs and $Q_{tw,pot}$ maneuvers were performed with 30 seconds between each MVC. In addition, to quantify activation of the quadriceps during the MVCs, a superimposed twitch technique was employed (2, 35). Briefly, 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 (%VMA). Peak force, maximal rate of force development (MRFD) and maximal relaxation rate (MRR) were analyzed for all $Q_{tw,pot}$ values (30).

Statistical Analyses

Two-way repeated measures ANOVA was used to compare the effect of antioxidant treatment on physiological parameters during exercise, with a Tukey posthoc analysis if a significant main effect were found. Student's paired *t* tests were used to compare the effect of the AOC in terms of antioxidant efficacy, end exercise physiological parameters, and indices of peripheral fatigue. Correlations between variables were evaluated using Pearson correlation coefficients. Statistical significance was set at $\alpha = 0.05$ for all tests. All group data are expressed as means ± standard error of the mean (S.E.M).

Results

Subject Characteristics

Eleven subjects took part in the initial stages of the study, whose data are reflected in Table 2. However, complete muscle function and exercise data are presented only for the 10 subjects who completed the entire study, with the exception of blood sample data from one individual. Interestingly, resting free radical concentration, assessed by EPR spectroscopy, was inversely correlated with FEV_1 and baseline MVC force (Figure 3).

Antioxidant Efficacy

Consumption of the AOC increased plasma ascorbic acid levels and reduced the EPR spectroscopy signal AUC (Figure 4), but did not alter any other markers of oxidative stress (TBARS: 5.1 ± 0.3 vs 4.7 ± 0.3 uM, MDA: 1.2 ± 0.1 vs 1.1 ± 0.1 uM) or antioxidant status (FRAP: 0.99 ± 0.1 vs 1.0 ± 0.1 SOD: 21.4 ± 5.5 vs 20.5 ± 5.3 U/ml, catalase: 22.4 ± 2.8 vs 27.7 ± 5.0 nmol/min/ml, PL vs AOC respectively) prior to exercise. Following exercise, FRAP was increased above the PL condition with the AOC (1.0 ± 0.1 vs 1.1 ± 0.1 mM, $p<0.05$, respectively), however, there were no other significant pre- to post-exercise changes.

Performance Trials

Exercise to exhaustion resulted in a decrease in quadriceps muscle function, as illustrated by reductions in all measured indices of fatigue (MVC, $Q_{tw,pot}$, MRFD, and MRR) and an increase in the iEMG signal. M-wave characteristics were maintained from pre- to postexercise, indicating preserved membrane excitability. In addition, the %VMA

was reduced by ~5% following both exercise trials. AOC consumption, however, did not alter the physiological response to exercise, resulting in a similar time to exhaustion and magnitude of end-exercise fatigue (Figure 5).

Isotime Trials

Following exercise of equal duration and intensity, similar levels of end exercise quadriceps fatigue were attained with either AOC or PL consumption (Figure 6). M-wave characteristics were again preserved from preexercise values, and the %VMA was reduced by ~3% in both conditions. The physiological response to exercise was not different between conditions and is documented in Figure 7.

Discussion

This study has documented the ability of an oral AOC (vitamins C, E and alpha-lipoic acid) to reduce the resting plasma free radical concentration in patients with COPD. A broad array of resting free radical levels, however, were also observed, which is in line with the diverse etiology of COPD, which were inversely correlated with FEV_1 as well as baseline MVC force. Indeed, the efficacy of the antioxidant cocktail in this population appeared to be affected by the heterogeneity of both the degree of lung dysfunction and subsequent free radical load in the current subjects. Therefore, despite clearly documented antioxidant effects, we did not observe any functional consequences in terms of KE exercise endurance time or end-exercise quadriceps fatigue. Collectively, these data suggest that this oral AOC is most efficacious when the oxidant load is elevated, and this susceptibility to oxidative stress may be related to pulmonary disease

severity, but an acute free-radical reduction does not necessarily impact skeletal muscle function in patients with COPD.

Oxidative Stress and Lung Disease

Oxidative stress has been implicated in a variety of pathophysiological roles in the etiology of COPD, including modulation of redox sensitive inflammation, mitochondrial dysfunction, and alterations in myofilament interactions (17, 25). Indeed, there are numerous instances where an elevated oxidant burden has been documented, both systemically (15) and in the lung (10, 37), in patients with COPD. Specifically, Barreiro et al (10) documented higher protein carbonyl groups, a marker of protein oxidation, in diaphragm muscle samples of patients with severe COPD, which were inversely correlated with FEV_1 across those patients with moderate to severe airflow obstruction. Similar negative correlations between other markers of oxidative damage and airflow limitation have also been observed in the exhaled breath condensate (37, 38) as well as in the blood (40) of patients with COPD. However, EPR spectroscopy is considered to be the most sensitive technique for the direct detection of free radicals (9), and although it has been employed previously in the bronchoalveolar lavage fluid of patients with COPD (41), the current study is the first to use the technique to directly assay the concentration of oxygen- and carbon-centered free radicals in the plasma of patients with COPD, reflective of the systemic free radical concentration.

Resting free radical concentration, across the range of disease severity in this study cohort, tended to be inversely correlated with FEV_1 ($r = -0.54$, $p = 0.08$, Figure 3), a finding that is in accord with previous literature, and corroborates the role of oxidative stress in the pathology of COPD. The measured resting free radical concentration also

tended to be negatively correlated with baseline MVC force ($r = -0.54$, $p = 0.09$, Figure 3), implying a detrimental relationship between pulmonary disease severity, systemic free radical load and skeletal muscle function. AOC administration significantly reduced the concentration of plasma free radicals, assessed by EPR spectroscopy, consequent to an increase in plasma ascorbate levels (Figure 4). However, upon secondary analysis, a dichotomous response to the AOC was observed (Figure 8). Indeed, in terms of reducing free radicals, the AOC seemed to be most efficacious in those patients with high initial levels, with minimal effects in those with the lowest concentrations, despite a universal increase in plasma ascorbate. This finding parallels previously documented paradoxical effects of the AOC in other populations (45, 54). Thus, the current data support a role for the AOC to combat an elevated oxidant burden in patients with COPD, which, perhaps, may only be a concern in those patients with significant disease progression.

Performance Time and Oxidative Stress

Evidence exists for a role of oxidative stress in modulating exercise performance (43). Germane to the current study, Koechlin et al. (27) documented an improvement in quadriceps exercise time to exhaustion following oral NAC administration in patients with COPD, which was accompanied by an amelioration of the exercise induced increase in plasma TBARs observed in the placebo condition. The effects of NAC, however, are not unique to patients with COPD, as beneficial effects have also been documented in young, healthy subjects (33, 44), although typically only following intravenous administration which can yield greater plasma NAC levels. Such effects on exercise performance have not typically been demonstrated with vitamins C, E, or alpha lipoic acid (8, 43); however, the current study, to our knowledge, is the first to assess the

potential for nonpharmacological doses of these vitamins in modulating exercise performance in COPD.

In contrast to the effects observed with NAC, the AOC did not change exercise time to exhaustion or the magnitude of quadriceps fatigue achieved at task failure (Figure 5). This was despite the striking similarities between the characteristics of the subjects in the study by Koechlin et al. (27) and the current study's cohort (i.e., moderate to severe COPD and an average age of 62 yrs of age). This may potentially be explained by the 4-day loading period employed by the aforementioned study, enhancing the ability for NAC to enter the skeletal muscle itself. However, the principal aim of the current study was to document the efficacy of an acute, oral AOC with no known side effects, in the context of rehabilitative medicine. Thus, in an attempt to reconcile these discordant responses, in light of our AOC efficacy data, we correlated the change in free radical concentration following AOC consumption with the change in endurance time, but, somewhat disappointingly, did not find evidence of a relationship. Therefore, these data suggest that acutely decreasing free radicals in patients with COPD with the AOC does not translate to improved exercise capacity.

Oxidative Stress and Fatigue

Exercising skeletal muscle has been documented to contribute to the elevated oxidant load in COPD (15, 16), which, in turn, has been suggested to contribute to limited exercise capacity in this population (17). Indeed, Couillard et al. (16) have previously documented an inverse relationship between the exercise-induced increase in muscle oxidative stress and quadriceps endurance time in patients with COPD. In the current study there was no evidence of a relationship between exercise performance and

plasma free radical concentration at any time point. Due to the inherent variability in exercise performance trials, we employed isotime exercise to allow comparisons of the magnitude of end-exercise fatigue following identical exercise bouts with PL and the AOC. As illustrated in Figure 7, the physiological responses to the isotime exercise bouts were remarkably similar, at the very least illustrating the potential to achieve reproducible physiological measurements in a historically varied population. Pertinent to peripheral fatigue development during KE exercise, the percent increase in the iEMG signal, an index of central motor drive to the muscle, was one of these nearly identical responses assessed in both trials (Figure 7). Accordingly, no differences in end-exercise peripheral fatigue were observed (Figure 6), which again were unrelated, by correlational analysis, to a change in free radical concentration with AOC consumption. Therefore, although the EPR data imply a relationship between chronic oxidative stress and muscle function in patients with COPD (Figure 3), acute amelioration of this oxidant load does not diminish the magnitude of end-exercise quadriceps fatigue following dynamic KE exercise.

Blood Flow, Vascular Function, Oxygen Delivery and Oxidative Stress

The preponderance of efficacy data for this AOC is derived from studies examining vascular function in populations predisposed to oxidative stress (21, 45, 53, 54). Specifically, our group has documented that the AOC can restore vascular function in the elderly as assessed by flow-mediated dilation (52) and submaximal handgrip exercise (21). In addition, this AOC has been observed to improve end-exercise muscle perfusion, evaluated during plantar flexion exercise utilizing nuclear magnetic resonance spectroscopy, resulting in an improvement in muscle oxidative capacity (53). Thus, it

would be reasonable to assume that in patients with COPD with consistently documented elevations in markers of oxidative stress (15, 16, 37), the vascular effects of the AOC would be more pronounced. Although evidence for the translation of improved vascular function to improved exercising muscle blood flow is relatively sparse, antioxidant driven increases in the bioavailability of the vasodilator nitric oxide, secondary to a reduction in oxidative stress, may improve skeletal muscle blood flow and potentially oxygen delivery (18, 24), which would likely improve exercise performance (1). Thus, in the current study, femoral artery blood flow was measured during KE exercise; however, no difference between the PL and AOC trials was observed (Figure 7). Collectively, these data are suggestive of similar oxygen delivery to the working muscle, although it is possible that the initial impact of the varied baseline free radical concentration and the subsequent heterogeneity of the AOC effects may have obscured this effect.

Conclusion

This study reveals a negative relationship between COPD severity, skeletal muscle function, and resting free radical concentration, as assessed by blood EPR measurements, and documents the efficacy of an oral AOC in reducing oxidative stress. Importantly, this study also reveals that low free radical levels prior to ingestion of the AOC in this population may temper these antioxidant effects. However, a translation to improved KE exercise performance is not supported by the current data. Thus, although a potentially beneficial role for the AOC can be inferred for patients with COPD, this acute reduction in free radicals does not appear to be related to KE exercise endurance or ameliorate peripheral fatigue development.

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Disclosures

There are no conflicts of interest to report.

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Table 2. Subject characteristics

Age (yr)	62 ± 3
Height (m)	1.73 ± 0.03
Weight (kg)	84.1 ± 7.4
BMI (kg/m^2)	27.9 ± 1.7
Quadriceps muscle mass (kg)	1.71 ± 0.2
Peak knee-extensor work rate (W)	28 ± 3
Male/Female	7/3
Pulmonary function	
Forced vital capacity, l (% predicted)	3.58 ± 0.2 (86.18 ± 4.7)
Forced expiratory volume in one s, l/s (% predicted)	1.81 ± 0.2 (57.09 ± 4.6)
FEV_1/FVC (%)	50.45 ± 4.9
Resting arterial blood gases (n = 8)	
Hemoglobin concentration (g/dl)	13.5 ± 0.5
Oxyhemoglobin (%)	91.9 ± 0.6
Partial pressure of oxygen (mmHg)	69.9 ± 2.0
Partial pressure of carbon dioxide (mmHg)	32.2 ± 2.0
Bicarbonate (mmol/l)	22.0 ± 1.4
pH	7.45 ± 0.01

Values expressed as mean ± S.E.M. FEV1/FVC = Forced expiratory volume in one second relative to forced vital capacity. BMI = Body mass index

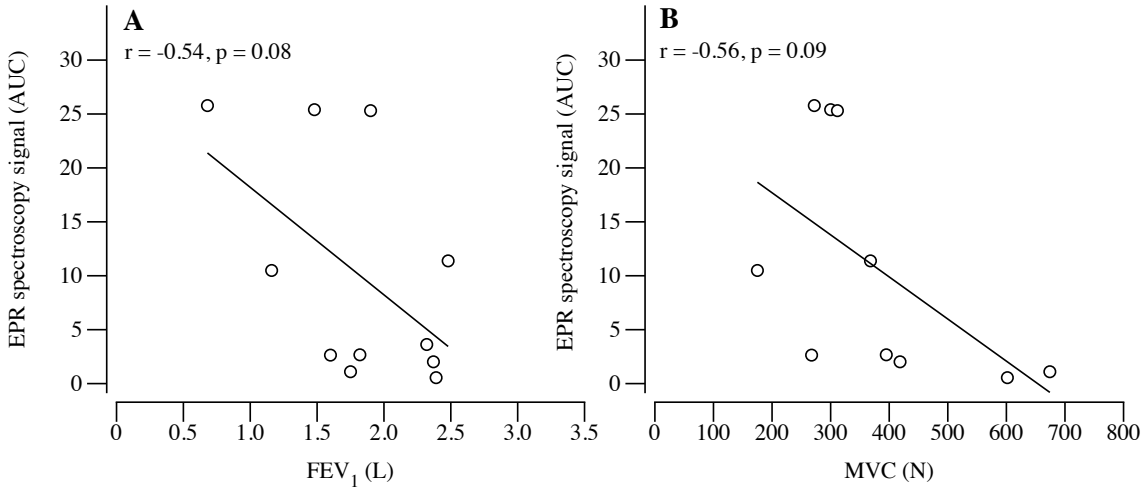

Figure 3. Relationships between forced expiratory volume in 1 second (FEV_1) and quadriceps maximal voluntary contraction (MVC) with resting free radical concentration assessed by electron paramagnetic resonance (EPR) spectroscopy. Resting free radical concentration (AUC, arbitrary units) tended to be moderately inversely correlated with A: FEV_1 ($p = 0.08$) and B: baseline quadriceps MVC ($p = 0.09$).

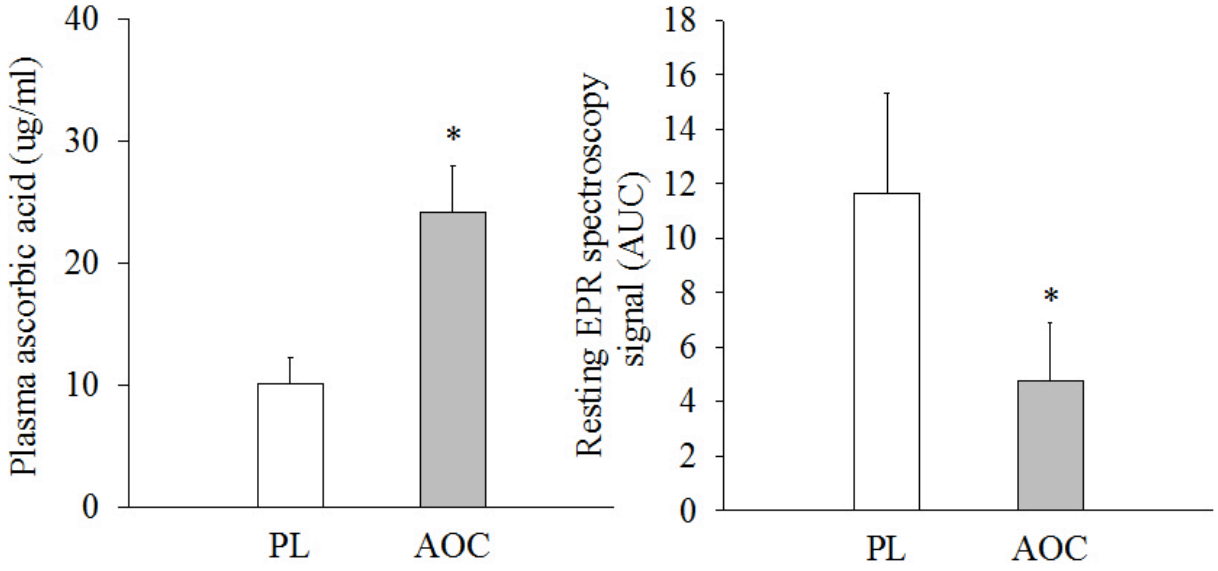

Figure 4. Resting antioxidant and oxidant status assayed in the plasma following placebo (PL) and antioxidant cocktail (AOC) consumption. Data are presented as mean ± S.E.M. AOC consumption resulted in an increase in plasma ascorbic acid (A) and a reduction in the free radical concentration assessed by electron paramagnetic resonance (EPR) spectroscopy area under the curve (AUC, arbitrary units) (B). *Significantly different from placebo condition, $p < 0.05$.

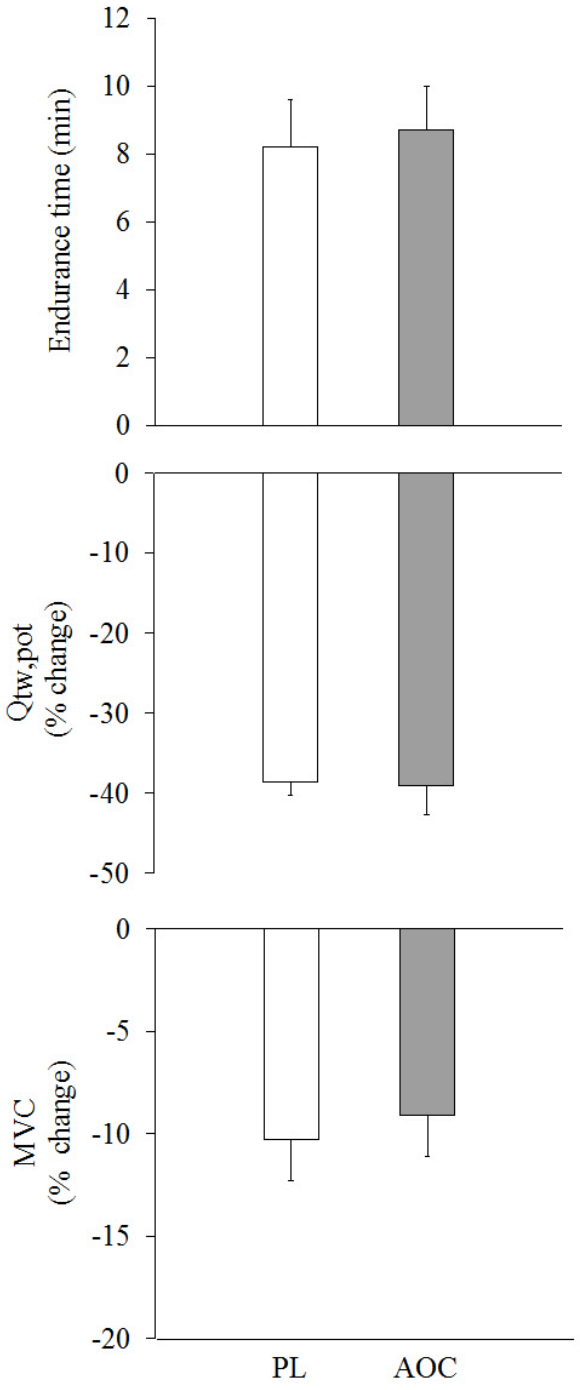

Figure 5. Endurance time and end-exercise quadriceps fatigue assessed following exhaustive knee extensor exercise with either the consumption of a placebo (PL) or an antioxidant cocktail (AOC). Data are presented as mean ± S.E.M. Quadriceps fatigue values represent the percent change from pre- to post-exercise. $Q_{tw,pot}$, potentiated twitch force; MVC, maximal voluntary contraction. There were no statistically significant differences.

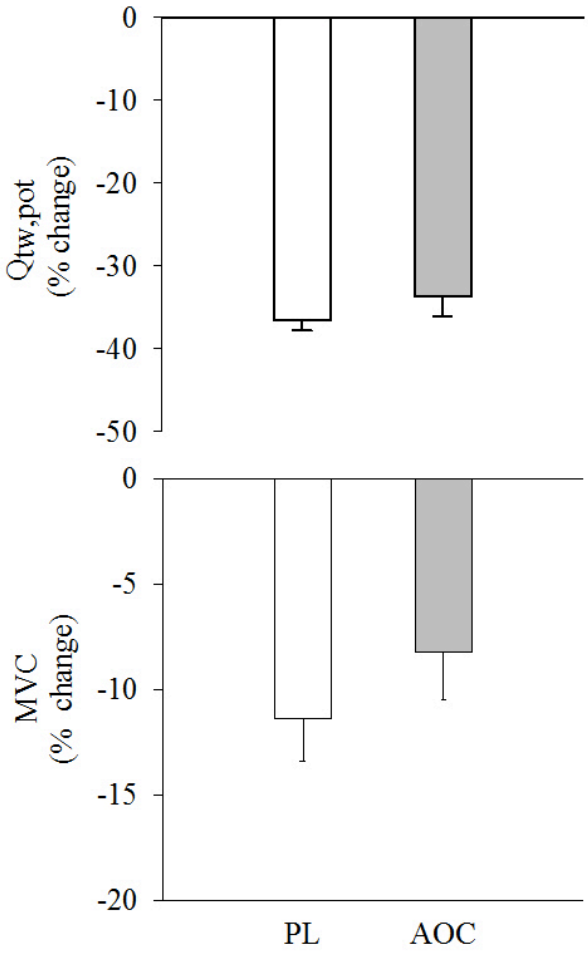

Figure 6. End-exercise quadriceps fatigue assessed following constant workload knee extensor exercise matched for intensity and duration (isotime) following consumption of either placebo (PL) or antioxidant cocktail (AOC) Data are presented as mean ± S.E.M and values represent the percent change from pre- to post-exercise. $Q_{tw,pot}$, potentiated twitch force; MVC, maximal voluntary contraction. There were no significant differences.

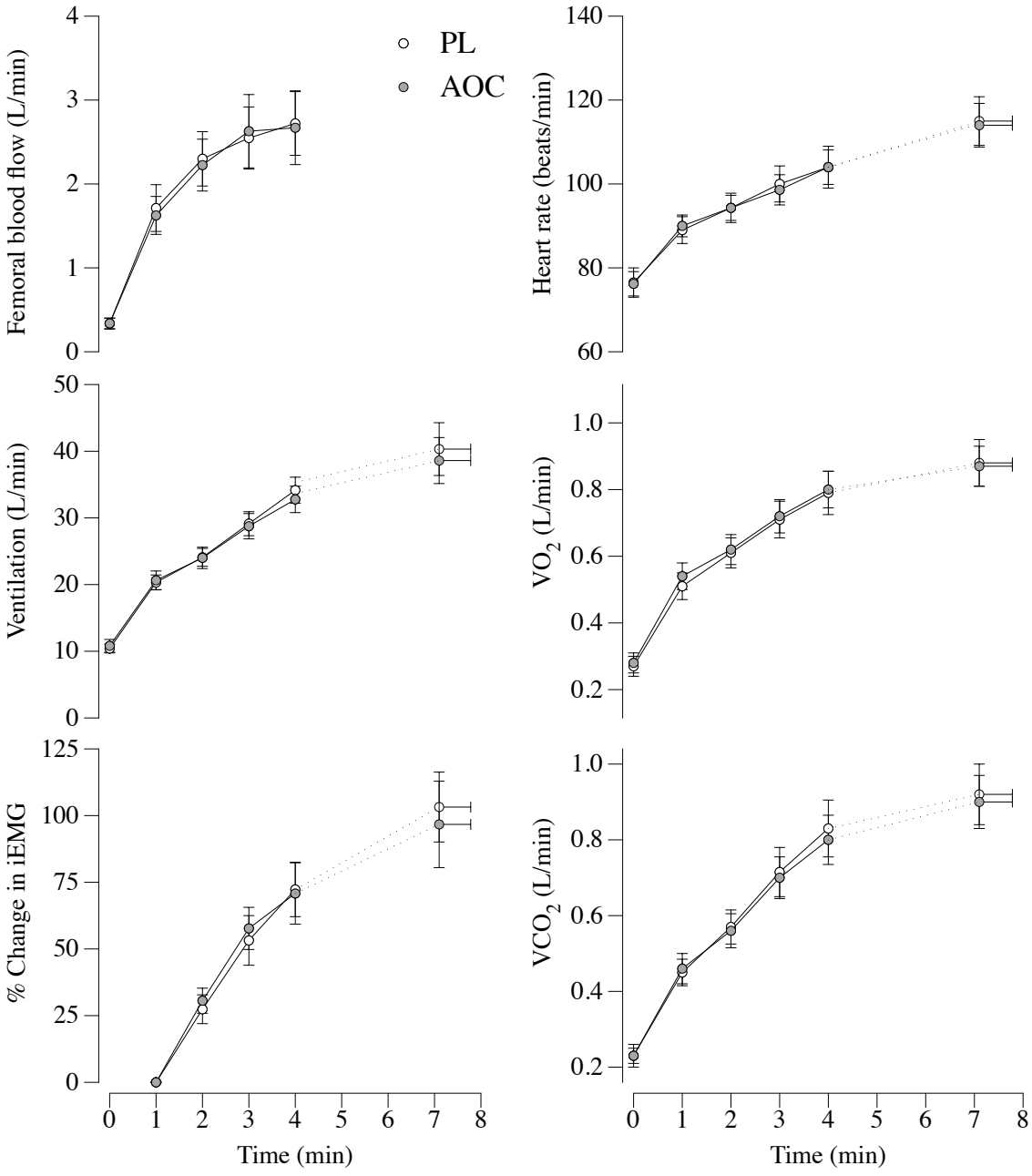

Figure 7. Physiological responses to constant workload knee extensor exercise matched for intensity and duration (isotime) following consumption of either a placebo (PL) or an antioxidant cocktail (AOC). Data are presented as mean ± S.E.M. End exercise values for femoral blood flow are not reported due to loss of signal. VE, ventilation; VO_2, oxygen consumption; VCO_2, carbon dioxide production; iEMG, integrated electromyogram. There were no statistically significant differences between interventions.

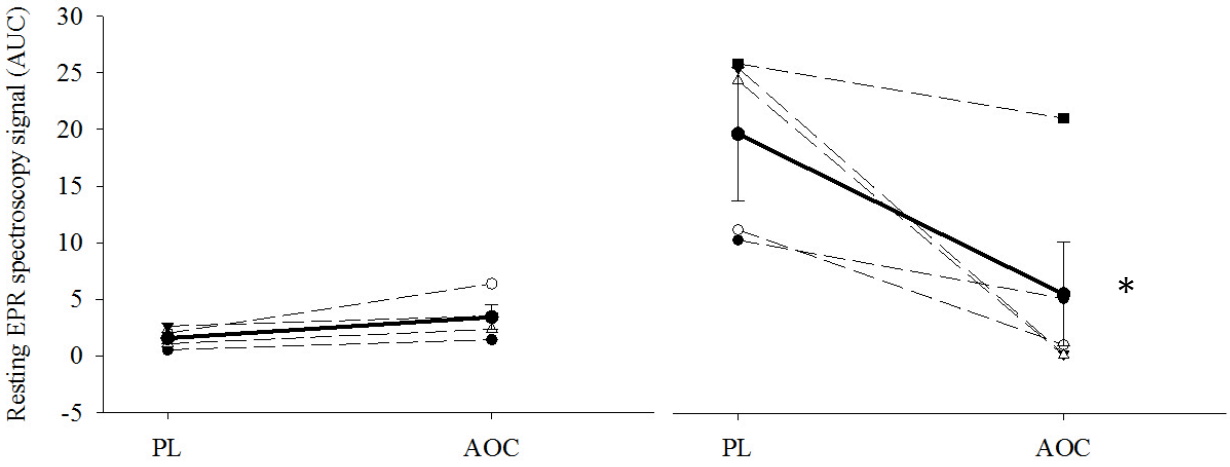

Figure 8. Individual changes in the α-Phenyl-*tert*-butylnitrone (PBN) spin adduct area under the curve (AUC, arbitrary unit) assessed by electron paramagnetic resonance (EPR) spectroscopy following the ingestion of both the placebo (PL) and antioxidant cocktail (AOC). Individual responses have been separated into two groups, those with low (left) and higher (right) initial values in the PL condition. As illustrated, the AOC appeared to only have an effect in those with high initial levels of oxidative stress, which is evidence that the baseline free radical load impacts the effect of the AOC. *Significantly different from placebo condition, $p < 0.05$.

CHAPTER 4

CONCLUSION

Elucidating the mechanisms contributing to skeletal muscle fatigue in health and disease is important both scientifically and in terms of rehabilitative medicine, which may enhance the quality of life in populations with limited mobility. Among these mechanisms, group III and IV afferent feedback from exercising muscle, which relays information regarding metabolic disturbance and the magnitude of peripheral fatigue, has been documented to substantially influence the voluntary termination of endurance exercise performance (1), and therefore, is highly germane to the study of exercise intolerance. Active muscle mass and oxidative stress have both been documented to alter skeletal muscle afferent activity (4, 5), and thus the manipulation of these two factors has the potential to alter exercise capacity. In addition, an excessive free radical load has been recognized to depress contractile function (2) and therefore accelerate the development of peripheral fatigue. Accordingly, first, endurance exercise performance as well as the magnitude of end-exercise quadriceps fatigue was examined in a healthy, young group of subjects to determine the effect of varying the amount of active muscle mass on muscle fatigue. Second, the effect of an oral AOC, with previously documented efficacy in attenuating free radicals (8, 9), on skeletal muscle fatigue was studied in a population with a heightened susceptibility to oxidative stress and well-documented exercise intolerance, patients with COPD.

In the first study, we sought to vary the volume of muscle mass active during dynamic constant workload, single-leg KE and BIKE exercise, to alter the ensemble magnitude of group III/IV afferent feedback (5), and examine the impact on end-exercise peripheral quadriceps fatigue in young, healthy subjects. With this approach, we observed far greater quadriceps compared to BIKE fatigue following KE exercise to exhaustion.

This effect was likely due to the constraint of muscle afferent feedback to the one quadriceps muscle during KE exercise such that, at task failure, the critical level of ensemble afferent feedback influencing the voluntary termination of exercise was eventually reached by a strong, local afferent signal in contrast to the sum of the more diffuse signals during BIKE exercise. Thus, the magnitude of peripheral fatigue in the quadriceps muscle, presumably necessary to elicit the strong local afferent signal, was greater following KE exercise. These data suggest that the CNS tolerates a greater degree of peripheral fatigue during small muscle mass exercise, and have important implications for rehabilitative medicine. Specifically, small muscle mass training appears to facilitate a greater local homeostatic disturbance and therefore may promote greater muscle adaptation.

The second study examined the impact of an oral AOC on free radical concentration, dynamic KE exercise performance, and peripheral quadriceps fatigue in patients with COPD. In this population with consistently documented elevations in oxidative stress (3, 6, 7), we observed a tendency for an inverse relationship between resting free radical concentration and airflow limitation, assessed by FEV_1, as well as baseline MVC force. The efficacy of the AOC, however, was dependent upon initial levels of free radicals, significantly decreasing free radicals in those with high initial levels, but having little effect in those patients with low baseline levels. Despite these antioxidant effects, no differences in endurance exercise performance or the magnitude of peripheral quadriceps fatigue were observed following AOC ingestion. Collectively, these data document the efficacy of the AOC to acutely reduce resting free radicals in patients with COPD, which may only be a concern in those patients with significant

disease progression. However, despite the suggestion of a relationship between chronic oxidative stress and skeletal muscle dysfunction in the study cohort, acutely reducing the free radical concentration with the AOC in patients with COPD does not necessarily impact skeletal muscle function during dynamic KE exercise.

In summary, the study of skeletal muscle fatigue is certainly of importance in the context of rehabilitative medicine, which is highly germane in populations with limited exercise capacity, such as patients with COPD. These studies have provided novel insight into the use of small muscle mass exercise to enable the attainment of a greater degree of peripheral quadriceps fatigue, potentially eliciting greater skeletal muscle adaptation. In addition, despite failing to document an effect of an AOC on peripheral fatigue in COPD, the second study has further substantiated the role of oxidative stress in the pathology of this lung disease and identified a beneficial antioxidant effect, most notably in those patients with more severe airflow obstruction. Further investigations addressing the role of free radicals and fatigue in patients with COPD, perhaps in patients with more homogeneously elevated levels of oxidative stress and perhaps with greater concentrations of antioxidants, are needed. However, the conclusions garnered from the current studies still have broad implications for improving rehabilitative medicine, and thus quality of life, in both health and disease, and have contributed to a better understanding of the factors influencing skeletal muscle fatigue.

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