EFFECTS OF N-ACETYLCYSTEINE ON FATIGUE, CRITICAL POWER AND MUSCLE ENERGY STORES

by

SARAH D. CORN

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Department of Kinesiology
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Major Professor
Thomas J. Barstow, PhD

Abstract

The accumulation of reactive oxygen species (ROS) has been linked to the development of muscular fatigue. Antioxidant administration has the potential to counteract the increased levels of ROS, leading to improvements in performance. N-acetylcysteine (NAC), a nonspecific antioxidant, is especially promising due to its ability to support the biosynthesis of glutathione, one of the primary endogenous antioxidants. Despite this, the effects of NAC on time to fatigue appear to be dependent upon the exercise intensity, with the more pronounced effects evident at submaximal exercise intensities. The purpose of this study was to determine the effects of an acute dose of NAC on whole body fatigue, critical power (CP) and *W'* during high-intensity exercise. It was hypothesized that pretreatment with NAC would result in *(1)* an increase in time to fatigue (TTF), CP and *W', (2)* NAC administration would attenuate changes in the EMG responses indicative of fatigue, and *(3)* speeding of the kinetics of the primary phase of VO_2 and a reduction in the slow component. Seven healthy, active males (age: 21.4 ± 1.6 years, weight: 89.1 ± 11.0 kg, height: 183 ± 5 cm) completed an incremental ramp test until exhaustion for the determination of peak VO_2 and power. Four tests were subsequently performed at power outputs corresponding to 80, 90, 100, and 110% P_{max} under NAC and placebo (PLA) conditions. NAC resulted in a significant increase in [tGSH] in red blood cells compared to baseline and PLA condition. TTF was significantly increased only in the 80% P_{max} trial ($p = 0.033$). CP was also significantly higher with NAC (NAC: 232 ± 28 W vs PLA: 226 ± 31 W; $p = 0.032$), but *W'* showed a tendency to decrease (NAC: 15.5 ± 3.8 kJ vs *W'*: 16.4 ± 4.5 kJ). The change in *W'* was negatively related to CP ($r = -0.96$), indicating that the increase in CP was associated with a

decrease in *W'*. EMG analysis revealed a tendency for MdPF and RMS to demonstrate less of a change with NAC. There were no significant differences in VO_2 kinetics, but an inverse relationship was observed between the change in τ_p and the magnitude of the slow component expressed both in absolute terms ($r = -0.632$, $p = 0.007$) and as a gain ($r = -0.751$, $p = 0.0005$). We conclude that NAC was effective in delaying fatigue and improving exercise performance at 80% peak power, although the exact mechanisms are still unclear.

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CHAPTER 1 - Introduction

Fatigue can be defined as a decline in the maximal force production of a muscle despite maximal effort. Exercise performance is limited by fatigue development in all populations from athletes to normal healthy individuals to those with clinical conditions. Understanding the mechanisms of fatigue and potential methods of alleviation may result in great benefits for these people. Oxidative stress, which is caused by an accumulation of reactive oxygen species (ROS), has been linked to fatigue development. The body produces small amounts of ROS at rest, which is required for optimal contractile function (171). Contrary to this, high levels of ROS can be toxic to cells. The bodyôs endogenous antioxidant system counteracts ROS and prevents accumulation of these substances during normal conditions (58). However, during exercise the production of ROS increases dramatically, overwhelming the bodyôs antioxidant capabilities. As a result, research has turned to exogenous antioxidants to supplement the bodyôs endogenous antioxidant system and hopefully prevent or delay the accumulation of ROS. Several antioxidants have been analyzed, but the most promising substances are thiol donors due to their ability to support the synthesis of glutathione, one of the primary endogenous antioxidants. N-acetylcysteine (NAC) is a thiol donor that has been shown to be effective in several conditions, but much research is still needed to elucidate the exact mechanisms and effects.

Background

Reactive oxygen species are free radicals that have been implicated as major contributors to fatigue development. These damaging molecules are produced constantly at rest via a variety of sources, a prominent source being errors in the mitochondrial electron transport chain (50, 149). Approximately 2% of mitochondrial oxygen uptake binds with unbound electrons that have

escaped from the electron transport chain to form reactive oxygen species (73). This low rate of production corresponds to the slow rate of oxidative phosphorylation in the mitochondria at rest. During exercise the amount of oxygen required to meet the bodyôs aerobic energy demands can increase 10- to 20-fold within contracting skeletal muscle. The rate of oxidative phosphorylation, and thus electron flux through the electron transport chain, may increase up to 100-fold (110), with a parallel rise in ROS concentrations (33, 40, 47, 65, 90, 97, 106, 115, 126, 127, 150, 170, 173, 184, 197, 211). As free radicals, these molecules are unstable and have detrimental effects, including 1) damage to cell membranes, lipids, and proteins, 2) genetic modifications, and 3) alterations in cellular redox status and blood flow (40, 110, 132, 182). Much of the myocyteôs intracellular machinery may be damaged by the ROS produced during exercise, which explains the substantial effects of ROS on muscle function and fatigue.

Aerobic organisms adapted to the oxidative stress resulting from ROS production by developing an endogenous antioxidant system (58). Antioxidants have the ability to neutralize free radicals and transform them into more stable compounds. However this in-dwelling defense mechanism is inadequate to fully compensate for the substantial production of ROS during exercise (195). Increasing the quantity of antioxidants available should then improve the bodyôs ability to defend itself during stressful conditions such as exercise, but data in this regard is ambiguous. The first exogenous antioxidants studied were dietary antioxidants such as vitamins A, C and E, which proved to be largely ineffective at improving exercise tolerance (67, 89, 103, 154, 169, 174, 215, 219). Attention then turned to thiol antioxidants due to their ability to support the biosynthesis of glutathione, one of the bodyôs primary endogenous antioxidants. N-acetylcysteine is one of these thiols that is particularly promising due to its multiple modes of

action. In addition to promoting the synthesis of glutathione (8, 38, 54, 198), NAC also directly scavenges free radicals (8, 38).

Despite the attractive theoretical potential of using NAC as a therapeutic supplement to delay the onset of fatigue, not all research has garnered positive results. In general, NAC supplementation has been ineffective during intense (130% VO_{2peak}) exercise (131) and high-frequency electrical stimulation protocols (174), but it has shown markedly improved function during prolonged submaximal exercise (126, 133) and low-frequency stimulation experiments (45, 111, 174, 190). It remains unknown if this crossover point is related to a significant physiological variable (for example the lactate threshold or serial recruitment of muscle fibers). An in-depth critique of the underlying factors is necessary to elucidate possible relationships. While this data may indicate completely different mechanisms of fatigue, there is not enough evidence to rule out other potential factors.

Researchers have attempted to explain the effects of NAC administration in several preparations, both *in vitro* and *in vivo*, but most have used isolated muscles or muscle groups. Only a few studies have implemented a whole-body exercise model such as cycling. These studies have resulted in equivocal data, showing no effects on time to fatigue (131), an improvement in time to fatigue with NAC administration (133), or a direct relationship between the improvements in time to fatigue with NAC and VO_{2peak} (132).

The few whole-body exercise studies that have been conducted typically evaluate all subjects at the same relative exercise intensity (70 and 90% VO_{2peak} are common). Even when attempting to obtain the most homogenous sample possible by using competitive cyclists, for example, no study has attempted to relate the chosen intensity with any physiological variable other than VO_{2peak}. The specified intensity may have dramatically different results in subjects

due to the relation to underlying physiological parameters of exercise (for example, 70% VO_{2peak} may be below the lactate threshold for one subject but above for another). The current study is the first systematic evaluation of the effects of NAC on time to fatigue at a range of exercise intensities. This protocol also permitted determination of critical power (CP) following either NAC or placebo administration. CP is a threshold demarcating the heavy and severe intensity exercise domains (163). Modeling the CP responses under both conditions allowed for subject responses to be compared with respect to an important physiological parameter.

The concept of critical power was first described in 1965 by Monod and Scherrer (140) when they noted an inverse relationship between the amount of work being done by a muscle and the time to fatigue. Further modeling revealed a hyperbolic relationship that yielded two important pieces of information: critical power and *W'*. Critical power refers to the maximum work rate that an individual can theoretically maintain indefinitely. It can be interpreted as the highest metabolic rate in which a steady state of VO_2 (163), lactate (163, 166) and phosphocreatine (35, 99) can be observed. It has been hypothesized that this maximal steady state metabolic rate is decided by a balance between glycolytic flux and oxidative metabolism (35). *W'* describes the finite amount of work that can be performed above the CP (140). Once this amount of work is depleted, power output must either stop or be reduced to below CP. An increase in time to fatigue would be evidenced as an increase in CP and/or *W'*, depending on the work rates that are affected.

Finally, the vast majority of research has used an intravenous infusion protocol to administer NAC. In order for antioxidant supplementation to become a mainstream practice, a more practical method of application is necessary. This problem may stem from the dearth of information regarding the pharmacokinetic properties of oral NAC administration, such as peak

concentration and time to peak concentration. To date, the pharmacokinetic studies regarding oral NAC (20, 21, 41, 153) have evaluated much smaller doses than are being used in current research (109, 126, 217). The available literature indicates a strong positive linear relationship between the dose administered and the time to peak concentration; however it is possible that a saturation point exists where increasing the dose of NAC will no longer result in increases in concentration. The peak concentration varies widely with the type of oral application. For example, a study by Borgstrom et al. compared four different oral sources (granulate dissolved in water, an effervescent tablet, a fast dissolving tablet and a slow release tablet; 21). The peak concentration of the slow-release tablet was significantly lower ($p < 0.05$) than the other three forms despite ingestion of equal amounts (600 mg in each condition).

Purpose

The aim of the present study was primarily to conduct a systematic evaluation of the effects of NAC on time to fatigue across a range of exercise intensities using a whole-body cycling protocol. Second, determination of individual CP and *W'* values with NAC and placebo will permit inter-subject comparisons at an equivalent physiological performance threshold. Third, possible mediators of ROS-induced fatigue will be assessed using electromyography and analysis of VO_2 kinetics. The electromyographic responses of active muscles are known to change as fatigue ensues, thus the EMG responses of the vastus lateralis and rectus femoris will be monitored throughout each exercise test to ascertain any effect of NAC on patterns of muscle motor unit recruitment. In regard to VO_2 kinetics, prolonged time constants and amplitude of the slow component are generally associated with reduced exercise tolerance. The VO_2 responses were modeled and compared to determine possible effects of NAC on the kinetic parameters.

Finally, NAC was administered orally to evaluate the efficacy of a practical mode of supplementation.

Hypotheses

Hypothesis 1: NAC supplementation will increase time to fatigue, critical power, and W' in a whole-body cycling protocol. Previous data indicate that NAC has the potential to increase time to fatigue, with the most dramatic effects at submaximal intensities. This would result in an elevated CP, and an increased *W'* would be manifested as an intensity-dependent response altering the curvature of the relationship.

Hypothesis 2: The electromyographic responses, as measured by median power frequency and root mean square, will be altered with NAC supplementation. Since all trials terminate at the same physiological end-point, EMG profiles at fatigue are expected to be identical under both conditions. However when trials are time-aligned the NAC trials should display less of a change in median power frequency (MdPF; a measure of the distribution of frequency content) and root mean square (RMS; an indicator of the recruited muscle activity for force generation) values.

Hypothesis 3: NAC will induce a speeding of Phase II VO_2 kinetics. Previous research in our lab has demonstrated a trend toward a decreased time constant for this phase (τ_p). Improved exercise tolerance (longer time to fatigue) is generally associated with a faster phase II time constant and a reduced amplitude of the slow component (28, 98). Additionally, nitric oxide, a potent ROS, competitively inhibits a key enzyme in the mitrochondrial electron transport chain (23), thus scavenging NO may allow for increased mitochondrial oxygen flux and a faster time constant.

Significance

Muscular fatigue is most commonly associated with athletic competition, yet it may be even more important in clinical settings. Every disease that exhibits fatigue and reduced exercise capacity related to oxidative stress has the potential to be alleviated using antioxidant supplementation. Reducing or delaying fatigue in diseased individuals could lead to numerous benefits, including improved disease status and quality of life.

CHAPTER 2 - Review of Literature

Reactive Oxygen Species

Exercise performance is known to be limited by fatigue. One of the predominant mechanisms associated with fatigue development is the accumulation of reactive oxygen species (ROS). Reactive oxygen species are a type of free radical, meaning that the outer orbital contains an unpaired electron, causing it to be reactive and unstable.

Sites of Production

A prominent source of ROS is through malfunctions in the electron transport chain (ETC) in the mitochondria (50, 149). Research has indicated that during normal, resting metabolism as much as 2-5% of the total electron flux escapes the ETC to form free radicals (22, 73). As expected, exercise causes an increase in oxygen uptake, with a concomitant increase in electron flux through the ETC. This increased flux of electrons may increase the rate at which free radicals are formed (104, 150).

Healthy skeletal muscles have been demonstrated to produce ROS (33, 47, 77, 150, 170, 211). ROS have been found in homogenates of muscle tissue (19, 47, 77, 119), in the cytosol (127, 148, 170), and in mitochondria located within the muscle cell (147, 211), as well as in the extracellular space of skeletal muscles (33, 156, 173, 196, 211). Reid et al. discovered that superoxide radicals that are produced within skeletal muscle cells are subsequently released into the extracellular space (170, 173).

The most common ROS found in aerobically-respiring systems are superoxide radicals, hydrogen peroxide, hydroxyl radicals, lipid alkoxyl and peroxyl radicals, and nitric oxide (195). Superoxide, hydrogen peroxide, and hydroxyl radicals are direct derivatives of oxygen and are part of a free radical cascade. They are formed by the addition of one, two or three electrons to oxygen, respectively. These particular ROS also stimulate the production of more free radicals (104).

Damage Caused by Oxidative Stress

Due to the unstable properties of ROS, these molecules tend to transfer electrons to, or reduce, other species (187). Antioxidants stop this chain reaction by either accepting the unpaired electron from the free radical or by donating one of its own electrons to stabilize the molecule. Oxidative stress is defined as a state in which the antioxidant system is overwhelmed by the amount of ROS present (184). Several disease states, such as congestive heart failure and kidney disease, as well as strenuous exercise exhibit oxidative stress. The detrimental effects of this condition include alterations in redox status and blood flow, damage to cell membranes and other biological substances such as lipids and proteins, and genetic mutations (40, 110, 132, 182). Based on the potential sources of ROS generation, it is important to note that mitochondrial proteins are damaged by oxidative modification (30, 78, 161).

The damage to the skeletal muscle cell in response to free radical exposure is well characterized. Damage due to ROS occurs throughout the muscle fiber. First, action potentials are affected by oxidative stress in the cell via disruptions in sarcolemmal potassium channels (185) and the Na^+-K^+ ATPase pump (130). The sarcoplasmic reticulum is also disturbed by the presence of ROS, primarily by alterations in calcium homeostasis (79, 80). Modifications occur in the calcium release channels (6, 74, 159) and the calcium ATPase (113, 180). Disruptions also

occur in troponin (167), tropomyosin (218), the active sites of actin (122), and myosin heavy chains (11, 39).

Effects of Exercise

During resting conditions, the body produces ROS at a low rate (31, 90, 157, 169, 170, 173, 198). Similarly, oxidative phosphorylation in the mitochondria occurs at a slow rate during rest, but during exercise the rate may increase as much as 100-fold (110). As a result, the production of ROS parallels this increase during strenuous exercise (33, 40, 47, 66, 90, 97, 106, 115, 126, 127, 150, 170, 173, 184, 197, 211). Furthermore, this increased rate of ROS production occurs only in the active muscles (150). Using cats, OõNeill et al. (150) administered L-phenylalanine, which is converted to *p-, m-,* or *o*-tyrosine following a hydroxylation reaction. This method allows for the quantification of hydroxyl radicals by measuring the concentration of these tyrosines (72). Following five minutes of intermittent static contractions of one triceps surae muscle and one minute of rest, production of isomeric tyrosines increased significantly. However, the contralateral muscle, which was not contracting, did not exhibit an increase in tyrosine concentration. These results indicated that the increased production of ROS seen during strenuous exercise is limited to the contracting musculature with minimal diffuse effects (150), although ROS produced within the myocyte have been shown to be released extracellularly (173).

In a study involving rats, Davies et al. (40) compared the effects of intense exercise to the effects of a vitamin E deficient diet. Homogenates collected from the muscle and liver of rats post-exercise showed signs of decreased control of mitochondria respiration when compared to non-exercised rats. Vitamin E deficiency resulted in similar findings. These findings imply that the mitochondrial inner membrane may become more permeable, or ñleaky,ò to protons and that

the efficiency of oxidative phosphorylation is diminished due to exhaustive exercise. Latency calculations comparing the vitamin E deficient diet to exhaustive exercise indicated that both conditions result in a decreased integrity of the sarcoplasmic or endoplasmic reticulum (40).

The relatively high levels of ROS resulting from strenuous exercise seem to be associated with damage and fatigue of the exercising muscle. Reid et al. (171) found that administration of antioxidants actually decreases contractile function of unfatigued muscle, indicating that a small amount of ROS is necessary for optimal contractile function. However, higher levels of ROS result in a decreased ability of the muscle to maintain tension over time (14, 45, 66, 97, 106, 118, 149, 170, 174, 183, 184, 197, 201). Fatiguing exercise has been demonstrated to result in changes in commonly used biochemical indices of oxidative stress, including glutathione status (7, 37, 66, 96, 97) and lipid peroxidation (7, 37, 106, 115, 152, 182, 185, 197, 199). Research also indicates there is a direct relationship between the amount of ROS produced and the resulting fatigue (40, 173), meaning that higher levels of ROS produce greater fatigue. Using a cat model, O'Neill et al. also demonstrated that the rate of hydroxyl radical production is directly proportional to the developed tension (150). Following exercise, ROS production immediately decreases (114).

Mechanisms of Inducing Fatigue

The effect of ROS on skeletal muscle function has been well characterized. The mechanisms by which these effects are produced are less clear, and the possibility of a complex combination of mechanisms certainly exists. Muscular fatigue has been linked to alterations in intracellular pH and energy metabolism, build-up of inorganic phosphate, disturbances in the ions necessary for action potential conductance (Na^+ and K^+), and calcium dysregulation or desensitization (53, 102) in addition to ROS accumulation. Since it has been clearly

demonstrated that contracting muscles produce high levels of ROS, it is important to determine if these other conditions occur in response to the accumulation of ROS or if they result from some other factor. Several sarcoplasmic reticulum regulatory proteins are responsive to changes in redox status, including both the Na^{+}-K^{+} and Ca^{++} ATPases, and the sarcoplasmic reticulum Ca^{++} release channel known as the ryanodine receptor (111, 133).

Sodium and Potassium

The muscle cell depends on the Na^{+}-K^{+} ATPase to restore the gradients of these ions and allow for propagation of an action potential. Strenuous exercise requires rapid cycles of depolarization and repolarization of nerves and myocytes. During exercise the Na^{+}-K^{+} ATPase activity is upregulated via translocation of pump subunits and modifications in ion affinity (102), resulting in an 18-22 fold increase in ATPase activity above rest (32, 51, 129). However, activity of the Na^{+}-K^{+} ATPase does not reach maximal potential following prolonged dynamic exercise in rat muscle (55) and in human skeletal muscle (57), and during exhaustive isometric contractions in humans (56).

The Na^{+}-K^{+} ATPase is redox sensitive (130), and accumulation of ROS may be a factor in the depression of Na^{+}-K^{+} ATPase activity (116, 185). Changes in sodium and potassium concentrations occur during muscular contraction despite the upregulation of the Na^{+}-K^{+} ATPase (130). ROS scavengers may prevent the deleterious effects of ROS on the Na^{+}-K^{+} ATPase, resulting in improved ion regulation and delayed fatigue (132).

Calcium

Calcium is an ion that is obligatory for excitation-contraction coupling and action potential generation. ROS induce changes such as calcium dysregulation (1) and diminished calcium sensitivity of the myofilaments (5, 142). These changes appear to be due to oxidation of

key proteins, which has been shown to occur in the sarcoplasmic reticulum of fatigued skeletal muscle (26, 27, 216) and in cardiac muscle (49).

ROS are highly reactive and have a strong tendency to oxidize other substances. Myofibrillar proteins contain sulfhydryl residues that can be oxidized, forming disulfides and thus diminishing the responsiveness to calcium (5, 46, 141). Similar reactions may also occur in the Ca^{++} ATPase located in the sarcoplasmic reticulum. The sulfhydryl groups in this ATPase are necessary for dephosphorylation (37), and oxidation of these residues would decrease the activity of the Ca^{++} ATPase and calcium reuptake (26, 49, 179). Finally, the ryanodine-sensitive calcium channel protein contains residues that are susceptible to oxidation (221). This reaction also yields a disulfide, which causes a configurational change of the channel and eliciting a rapid outflow of calcium from the sarcoplasmic reticulum (205). These changes result in high cytosolic calcium concentrations by opening sarcoplasmic reticulum release channels and inhibiting the Ca^{++} ATPase reuptake pump (111). Reduction of the disulfides can reverse these detrimental results (26, 49, 205) and promote calcium storage in the sarcoplasmic reticulum (111).

As previously described, a low level of ROS is necessary to achieve optimal contractile performance (171). This level of ROS found in unfatigued muscle promotes excitation-contraction coupling by enhancing calcium release. ROS scavengers would thus restrain excitation-contraction coupling and reduce the contractile properties of unfatigued muscle (111).

These findings imply that strenuous, prolonged exercise leads to the depletion of sequestered calcium and the desensitization of myofibrillar proteins to calcium. Although reversal of these conditions may impair contractile function of unfatigued muscle, it may create an optimal environment for prolonged exercise.

Antioxidant Supplementation

An endogenous antioxidant system exists in aerobic organisms to counteract ROS accumulation (58). In addition to these endogenous antioxidants, nutritional antioxidants are also necessary in order to counteract oxidative stress (104). These endogenous and exogenous antioxidants work in a synergistic manner (36, 68, 69, 71, 123, 128, 182, 187, 214) in a chain reaction format (188). The chief components of the endogenous antioxidant system include the superoxide dismutase, catalase, and the glutathione-glutathione peroxidase system (133, 184).

Several conditions can render this antioxidant system inadequate, resulting in an inability to prevent ROS accumulation. These conditions include insufficient intake of nutritional antioxidants or extreme consumption of pro-oxidants, chemical or UV exposure, injuries and wounds that elicit an immune response, and severe exercise (195). The high levels of ROS produced during exercise overpowers the bodyôs endogenous antioxidant system, resulting in increased levels of ROS in the body tissues (10, 40, 90).

Based on this information, it follows that supplementing the bodyôs antioxidant system should result in an attenuation of the ROS accumulation. Several antioxidants have been tested, including glutathione, N-acetylcysteine (NAC), Ŭ-lipoic acid, and vitamins A, C, and E (187). However, the efficacy of these supplemental antioxidants is dependent on several factors, such as the antioxidant and dosage tested, the type of exercise chosen to induce fatigue (174), and on the temperature of the muscle preparation (142). Dietary antioxidants have largely proven to be ineffective in delaying fatigue associated with ROS accumulation, even when the biochemical indices of oxidative stress were lessened (67, 89, 103, 154, 169, 174, 215, 219). Oddly enough, deficiencies of these antioxidant vitamins may impair an individualôs endurance capacity

although supplementation was ineffective (40, 65). This disparity may be due to something as simple as the right combinations and doses of these vitamins having not been discovered.

Despite the fact that severe exercise overpowers the antioxidant system, the body exhibits amplified antioxidant activity after exercise in skeletal muscle (63), in the liver (105), and in the blood (105, 135, 151, 175). Exercise training actually serves to strengthen the bodyôs antioxidant system (104, 125, 139), typically resulting in a 15-50% increase in antioxidant capacity (104). Research has shown that the maximal potential of several antioxidants corresponds to the aerobic capacity of various tissues (95).

The preventative application of antioxidants that specifically scavenge ROS can result in delayed fatigue (142, 170, 200). Muscular fatigue can be defined as a decline in force output despite maximal effort. Administration of specific antioxidants may slow the decline in force output and thus delay fatigue (14, 170, 190, 199), via direct effects on the muscle fiber (45, 111, 170, 199). However, pretreatment with antioxidants actually decreases the contractile properties of unfatigued muscle fibers, including the twitch response and tetanic force production. This effect has been demonstrated using catalase and superoxide dismutase (168, 171), and dimethyl sulfoxide (172, 177).

Glutathione

One class of antioxidants that has proven to be effective against ROS accumulation is thiols. Most thiols can serve as reducing agents, meaning that their negative standard reduction potentials allow them to accept electrons (187). A sufficient thiol redox status is necessary to maximize the antioxidant capacity (110). The bodyôs endogenous glutathione and glutathione-peroxidase (GPX) are of particular importance. This antioxidant system, which makes up the bodyôs primary defense against ROS accumulation (192) has two mechanisms of action in the

prevention of ROS build-up: (1) direct interactions with ROS, and (2) detoxification of ROS via the GPX-catalyst. Both of these mechanisms require the oxidation of GSH to GSSG (187); thus maintaining the stores of GSH is of utmost importance. The enzyme glutathione reductase is responsible for converting GSSG back to the more useful GSH (4, 186). Cells typically contain rather high levels of glutathione, on the order of 0.1-10 mM. During resting conditions, the vast majority (>99%) is in the form of GSH (134). GSH is unique not only in its prominent ability to prevent ROS accumulation, but also in the fact that it boosts the functional capacity of other exogenous antioxidants, including vitamins E and C (187).

An acute bout of fatiguing exercise results in oxidation of GSH, causing an increase in the levels of GSSG and a decrease in GSH. This change in redox status following exercise has been documented in muscle (121, 184), liver (121), blood (66, 131, 184), plasma, and lungs (184). In response to training, the body adapts to the increased amounts of ROS by augmenting the GSH and GPX stores (110, 182). This adaptation serves to increase the cellôs ability to effectively counteract the detrimental effects of ROS accumulation.

Despite the proven value of glutathione in the face of oxidative stress, administration of glutathione has not been successful due to its low bioavailability (110). However, synthesis of glutathione occurs intracellularly (134), so provision of the necessary substrates should allow for increased production. In order to maximize availability, levels of cysteine should be maintained as this substance is rate-limiting in the formation of glutathione (181). Several cysteine donors have been examined, including N-acetylcysteine (NAC), cysteamine, lipoic acid, and 2-oxothiazoliding 4-carboxylate. Of these, NAC and Ŭ-lipoic acid have the greatest potential to be successful due to the proven success of trials and their clinical safety (187).

N-Acetylcysteine

As previously alluded to, NAC acts as a reduced cysteine donor to aid in GSH resynthesis (8, 38, 54, 176). NAC also has the ability to directly scavenge many ROS, including hydrogen peroxide (H_2O_2), hydroxyl radicals (·OH), and hypochlorous acid radicals (HOCl). NAC is a very potent scavenger of HOCl and ·OH, but reacts slowly with O_2· and H_2O_2 (8). Finally, the cysteine residue itself is a scavenger of free radicals (38). It is unknown whether NAC is transported across the sarcolemma to act intracellularly. However, active uptake of the cysteine residue into the cell does occur following dissociation of the NAC molecule (13), allowing for the intracellular synthesis of GSH. Evidence also clearly indicates that the ROS that are produced within the myocyte are released from the cell (73, 173), where they can be scavenged by extracellular antioxidants.

NAC has been proven to be successful in attenuating fatigue in several models involving animals and humans, respiratory and skeletal muscles, and electrical stimulation and voluntary exercise. Shindoh et al. (190) induced fatigue of the diaphragm in the rabbit using electrical stimulation following pretreatment with NAC or placebo. The rate of fatigue development was much slower in NAC-treated animals versus controls in both high- and low-frequency stimulation protocols. NAC pretreatment reduced the rate of decline of force produced during high-frequency stimulation (100 Hz) by 60% and by 40% during low-frequency (20 Hz) stimulation.

A study by Khawli and Reid (111) examined the effects of NAC on unfatigued muscle in rats. Diaphragm fiber bundles were excised and electrically stimulated to measure twitch characteristics and tetanic force development. Most twitch characteristics, including maximal tetanic contraction (P_0), time to peak tension (TPT), and P_t/P_0 (P_t = maximal twitch force), were

significantly reduced after treatment with NAC. Maximal tetanic force was also reduced following stimulation to fatigue at 15 and 30 Hz with no effects at higher frequencies. However, NAC treatment slowed the decline in force production during these trials.

Reid et al. built upon these findings by applying them to skeletal muscle in humans. The tibialis anterior muscle was stimulated at 10 Hz and 40 Hz. NAC treatment delayed fatigue in the low-frequency (10 Hz) protocol, but did not affect fatigue development following high-frequency (40 Hz) stimulation, the maximal voluntary contraction of unfatigued muscle, or contractile properties (174).

A series of experiments conducted by Medved and colleagues have made significant contributions toward the application of these principles in human voluntary exercise models. In the first study subjects cycled at 130% $VO_{2\ peak}$ for three 45-second bouts separated by 135 seconds of rest followed by a fourth bout at the same intensity until volitional fatigue. Blood redox status was changed, but no improvements were seen in time-to-fatigue in the NAC trials compared to the control (saline) trials. In addition to the changes in glutathione status, the researchers also found that NAC impaired plasma K^+ regulation (131). Based on these results, a follow-up study was conducted using a prolonged cycling protocol. This protocol involved cycling at 100 rpm at a work rate corresponding to 70% $VO_{2\ peak}$ for 45 minutes, then 90% VO_{2peak} until fatigue. Interestingly, the average time-to-fatigue for the group did not increase with NAC administration. To further examine this data, results were expressed as a ratio of the percentage of change in time-to-fatigue relative to control trials versus $VO_{2\ peak}$. It was found that the effects of NAC were dependent on $VO_{2\ peak}$ so that subjects with a higher maximal aerobic capacity saw greater increases in time-to-fatigue than subjects with lower maximal aerobic capacities (132). Using trained subjects with similar $VO_{2\ peak}$ values (65.6 ± 2.2 ml/kg/min), a

subsequent study showed an increase in time-to-fatigue of 26.3 ± 9.1% after NAC administration when cycling at 92% VO_{2peak} (133).

Two potential mechanisms of how ROS induced fatigue were previously discussed, including impairments in sodium, potassium and calcium regulation. Medved et al. examined the effects of a prolonged cycling protocol on potassium regulation. A smaller change in $[K^+]$ was seen at fatigue in the NAC trials versus the control trials, indicating that NAC does improve potassium regulation (132). McKenna et al. used a K^+-stimulated 3-O-methyflurorescin phosphatase activity assay to assess the activity of the Na^+-K^+-pump following a fatiguing bout of submaximal cycling exercise in humans. The decline from preinfusion activity levels at 45 minutes was ~12% in NAC trials compared to a 22% decline in control trials, with similar results seen at fatigue (130). These data indicate that the redox status of the Na^+-K^+-pump is potentially a contributor to ROS-induced fatigue.

The second mechanism discussed was alterations in calcium regulation or sensitivity. Studies have examined the effects of ROS scavengers, including Tiron (141, 142) and dithiothreitol (141). These substances were shown to be effective in improving the changes in calcium sensitivity. However, administration of NAC has yet to be evaluated in this context.

Indices of Performance

Due to the varied effects of ROS and NAC administration on fatigue at different exercise intensities, it is conceivable that it may be related to some underlying physiological phenomenon. To date, research has only attempted to relate the improvements in performance seen with NAC to VO_{2peak}, but other parameters such as the lactate threshold or critical power should be considered.

Critical Power

Research has clearly established that the duration of high-intensity dynamic exercise has an inverse relationship with power (85, 88, 140, 143). Critical power (CP) is defined as the maximum power output that can be maintained for a prolonged period of time without fatigue (140). CP distinguishes the heavy-intensity exercise domain from the severe-intensity domain, and allows for the estimation of the tolerable duration of exercise at higher intensities (162).

W' refers to the finite amount of work that is able to be performed above CP, regardless of the rate of expenditure (85, 88, 140, 143). The energy stores related to *W'* consist of stored oxygen, a source of high-energy phosphates, and the energy produced from anaerobic glycolysis (44). Miura and colleagues have shown that *W'* can be increased via creatine loading (137) and decreased with glycogen depletion (138) without altering CP. It is not possible to replenish these energy stores during exercise above CP; the work rate must be decreased to below this threshold once the energy is exhausted if exercise is to be continued (34).

This relationship between CP and *W'* can be described by plotting power output versus the time to fatigue, resulting in a hyperbolic curve. This relationship can then be determined from the following three equations:

Nonlinear power-time model: $\text{time} = W' / (\text{power} - CP)$

Linear power-1/time model: $\text{power} = CP + (W' \cdot 1/\text{time})$

Linear work-time model: $\text{work} = W' + (CP \cdot \text{time})$

In the nonlinear power-time model, CP is determined as the asymptote of the relationship and the degree of curvature refers to *W'*. This can easily be transformed to a linear model by plotting power vs. 1/time. In this model, CP corresponds to the y-intercept and *W'* is represented by the slope of the line.

These models describe this relationship well, providing that extremes of power output and duration are avoided (82). The models are not accurate at these extremes due to the inability of the muscle to generate sufficient force for the highest power outputs and the limitations of substrate availability or thermoregulation requirements for exercise of markedly sustained duration (163). For activities that are adequately described by the models, CP and *W'* are physiological performance measures that allow for prediction of mode-specific performance. These measures are particularly attractive because they combine mechanical efficiency and energy production variables, and do not require invasive methods or expensive equipment. However, estimation of CP and *W'* typically requires several tests, and attractiveness of the procedure declines as the number of tests required increases (82). A 3-minute all-out test to determine CP has recently been developed and validated. This method requires only one test, thus improving the practicality of this measurement (24, 208, 209).

In review, CP is identified as the highest power output that can be sustained theoretically indefinitely, and *W'* refers to a finite amount of work that can be performed above CP, presumably reflecting specific energy stores. Based on these definitions, power outputs maintained below CP should have the capability of achieving a steady state, allowing for exercise to continue for a long period of time. It then follows that power outputs above CP should result in depletion of the *W'*, limiting the duration of exercise that can be maintained at this intensity. As a result, VO_2 does not display a steady state, and it approaches or may even exceed VO_2max (163). The slow component of oxygen uptake is evident above the lactate threshold and is responsible for the continued rise in VO_2 above that predicted from exercise performed below the lactate threshold (60, 83). Between the lactate threshold and CP, the slow component will achieve a delayed steady state; at exercise intensities greater than CP, VO_2

continues to increase until attainment of VO_{2max} with no evident plateau. Due to these characteristics of exercise above and below CP, this measure can be used to differentiate the heavy and severe intensity domains. The term ñheavy exerciseò refers to power outputs between the lactate threshold and CP and ñsevere exerciseò indicates power outputs greater than CP (163, 207).

Exercise at CP should theoretically be able to continue forever. However, studies typically indicate exercise lasts only 30-60 minutes at this level, and some studies indicated a duration of 10-30 minutes (82, 87, 91, 158). During extended heavy exercise, slow- and fast-twitch oxidative fibers are used primarily, but fast-twitch oxidative/glycolytic and fast-twitch glycolytic fibers are secondarily recruited as the initial fibers are depleted of glycogen (213). Additionally, several factors may skew the estimation of CP and the following performance at CP, including pedal cadence, test termination criteria, training status, relative lactate threshold and muscle fiber type distribution (15, 82). Barker et al. (15) evaluated the effects of different pedaling cadences in sprinters (presumed to have primarily fast-twitch muscle fibers) and cross-country runners (primarily slow-twitch fibers). The endurance athletes consistently displayed a greater mean CP than sprinters. Pedaling at 60 rpm resulted in a 9% higher CP compared to 100 rpm ($p < 0.05$); however, the VO_2 equivalent to CP was the same between the two pedaling cadences.

Research has attempted to elucidate the underlying mechanisms responsible for CP. It is significantly different from but related to both the lactate threshold and VO_{2peak} (for review, see reference (82), occurring at approximately 46% of the difference between these measures (162). Relationships of CP with the maximal lactate steady state (MLSS) and the electromyogram fatigue threshold (EMG_{FT}) are less clear.

The MLSS refers to the highest power output that still results in an eventual plateau of blood lactate concentration. Power outputs below MLSS result in a balance between lactate production and removal, but for power outputs above MLSS production of lactate exceeds the elimination; thus the power associated with MLSS (P-MLSS) forms a boundary above which [La], VO_2 and $[H^+]$ progressively increase without achieving a steady state. For this reason, P-MLSS, like CP, has been used to demarcate the boundary between heavy- and severe-intensity exercise. Pringle and colleagues (166) tested the possibility that these two measures were actually reflecting the same phenomenon. They determined that the CP was significantly higher than P-MLSS (CP: 71 ± 3% P_{max} versus P-MLSS: 65 ± 3% P_{max}; $p < 0.05$), although a strong correlation existed between the measures ($r = 0.95$, $p < 0.01$). Despite this finding, it is still possible for CP and P-MLSS to be measures of the same underlying mechanism, but variability in the methods of quantifying both CP and P-MLSS may obscure the relationship.

An increase in the integrated EMG (iEMG) from working muscle is evidenced simultaneously with the slow component of VO_2 during severe exercise. This observation has led to the hypothesis that the serial recruitment of type II motor units, which may be less efficient, is related to the slow component of VO_2 (16, 162, 178, 191). The size principle states that the motor units recruited secondarily will be larger and thus of a higher threshold (17, 18, 212), which would result in a greater iEMG signal. The increase in iEMG could also be attributed to an increased firing rate of the already activated motor units in order to compensate for fatigued or impaired motor units (48). These observations led to the concept of the EMG_{FT}, which refers to the highest power output that can be maintained without a systematic increase in iEMG (145). In testing this theory, Moritani et al. (145) asked subjects to cycle at their predetermined EMG_{FT} as well as 20 and 40 W below and 40 W above EMG_{FT}. In the trials conducted at or below EMG_{FT},

a steady-state in both iEMG and VO_2 was observed; however progressive increases in both parameters were evident in the supra-EMG_{FT} trials. These results were taken in support of the EMG_{FT} concept.

Given that the EMG_{FT}, P-MLSS, and CP seem to outline the highest power output in which a steady-state exists, it is plausible that the concepts refer to the same physiological phenomenon. Pringle et al. (166) evaluated the relationships between these variables, and the results pertaining to CP and P-MLSS have been discussed previously. In the study, the EMG_{FT} could be determined in only four of the eight subjects, and it was not related to either CP or P-MLSS in these subjects. Le Chevalier and colleagues (120) have reported data indicating that the EMG_{FT} and CP are not significantly different in subjects performing knee extension exercise. In opposition, deVries et al (42) found that the EMG_{FT} was significantly (~12%) higher than CP, although a significant correlation ($r = 0.87$) was found. However, many researchers have indicated difficulty measuring EMG_{FT}. For example, Takaishi et al. (202) reported a progressive increase in iEMG in the absence of a VO_2 slow component. The controversy regarding the reliability of this measurement precludes any significant conclusions made from these data.

W'

W' refers to a finite amount of work that is able to be performed above CP. Although the precise underlying mechanisms of *W'* are unknown, it is thought to be an energy store composed primarily of anaerobic glycogenolysis and phosphagen stores, with a minor contribution from stored oxygen (140, 143, 163). Miura and colleagues have clearly demonstrated that creatine loading results in an increase in *W'* (137) while glycogen depletion results in a decrease (138). Anaerobic power is commonly measured using a 30-s Wingate test, intermittent high-intensity exercise, and by determining maximum O_2 deficit. Each of these measures has shown to be

moderately correlated with *W'* (94, 146). However, 30-s Wingate tests may be too short in duration to completely exhaust the anaerobic energy sources, resulting in a substantial amount of energy available at conclusion of the test (107, 206). The estimated value of anaerobic power determined from these tests may also be inflated due to the unavoidable contribution of aerobic energy, which may account for 9-28% of the work performed (84, 108, 189, 194). This error is avoided when using *W'* to quantify anaerobic power as values are unaffected when subjects breathe a hypoxic gas mixture (143). If aerobic energy source made a significant contribution to *W'*, measurements of this parameter would be expected to decline with hypoxia.

Recently a single 3-minute all-out cycling test to measure CP and *W'* has been developed and validated. In these tests, subjects exercise maximally against a constant work load. CP is then calculated as the mean power output over the last 30 seconds of the test, and *W'* is estimated by the power-time integral (24, 209). In a study to determine the effects of manipulations in the resistance applied and pedal cadences, Vanhatalo et al (210) found that applying a work rate equal to either 100% or 130% of peak power had no effect on CP. Subjects also performed tests at their preferred cadence as well as 10 rpm faster and slower. CP and *W'* were both found to be affected by these changes in pedal cadence. Finally, this test was able to detect changes in critical power that were induced by a four-week high-intensity interval training program, a result that was validated by traditional CP testing (208).

Effects of Exercise Training

Theoretically, training programs can be designed to selectively enhance either CP or *W'*. CP may be targeted with endurance training while high-intensity training should specifically improve *W'*, although research has been ambiguous. In support of this theory, Jenkins and Quigley conducted two training studies. In the first, twelve males trained for 30-40 minutes at

CP three days/week for 8 weeks, resulting in a 30% increase in mean CP (92). The training protocol for the second study consisted of five all-out, one-minute cycling bouts against 0.736 N/kg with five minutes of rest between bouts. Subjects again trained three days/week for eight weeks. A 49% increase in *W'* was evident, with no changes in CP (93).

On the contrary, Poole and colleagues (164) implemented a training regimen of cycling designed to elicit increases in the lactate threshold and VO_{2max}. Subjects performed ten 2-minute cycling bouts at 105% of P_{max} separated by 2-minute rest periods. Training sessions occurred 3 days/week for 7 weeks. CP significantly increased in all subjects. Despite achieving higher VO_2max values following training, there were no significant changes in *W'*. The training resulted in an upward shift of the power-time relationship without any alterations in the curvature. Based on the training theory of Jenkins and Quigley (92, 93), one would have expected an increase in *W'* with minimal changes in CP following this high-intensity training.

Finally, Gaesser and Wilson (61) contrasted two training programs, one aimed at increasing CP and the other *W'*. Subjects in both groups trained 3 days/week for 6 weeks on a stationary cycle. The program designed to increase CP consisted of 40 minutes of cycling at 50% of VO_2max, and the *W'* regimen involved ten 2-minute bouts at 100% VO_2max. Interestingly, both groups displayed a significant increase in mean CP (15% for the *W'* training and 13% for the CP) but no significant changes in mean *W'*.

The properties of the CP-*W'* relationship have several implications for athletic competition. They allow for determination of an athleteôs ideal pace for completing a race without premature fatigue. Contrary to popular belief, Fukuba and Whipp (59) demonstrated that running below critical velocity (CV; equivalent to critical power) at any time throughout a race will result in a slower time than if pace had been consistently maintained at CV.

Summary

This paper investigates the role of reactive oxygen species (ROS) in the development of muscular fatigue and the use of antioxidants to minimize the build-up of ROS. Evidence has linked the accumulation of ROS to fatigue development for many years, but the exact mechanisms have yet to be elucidated. Potential mechanisms include dysregulation of sodium and potassium, malfunction of calcium release channels and the sarcoplasmic calcium ATPase, and reduced sensitivity of the myofilaments to calcium.

Antioxidants have the ability to diminish the effects of ROS by chemically transforming them to stable compounds. Several studies of the effects of NAC have demonstrated reduced indicators of oxidative stress, and improved ion regulation and sensitivity, yet NAC appears to have an intensity-dependent effect on time to fatigue.

Finally, critical power and *W'* are commonly used indices of exercise performance, although they have not yet been applied in this circumstance. These measurements allow for both analysis of fitness status and prediction of future performance.

The current study addresses the discrepancies highlighted here. A cycling protocol will be used to conduct a systematic evaluation of the effects of NAC on time to fatigue across a range of intensities and to determine CP and *W'*. EMG activity of the major cycling muscles will allow for assessment of fatigue and potential alterations in muscle motor unit recruitment patterns. Finally, the efficacy of an oral dose of NAC will be evaluated rather than the standard intravenous infusion.

CHAPTER 3 - Methodology

Subject Characteristics

Seven healthy males ages 20-24 yr were recruited for participation. All were free of: (1) pulmonary and cardiovascular diseases as determined from a medical history questionnaire, and (2) from physical injuries that may hinder physical performance. Subjects were active and encouraged to maintain this activity level throughout the duration of the experiment. Participants were requested to abstain from alcohol, caffeine and vigorous exercise for a 24-hour period prior to testing and to avoid consuming food for two hours prior to testing. Written informed consent was obtained from each subject. All procedures were approved by the Kansas State University Institutional Review Board for Research Involving Human Subjects.

Experimental Design

A double-blind crossover design was used in this study. A total of nine tests were required per subject, with at least 48 hours between consecutive tests and no more than three tests per week. All trials were performed at the same time of day (± 2 hours) to eliminate the influence of circadian rhythms.

Experimental Protocol

Subjects reported to the Human Exercise Physiology Laboratory at Kansas State University for testing. During the first testing session subjects performed an incremental ramp test to fatigue on a electromagnetically braked cycle ergometer (Lode Corival model 844, Corival, Lode BV, Groningen, Netherlands) to determine VO_{2peak} and peak power. The test

consisted of four minutes of unloaded cycling followed by an incremental ramp increase in power output at a rate of 25 $W \cdot min^{-1}$ (5 W increase every 12 seconds) until exhaustion. The work of Poole and colleagues indicates that the previously accepted secondary indicators of VO_{2max} are not valid (165), so a validation test was performed on the same day as the ramp test. Following twenty-five minutes of rest and a four minute warm-up at 20 W, subjects exercised at 105% of the previously determined P_{max} until fatigue. If the VO_{2peak} achieved during the validation was not significantly different than that from the ramp test, it was accepted as the true $VO_{2max.}$ The highest 15-second value was taken as the peak response. Seat height was measured and reproduced for each subject throughout all trials, and pedal cadence was maintained at 60-70 rpm.

In order to determine the effects of NAC on CP and *W'*, subjects returned for a series of constant-load cycling tests until exhaustion at 110, 100, 90, and 80% of P_{max}. These intensities were chosen in order to elicit fatigue in 2-15 minutes. This series of tests were performed in random order, the only stipulation being that the 80% P_{max} workload was not performed first. The rationale for this was to allow subjects to experience fatigue at the higher intensities first, where motivation was thought to play less of a role in performance. These tests were performed after administration of N-acetylcysteine or placebo (PLA), the order of which was also randomly assigned within each pair of tests. This crossover design allowed each subject to serve as his own control, and treatment versus placebo condition was blinded to both the subject and investigator administering the exercise tests. A senior investigator remained un-blinded to administer the supplements. A 70 mg/kg dose of NAC (Physiologics, Northglenn, CO) was administered orally (caplets) 60 minutes before the onset of exercise. This dose was chosen based on data indicating that higher doses of NAC did not produce significantly greater changes in antioxidant status or

muscular performance (Ferreira, personal communication). PLA consisted of the same number of caplets of cornstarch in identical pill casings.

Venous Blood Sampling

A 22gauge, in-dwelling catheter was placed in an antecubital vein of each subject at the beginning of each testing session. Baseline blood samples were drawn before administration of NAC, two minutes before the onset of exercise (pre-exercise), and two minutes after exercise termination (post-exercise). Each sample was drawn into a 3cc syringe and placed in a tube treated with EDTA. After each draw, the catheter was flushed with heparinized saline to keep the line patent.

All samples were analyzed for total glutathione (tGSH) using a colorimetric assay kit (Sigma-Aldrich). Technical failure precluded determination of [GSH]. Samples were immediately centrifuged (4°C) and the plasma fraction was discarded. The remaining red blood cells were washed twice with phosphate buffered saline. A 200 µl aliquot of the red blood cell pellet was combined with an equal volume of 5% sulfosalicylic acid to lyse the cell membranes. The samples were then centrifuged at 10,000 x g and the supernatant was transferred to clean microcentrifuge tube and frozen at -80°C until subsequent analysis.

A fraction of each sample was first used to calculate tGSH, and all samples were assayed in duplicate. Due to the high intracellular concentrations of glutathione, samples were diluted 50-fold with 5%-sulfosalicylic acid. Glutathione reductase was added to convert all oxidized glutathione in the sampled to the reduced form. The reduction of 5,5ô-dithiobis-2-nitrobenzoic acid (DTNB) by GSH results in 5-thio-nitrobenzoic acid (TNB), a yellow product that is measured spectrophotometrically at 412 nm. Absorbance was measured at one minute intervals for five minutes. The concentration of tGSH was calculated by the following equation:

$$[tGSH]\ (nmoles/ml\ sample) = \frac{\Delta A_{412}/min(sample) \times dilution\ factor}{\Delta A_{412}/min(1nmol\ standard) \times volume}$$

Electromyography

Surface electromyography electrodes were placed on the right vastus lateralis and rectus femoris muscles to record the electrical activity of the muscle using a commercially available data acquisition and analysis system (Bagnoli-4 EMG System, Delsys Inc, Boston MA). These muscles were chosen as they are the primary muscles recruited during cycle ergometry exercise. Each site was shaved and thoroughly cleaned with alcohol to reduce inter-electrode resistance, and each site was marked and reproduced for all future trials. A reference electrode was placed over the head of the ulna or fibula. This EMG system uses a pre-amplified (gain=10) single differential electrode that incorporates two silver bars (1 x 10 mm) spaced 10 mm apart. The raw EMG signal was passed through a frequency window of 20-450 Hz. A sampling rate of 1024 Hz was used for the analog signal, which was saved from each study for off-line analysis.

The EMG data was analyzed using commercially available software (EMGworks, Delsys Inc). The root mean square (RMS) and the median power frequency (M_DPF) were calculated over 10 second windows at 30 seconds and end-exercise (EE) in all trials. Additionally, calculations were also made in the longer trial of each pair (NAC and PLA) at a time point corresponding to fatigue in the shorter trial (termed EEô). RMS quantifies the recruited muscle activity for force generation, and M_DPF describes the distribution of frequency content, which is commonly used to monitor the rate at which muscles fatigue.

VO_2 Kinetics

Breath-by-breath pulmonary gas exchange variables (VO_2, VCO_2, V_E, R) and heart rate (HR) were measured and recorded using a metabolic measurement system (Cardio2, Medical Graphics Corporation, St. Paul, MN). The O_2 and CO_2 analyzers were calibrated prior to each test using gases of known concentrations that spanned the expected range of expired gases, and volume was calibrated with a 3.0 L syringe. Heart rate was monitored during each test using an electrocardiogram with electrodes placed in a modified lead I arrangement.

The breath-by-breath data was converted to second-by-second values and time aligned to the start of exercise. A low-pass frequency filtering process was used to reduce the breath-by-breath variability for all VO_2 data using a method similar to that described by Ferreria et al. for blood flow (52, 75, 76). Briefly, this process eliminates the higher-frequency noise while preserving the frequencies essential for modeling the three phases of the VO_2 response. The default low-pass function of SigmaPlot was modified to achieve this (lowpass.xfm, SigmaPlot 2001, Systat Software). The following equation was used to fit the resulting VO_2 response for each phase:

$$VO_2 = VO_{2(b)} + A_i \cdot (1\text{-}e^{-(\text{Ű TDi})/\text{Ű}}) \quad \text{(Phase 1)}$$

$$+ A_p \cdot (1\text{-}e^{-(\text{Ű TDp})/\text{Ű}}) \quad \text{(Phase 2)}$$

$$+ A_s \cdot (1\text{-}e^{-(\text{Ű TDs})/\text{Ű}}) \quad \text{(Phase 3)}$$

In this equation, A, TD, and Űrefer to the amplitude, time delay, and time constant, respectively. The subscripts b, i, p, and s denote baseline cycling (20W), and initial, primary, and slow components. The data from each trial were fit using either a two-exponential model (Phases 1 and 2) or three-exponential model (Phases 1-3) depending on the presence of a slow component.

Critical Power and *W'*

Critical power (CP) and *W'* (*W'*) were modeled by fitting power output and time to fatigue to both a 2-parameter hyperbolic model (power output versus time to fatigue) and a linear model (power output versus 1/time to fatigue).

Hyperbolic model: time = *W'* / (power ï CP)

Linear model: power = CP + (*W'* · 1/time)

The parameters of the model were used for statistical comparison with other variables and parameters.

Statistics

The differences in CP and *W'* between NAC and PLA trials were evaluated using paired t-tests. Repeated-measures ANOVA was used to evaluate the differences between NAC and placebo trials for all measures, and Bonferroni Multiple Comparison Tests were used post-hoc to identify where significant difference existed. Relationships between variables were analyzed using a Pearson Product Moment Linear Correlation Analysis. Statistical significance was declared when $p < 0.05$.

CHAPTER 4 - Results

Subject Characteristics

Subject characteristics are reported in Table 1. Subjects were active (neither sedentary nor competitively trained) and were asked to maintain their activity levels throughout the duration of the study. All subjects were free of cardiovascular and pulmonary diseases and physical injuries that may affect performance as determined from a medical history questionnaire. None of the subjects reported adverse effects from the protocol.

Table 1: Subject Characteristics

Subject	Age (yr)	Weight (kg)	Height (cm)	BMI (kg/m^2)
1	20	81.5	180	25.2
2	20	82.0	181	25.0
3	23	111.5	183	33.3
4	21	82.0	185	24.1
5	24	82.5	183	24.8
6	22	93.0	178	29.4
7	20	91.5	193	24.6
Mean	**21.4**	**89.1**	**183**	**26.6**
Std. Dev.	**1.6**	**11.0**	**5**	**3.4**

Incremental Ramp Test Data

Peak metabolic variables are displayed in Table 2. Subjects completed a validation test following the ramp test, and none of the subjects achieved a significantly different VO_2 in this test. All values are reported as mean ± SD.

Table 2: Parameters of Incremental Ramp Test

Power (W)	**327 ± 43**
VO_2 (L/min)	**3.87 ± 0.55**
VO_2 (ml/kg/min)	**44.1 ± 8.6**
VCO_2 (L/min)	**4.65 ± 0.60**
VE (L/min)	**136 ± 27**
VE/VO_2	**34.8 ± 3.3**
VE/VCO_2	**29.1 ± 4.0**
RER	**1.20 ± 0.08**
HR (bpm)	**179 ± 7**

Blood Glutathione

Pretreatment with NAC resulted in a significant increase in the concentration of total glutathione one hour after ingestion (2.59 ± 1.85 mM at one hour versus 2.05 ± 1.78 mM at baseline; $p = 0.025$; Figure 5). This was also significantly higher than the placebo condition at the same time point (PLA: 1.58 ±0.91; $p = 0.042$; Figure 5). By the end of exercise, however, there was no difference in [tGSH] between PLA and NAC conditions ($p = 0.473$). Due to technical failure, the blood samples were not able to be assayed for reduced glutathione.

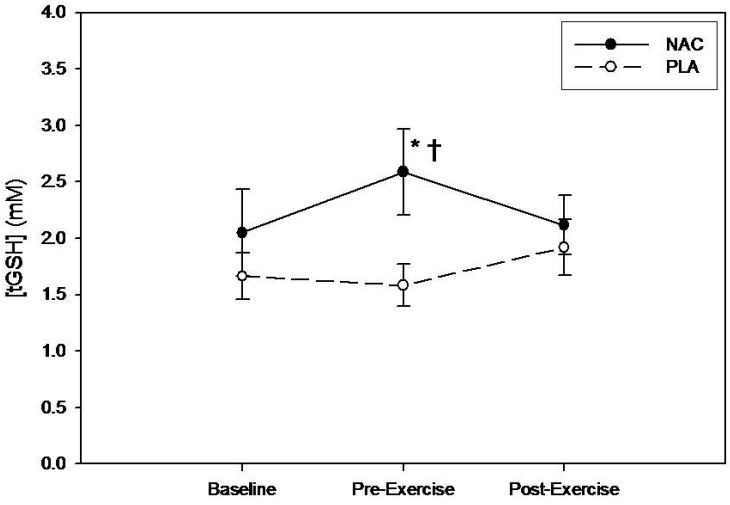

Figure 1: Concentration of total glutathione in red blood cells. Data are presented as group mean ± SE. Solid lines and circles describe the NAC condition and the open circles and dashed lines depict PLA. * significantly different from baseline (p = 0.025); Àsignificantly different from PLA (p = 0.042)

Time to Fatigue, Critical Power, and *W'*

Figure 1 describes the time to fatigue response at each exercise intensity under the NAC and placebo conditions. Repeated measures ANOVA revealed a significant difference for the interaction of the drug administered (NAC or PLA) and power output (p = 0.033; Table 3).

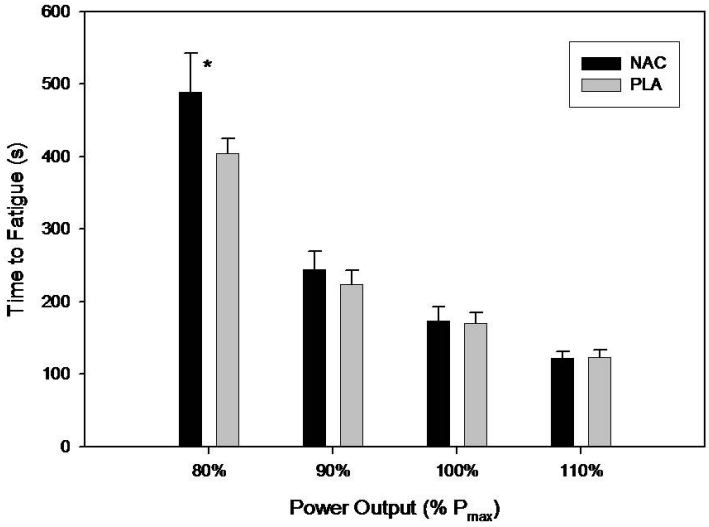

Figure 2: Comparison of time to fatigue at each exercise intensity between the NAC condition (black bars) and PLA (gray bars). * significantly different from PLA, p = 0.033

Table 3: Group mean responses for time to fatigue

Power (%P_{peak})	TTF – NAC (s)	TTF – PLA (s)
80%	489 ± 140*	405 ± 53
90%	253 ± 44	250 ± 55
100%	174 ± 50	170 ± 39
110%	124 ± 23	125 ± 25

* significantly different from PLA ($p < 0.05$)

The critical power and *W'* results for a representative subject are displayed in Figures 2a (hyperbolic model) and 2b (linear model). The group results are shown in Figures 3a and 3b and Table 4. Both the hyperbolic and linear critical power models fit the data well ($r > 0.95$ in all cases). There were no significant differences between the models for critical power or *W'* in either condition (Table 4). Five of the seven subjects achieved a higher CP in the NAC compared to the PLA condition, while one subject decreased and one experienced no change in CP. For the

group, pretreatment with NAC resulted in a significant increase in critical power as compared to placebo (232 ± 28 W versus 226 ± 31 W; p = 0.032, Figure 4). When expressed relative to the peak power output achieved during the ramp test, critical power increased from 69.1 ± 4.1% in the placebo condition to 71.1 ± 4.0% with NAC (p = 0.029).

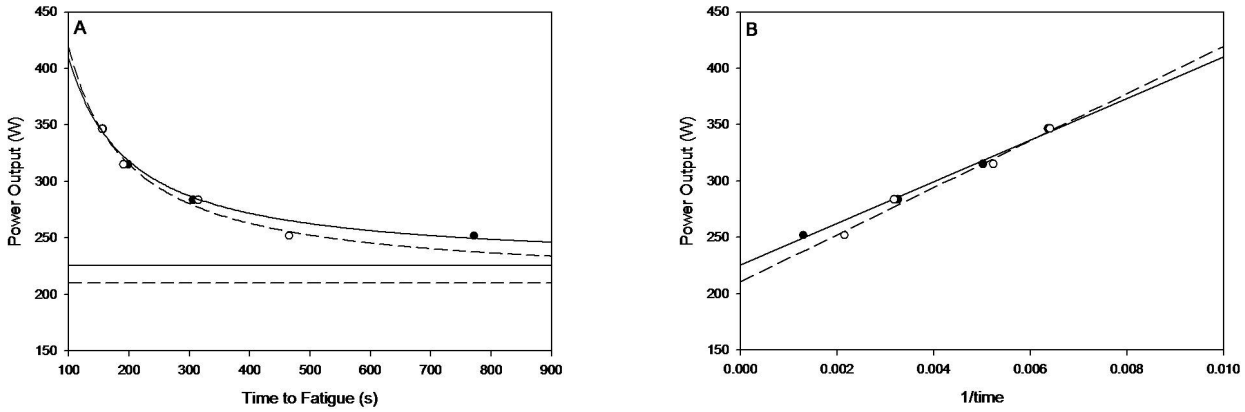

Figure 3: Hyperbolic (A) and linear (B) critical power models for a representative subject. NAC condition is denoted by solid circles and lines, and PLA is shown as open circles and dashed lines. In Figure 2A, the horizontal lines are the asymptote of each relationship, denoting critical power.

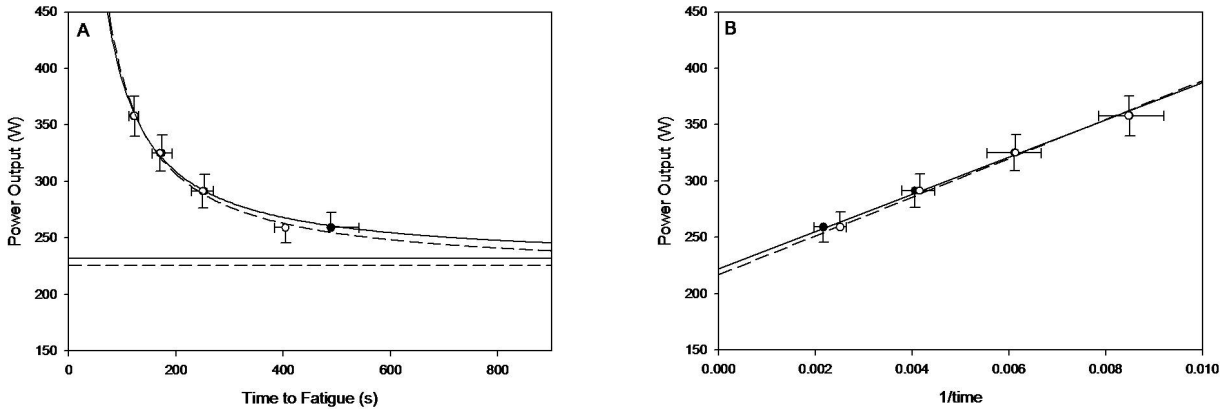

Figure 4: Hyperbolic (A) and linear (B) critical power models representing group mean ± SE. Solid circles and lines denote NAC and open circles and dashed lines refer to PLA.

Table 4: Individual CP and *W'* values derived from the linear model

Subject	CP – NAC (W)	CP – PLA (W)	*W'* – NAC (kJ)	*W'* – PLA (kJ)
1	248	239	12.9	15.0
2	211	203	12.6	14.1
3	194	188	13.7	14.1
4	230	233	15.4	14.2
5	226	211	18.4	20.9
6	282	282	12.7	12.1
7	232	223	22.8	24.4
Mean	**232***	**226**	**15.5**	**16.4**
Std Dev	**28**	**31**	**3.8**	**4.5**

* significantly different from PLA (p = 0.032)

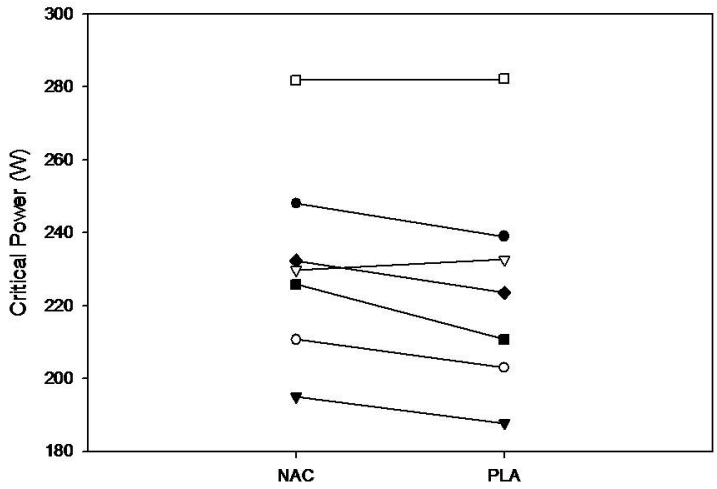

Figure 5: Comparison of critical power under the NAC and PLA conditions.

Changes in *W'* were not significant with NAC supplementation, (16.4 ± 4.5 kJ PLA versus 15.4 ± 3.8 kJ NAC; p = 0.10). The same five subjects that increased CP with NAC pretreatment experienced a decrease in *W'* while the other two subjects showed an increase *W'* (subjects 4 and 6, Table 4).

Electromyography

EMG data for the vastus lateralis and rectus femoris muscles are presented in Figures 6 and 7, respectively, and Table 5. EEôrefers to fatigue of the shorter trial and the corresponding time point of the longer trial. There were no differences between PLA and NAC for either measure. However, in the rectus femoris NAC prevented the significant decline in MdPF at both EEôand EE endpoints. Root mean square (RMS) values were significantly increased at both EEô and EE compared to 30 s in both the rectus femoris and the vastus lateralis with placebo ($p < 0.05$). With NAC, the increase in RMS seen in the vastus lateralis was not significantly different from the 30 s value.

Table 5: Electromyography Responses

Vastus Lateralis	NAC	PLA	p-value (NAC vs. PLA)
30s ï MDF	61.5 ± 15.8	63.2 ± 5.8	
EEôï MDF	65.0 ± 13.3	59.6 ± 6.5	0.098
EE ï MDF	59.3 ± 17.6	59.5 ± 6.8	
30s ï RMS	0.27 ± 0.28	0.27 ± 0.26	
EEôï RMS	0.34 ± 0.26	0.43 ± 0.37*	
EE ï RMS	0.34 ± 0.26	0.43 ± 0.37*	
Rectus Femoris			
30s ï MDF	64.3 ± 11.9	61.2 ± 10.1	
EEôï MDF	61.3 ± 10.9	56.9 ± 12.0*	0.084
EE ï MDF	61.4 ± 11.9	57.7 ± 11.3*	0.094
30s ï RMS	0.16 ± 0.11	0.19 ± 0.14	
EEôï RMS	0.31 ± 0.19*	0.36 ± 0.18*	
EE ï RMS	0.30 ± 0.16*	0.37 ± 0.17*	

Data are presented as mean ± SD. * significantly different from 30 s ($p < 0.05$)

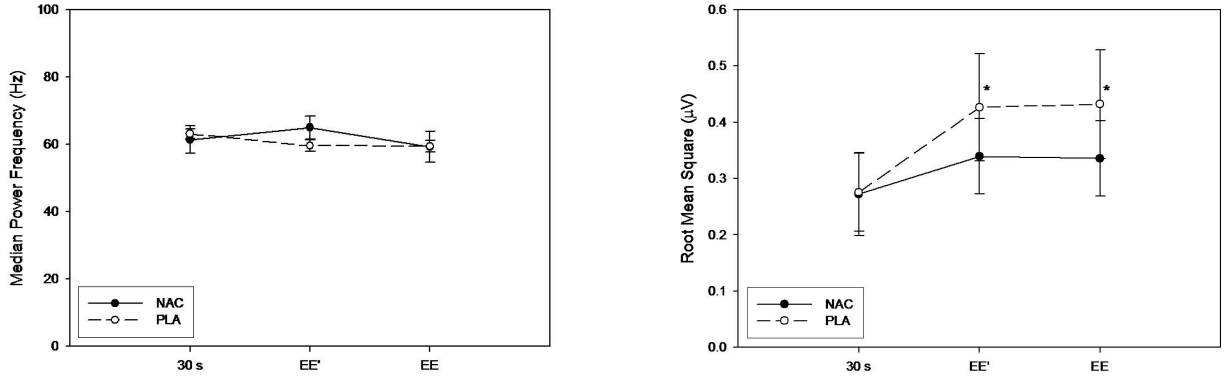

Figure 6: Group EMG data for the vastus lateralis muscle. NAC condition is depicted by the solid lines and circles and PLA by the dashed lines and open circles. NAC prevented the significant rise in RMS from 30 s to end-exercise. *significantly different from 30 s ($p < 0.05$)

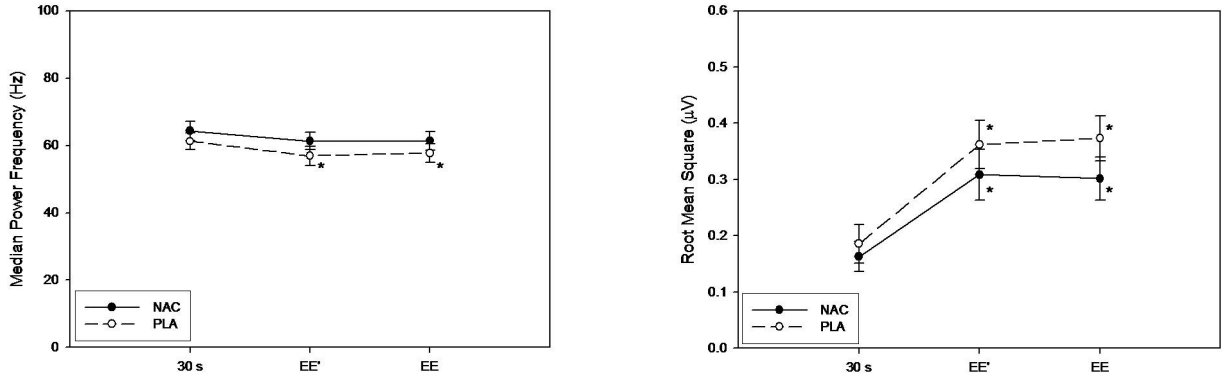

Figure 7: Group EMG data for the rectus femoris muscle. NAC condition is depicted by the solid line and PLA by the dashed line and open circles. NAC prevented the significant decline in median power frequency seen with PLA. *significantly different from 30 s ($p < 0.05$)

VO_2 Kinetics

All trials were modeled with both 2- and 3-exponential models, with the best fit accepted as determined from residual sum of squares. The VO_2 kinetic parameters are presented in Table 6. None of the relevant kinetic parameters demonstrated significant differences between the NAC and PLA conditions.

Table 6: VO_2 Kinetic Parameters

Intensity (% P_{peak})	Condition	TTF (s)	BSL VO_2 (L/min)	A_p (L/min)	A_p' (L/min)	τ_p (s)	TD_p (s)	G_p (L/min/W)
80%	NAC	489 ± 140*	0.742 ± 0.112	1.64 ± 0.20	1.88 ± 0.26	31.1 ± 17.5	15.1 ± 6.2	7.21 ± 1.26
	PLA	404 ± 53	0.786 ± 0.090	1.58 ± 0.29	1.72 ± 0.38	22.6 ± 6.7	17.8 ± 2.6	6.50 ± 1.00
90%	NAC	253 ± 44	0.822 ± 0.090	1.66 ± 0.31	1.95 ± 0.42	25.3 ± 8.5	17.5 ± 3.3	6.46 ± 1.41
	PLA	250 ± 55	0.803 ± 0.096	1.66 ± 0.20	1.90 ± 0.26	22.0 ± 6.6	20.2 ± 1.8	6.56 ± 1.15
100%	NAC	174 ± 50	0.712 ± 0.179	1.78 ± 0.36	1.70 ± 0.36	27.6 ± 10.6	16.6 ± 4.9	5.23 ± 1.31
	PLA	170 ± 39	0.813 ± 0.084	1.59 ± 0.49	1.64 ± 0.48	19.9 ± 10.0	18.4 ± 2.4	4.89 ± 1.50
110%	NAC	123 ± 23	0.798 ± 0.061	2.04 ± 0.40	1.80 ± 0.50	28.2 ± 4.3	14.7 ± 2.1	5.16 ± 1.30
	PLA	125 ± 25	0.803 ± 0.089	1.96 ± 0.29	2.31 ± 0.49	27.4 ± 6.9	17.8 ± 2.4	5.89 ± 1.30

Intensity (% P_{peak})	Condition	TTF (s)	A_{sc} (L/min)	A_{sc}' (L/min)	τ_{sc} (s)	TD_{sc} (s)	G_{sc} (L/min/W)
80%	NAC	489 ± 140*	1.12 ± 0.55	0.884 ± 0.388	280 ± 169	96.9 ± 19.6	2.93 ± 1.06
	PLA	404 ± 53	1.68 ± 1.09	0.957 ± 0.290	344 ± 250	72.7 ± 28.5	3.70 ± 1.26
90%	NAC	253 ± 44	1.43 ± 1.02	0.679 ± 0.274	224 ± 141	93.0 ± 35.9	2.35 ± 1.01
	PLA	250 ± 55	2.88 ± 4.06	0.729 ± 0.190	434 ± 559	81.7 ± 18.8	2.41 ± 0.64
100%	NAC	174 ± 50	1.12 ± 0.72	0.398 ± 0.237	209 ± 229	81.0 ± 21.9	1.32 ± 1.03
	PLA	170 ± 39	1.57 ± 0.79	0.882 ± 0.514	145 ± 88	55.9 ± 40.3	2.85 ± 1.95

*Significantly different from PLA ($p < 0.05$). Number of trials fit with a 3-exponential model: 80% NAC, N = 6; 80% PLA, N = 7; 90% NAC, N = 7; 90% PLA, N = 7; 100% NAC, N = 4; 100% PLA, N = 5.

Correlations

There was a very strong negative correlation between the change in *W'* and the change in CP (r = 0.964; Figure 8), indicating that the largest increases in CP with NAC treatment were associated with the largest decreases in *W'*. CP was correlated with P_{peak} for both NAC (r = 0.904, p = 0.005) and PLA (r = 0.891, p = 0.008, Figure 9), but the change in CP with NAC was not (r = 0.316). The change in CP with NAC was not related to VO_{2peak} (r = 0.182) or P_{peak} (r = 0.355), nor were the changes in *W'* (r =0.164 for VO_{2peak} and r = 0.305 for P_{peak}; all p > 0.05).

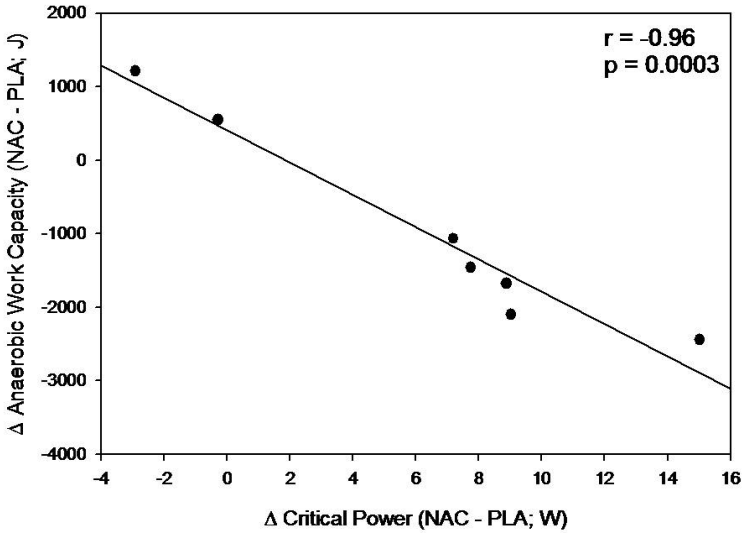

Figure 8: Correlation between the change in critical power and the change in W' following NAC pretreatment. Changes are calculated as NAC – PLA.

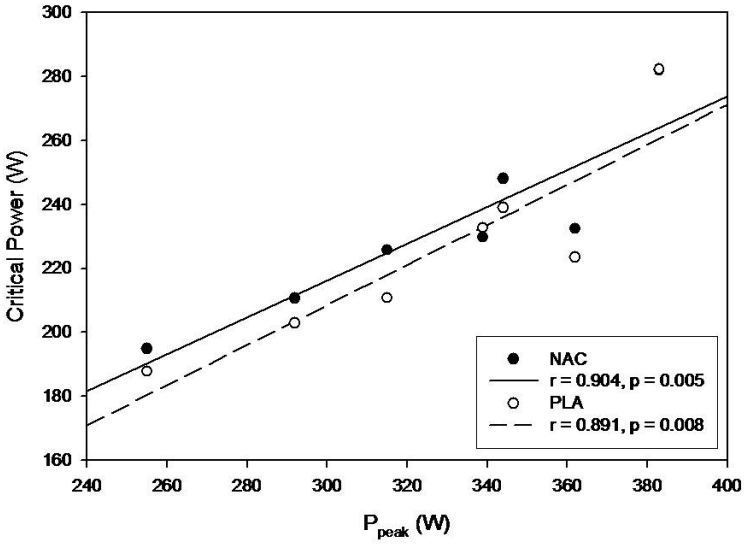

Figure 9: Correlations between critical power and P_{peak}. Solid line and circles denote NAC (r = 0.904; p = 0.005) and dashed line and open circles denote PLA (r = 0.891; p = 0.008).

There was no correlation between the change in time to fatigue with NAC administration and the change in τ_p (r = 0.123) or with the change in A_{sc} (r = 0.129). However, an inverse relationship was evident between the change in τ_p and the change in the amplitude of the slow component expressed either in absolute terms (A_{sc}, r = -0.632, p = 0.007, Figure 10) or as a gain (G_{sc}, r = -0.751, p = 0.0005, Figure 11). Delta values were calculated as NAC ï PLA.

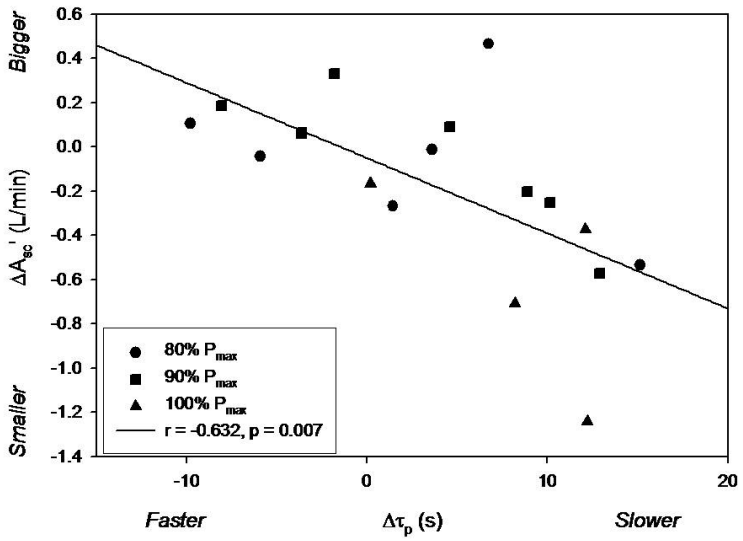

Figure 10: Inverse correlation between Δτp and ΔAsc'.

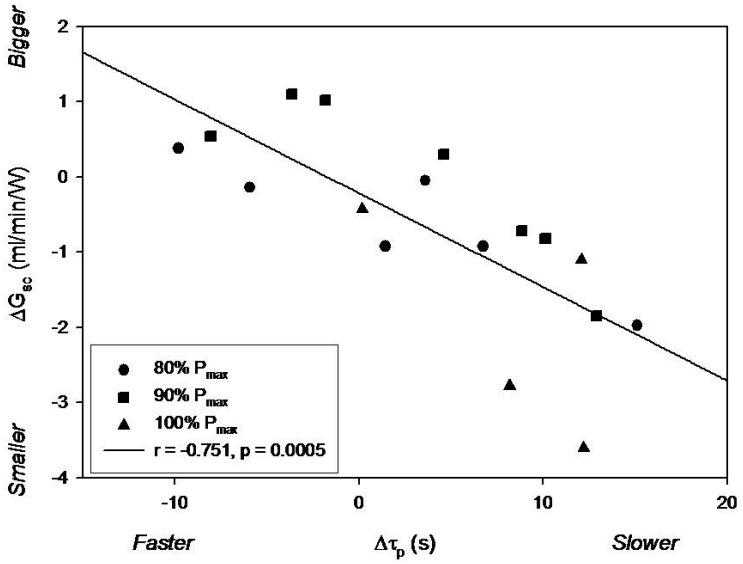

Figure 11: Inverse correlation between Δτp and ΔGsc.

CHAPTER 5 - Discussion

Major Findings

The intent of this study was to determine the effects of an acute oral dose of N-acetylcysteine on whole-body fatigue, specifically time to fatigue, critical power and *W'*. Changes in time to fatigue with NAC were intensity-dependent, with significant improvements seen only in the 80% P_{max} trials. The findings support the hypothesis that NAC would increase critical power, but contradicted the hypothesis of an increase in *W'*; rather, the changes in *W'* were not significantly different. It was also hypothesized that NAC administration would attenuate changes in the EMG responses indicative of fatigue. Consistent with this, NAC prevented significant changes throughout exercise in MdPF for the rectus femoris and in RMS for the vastus lateralis. Finally, a speeding of Phase II VO_2 kinetics and a reduction in the magnitude of the VO_2 slow component was predicted, but the results did not support this hypothesis.

Blood Glutathione

In this study, blood samples were analyzed to validate that the NAC administration resulted in an increase in antioxidant capacity. Glutathione is one of the foremost endogenous antioxidants, and its biosynthesis is augmented by N-acetylcysteine (38, 54, 176). It is for these reasons that it chosen as a proxy measurement for antioxidant capacity. Red blood cells were isolated and lysed in order for the cell contents to be assayed due to the low concentrations of glutathione in plasma found in previous studies conducted in our laboratory (109, 217). There was a significantly greater quantity of total glutathione in the pre-exercise blood sample in the

NAC versus placebo trials, with no differences at baseline or post-exercise. These changes were consistent with previous findings involving infused NAC (131). The blood samples were unable to be analyzed for reduced glutathione due to technical failure, but nonetheless it is likely that the increased levels of total glutathione were due to an increase in reduced rather than oxidized glutathione. Medved and colleagues (131) measured whole-blood NAC and reduced and total GSH before and after cycling exercise. Similar significant increases were found for these measurements, indicating that NAC was responsible for the increases in GSH.

Reid (169) proposed a model that could potentially explain why five of the seven subjects displayed an increase in critical power while two showed either no change or a slight decrease. The model describes isometric force development as a function of cellular redox status, although the principle can also be applied to time to fatigue and other indicators of performance. This model hypothesizes that there is an optimal redox state, where any deviation from this optimum results in a decrease in performance. Antioxidants and exercise (specifically the production of ROS) act on the relationship in opposite directions. The key to using antioxidant supplementation to maintain or enhance performance is finding the proper dose of antioxidant to maintain the optimum concentration of ROS during exercise. The complexity of the issue arises in determining the optimal redox state and dose to achieve this redox state, which may be unique to each individual and exercise condition.

Critical Power and *W'*

In this study, critical power averaged 226 ± 31 W in the placebo condition and increased to 232 ± 28 W with NAC administration. Expressed relative to the peak power attained in the ramp test, this translates to 69.1 ± 4.1% and 71.1 ± 4.0% for the respective conditions. In contrast, *W'* decreased from 16.4 kJ to 15.4 kJ with NAC. These values lie within the range of

previously published reports of critical power and *W'*. Reported values for critical power range from approximately 170 to 315 watts (59 to 74 % P_{max}), and *W'* values vary from 12 to 22 kilojoules (for review see (82). The wide variation in these values may be due to differences in physiological variables such as fitness level/training status and fiber type distribution, and/or testing parameters such as pedal cadence and test termination criteria.

Previous research has not evaluated the effects of NAC on critical power and *W'*, but data regarding time to fatigue have yielded inconclusive results. Pretreatment with NAC has been shown to have a improve exercise performance in protocols using low-intensity electrical stimulation (45, 111, 174, 190) and prolonged low-intensity exercise (126, 133). Contrary to this, no improvements in performance with NAC have been found using high-intensity stimulation (174) or high-intensity exercise (131) scenarios. NAC has even been shown to depress the contractile function of unfatigued muscle fibers excised from the diaphragm (111). The current study demonstrated a significant lengthening of time to fatigue with NAC administration when subjects cycled at 80% of peak power, but no differences were evident at any of the other exercise intensities. Due to the serial recruitment of type II muscle fibers with increasingly difficult exercise, this may indicate that NAC acts in a fiber-type specific manner, although no definite conclusions can be made in this regard.

The finding that NAC pretreatment resulted in a decrease in *W'* raises some issues. *W'* is thought to reflect energy stores consisting of stored oxygen, high-energy phosphates, and the energy produced from anaerobic glycolysis. It does not seem likely that an acute dose of NAC would have decreased these stores, although it cannot be ruled out since *W'* is not entirely understood. However, it is also possible that the observed decrease in *W'* occurred as an artifact of the mathematical modeling employed. From the hyperbolic relationship between time to

fatigue and power, by definition *W'* is a constant amount of work that can be performed above CP, independent of the rate at which it is expended. The finding that time to fatigue was not significantly different between NAC and PLA at the three highest intensities suggests that NAC may have altered the hyperbolic characteristic of *W'*.

Putative Mechanisms

Several mechanisms have been proposed to explain the effects of reactive oxygen species on fatigue, such as dysregulation of key ions and inhibition of essential components of the electron transport chain. The Na^{+}-K^{+} ATPase, Ca^{++} ATPase, ryanodine receptor and myofibrillar proteins all contain sulfhydryl residues that dimerize when they are oxidized. This dimerization causes a conformational change in the protein, leading to dysregulation of the ions. In a study of endurance athletes cycling at 92% VO_{2peak}, McKenna et al. (130) clearly demonstrated that Na^{+}-K^{+} ATPase activity was maintained with NAC as measured by 3-O-methyfluorescin phosphatase analysis of vastus lateralis biopsies. ATPase activity showed a smaller decline in the NAC trials compared to control at 45 minutes; however there were no differences at fatigue. Importantly, time to fatigue in with NAC was 23.8 ± 8.3% longer than control.

The effects of oxidative stress on calcium sensitivity were first demonstrated by Andrade et al. (5) using intact flexor digitorum longus fibers from a murine model. Exposure to hydrogen peroxide yielded no changes in peak [Ca^{++}] despite a dramatic decrease in tetanic force. These effects could be reversed through the application of dithiothreitol, a reducing agent. Similar effects were shown by Moopanar and Allen in two studies of muscle-derived oxidants (141, 142). In both cases, the effect was traced to a decrease in calcium sensitivity in the myofilaments.

The current study used two measurements to evaluate potential mechanisms for any effects of NAC on fatigue. Electromyography was used to ascertain any effect of NAC on patterns of muscle motor unit recruitment, and analysis of VO_2 kinetics was implemented to evaluate the possibility of an altered metabolic response with NAC pretreatment.

Electromyography

It was hypothesized that NAC administration would exert its effects to prolong exercise endurance by altering patterns of motor unit recruitment detected by EMG. Although a comprehensive study had not been conducted, previous research hinted that the effects of NAC on time to fatigue were dependent on exercise intensity. Since the contribution of type II fibers to exercise is relatively larger at higher intensities (212), this may indicate that NAC acts in a fiber-type specific manner. To assess this possibility, median power frequency and root mean square calculations were performed on EMG data of the vastus lateralis and rectus femoris muscles. The values at end-exercise were expected to be similar since fatigue should have occurred at the same physiological end-point. However, when end-exercise of the shorter trial was compared to the same time point of the longer trial, it was predicted that a marked attenuation would be evident in the longer trial. Root mean square is expected to increase over time, reflecting the recruitment of additional motor units and/or an increased activation of the already recruited motor units. In the vastus lateralis, root mean square values were significantly higher at EEô and EE compared to 30 s in the placebo condition, with no significant changes in root mean square of the NAC trials. In the rectus femoris, a significant increase was also seen in the NAC trials although the increase was substantially less than that seen in the placebo condition. Despite this, there were no significant differences in RMS between NAC and placebo at any time point. Median power frequency indicates the distribution of frequency content, allowing for an estimation of fiber

recruitment patterns. Type II fibers would be expected to initially contribute a higher frequency content than type I muscle fibers (62, 117, 203), but also to show a greater decline with fatigue (144). The observation that median power frequency was maintained at EEô in the rectus femoris may indicate a delayed fatigue of type II muscle fibers.

VO_2 Kinetics

Increased exercise tolerance, as with endurance training, is typically associated with improved VO_2 kinetics, specifically a faster Ű and a decrease in the magnitude of the slow component (28, 98). A faster Ű allows a steady state of VO_2 to be achieved more rapidly. The faster this adjustment occurs, the smaller the O_2 deficit that will be incurred at exercise onset, and the less the depletion of anaerobic energy stores. The magnitude of the slow component and the associated metabolic changes have also been associated with the fatiguing process (25).

To provide a mechanistic basis for VO_2 kinetics, Meyer (136) proposed an electrical analog model relating Ű to mitochondrial volume (representing the inverse of a resistor) and PCr (reflecting a capacitance). Through manipulations of mitochondrial content and concentration of total creatine in an in vitro preparation, Glancy et al (64) demonstrated that increases in mitochondrial content and decreases in available creatine resulted in a faster time constant for increases in oxygen utilization, consistent with this model.

The model proposed by Meyer (136) named mitochondrial density and availability of PCr as the primary determinants of Ű. However, other manipulations, such as activation of pyruvate dehydrogenase (PDH) using dichloroacetate (204) and the administration of N^{x}-nitro-L-arginine methyl ester (L-NAME; 100, 112), have been shown to alter VO_2 kinetics, albeit to a small degree. Activating PDH has the potential to speed VO_2 kinetics by increasing the rate of flux of pyruvate into the mitochondria as substrate for oxidative phosphorylation. In practice, however,

this strategy has led to inconclusive results (12, 204). Other studies have employed L-NAME to block nitric oxide synthase (NOS) and thus decrease the production of nitric oxide (NO). NO is a potent reactive nitrogen species that competitively inhibits cytochrome c oxidase (23), the terminal enzyme of the electron transport chain. Reducing this inhibition of cytochrome c oxidase is believed to allow for greater mitochondrial oxygen flux. Consistent with this, the previous studies demonstrate a faster τ_p with L-NAME (100, 112). It is possible that NAC affected one of these other determinant(s) of VO_2 kinetics, leading to the non-significant decrease in τ_p observed in the previous study.

Researchers have attempted to manipulate VO_2 kinetics using interventions such as training and administration of drugs. Numerous training studies have demonstrated a speeding of $\dot{V}_p$ (28, 70, 81, 160) and/or a reduction in the slow component (9, 28, 29, 164, 220). Carter et al. (28) found that six weeks of endurance training, employing a combination of continuous and interval training, significantly reduced the magnitude of the slow component. No differences were seen in $\dot{V}_p$ for the group. However, when subjects were stratified into groups based on their initial fitness, the low fitness group demonstrated a significant speeding of $\dot{V}_p$ post-training. Muscle mitochondrial density has been shown to be elevated with training (2, 86), which may be responsible for the faster kinetics observed. It was also proposed by Carter et al. (28) that these changes may result in fewer type II fibers being recruited, thus potentially reducing the amplitude of the slow component.

The slow component of VO_2 is thought to reflect the recruitment of type II muscle fibers (16, 162, 178, 191), which are generally regarded as being less efficient than type I fibers. Anderson and Neufer (3) recently reported that type II fibers produce relatively more ROS and have a lesser degree of antioxidant defense compared to type I fibers. These observations led to

the hypothesis that NAC administration may reduce the amplitude of the slow component due to speeding of primary phase kinetics in the previous study (217).

A pair of studies previously performed in our laboratory evaluated the effects of NAC pretreatment (1800 mg) on respiratory muscle fatigue and VO_2 kinetic parameters (109, 217). Subjects cycled at power outputs designed to elicit ~85% VO_{2peak}, with power outputs ranging from ~60 to 75% P_{peak}. To assess the effects of NAC on respiratory muscle fatigue, subjects cycled at their predetermined power output for six 5-minute bouts separated by two minutes of rest to conduct pulmonary function tests. In the NAC trials, maximal inspiratory pressure (PI_{max}) demonstrated less of a decline over the course of the protocol than control trials, indicating that respiratory muscle fatigue was delayed (109). To evaluate possible changes in VO_2 kinetics due to NAC pretreatment, subjects cycled at the same predetermined power output until fatigue (217). Time to fatigue did not change with NAC administration (NAC: 1047 ± 136 s versus CON: 1263 ± 334 s; p = 0.07). τ_p was slightly faster (p = 0.145) and A_{sc}ô was slightly larger (p = 0.179) with NAC. Although not significant, these findings were consistent with those of Jones et al. (101) using L-NAME to block the production of NO. These observations were inversely correlated (r = -0.78), meaning that subjects who displayed the largest decrease in τ_p values with NAC had the largest increases in A_{sc}ô(217). Results from the present study are just the opposite, i.e. a slower τ_p was associated with a smaller slow component with NAC. However, when the current data was combined with that of the previous study (217), a continuous relationship is observed (Figure 12). To account for the range of power outputs employed in the two studies, data were also expressed as a gain ($G_{sc} = A_{sc}$ô/ WR). These differences between the two studies may in part be due to differences in the dosage used. Martinez and Martinez (124) found that NAC affected cytochrome c oxidase in a dose-dependent manner, where low doses of NAC

increased activity but high doses reduced it. In the present study, Ű was slightly longer in the NAC condition, which is inconsistent with previous reports from our lab (217). However, the dose used in this study was much larger than the dose used in the study by Wicker (217) of the effects of NAC on VO_2 kinetics, which may account for the inconsistency in τ_p in accordance with the findings of Martinez and Martinez (124)

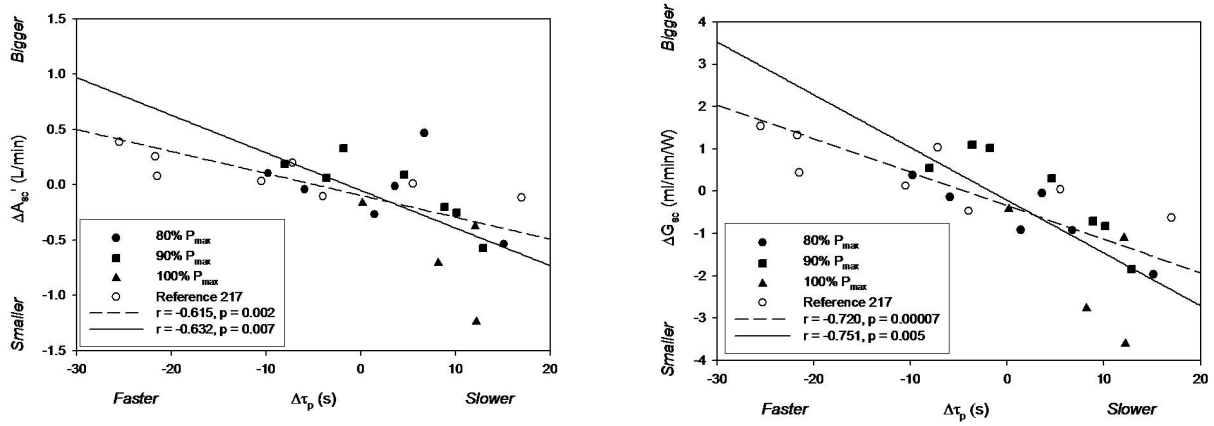

Figure 12: Inverse correlation of Δτp with ΔAsc' and ΔGsc. The solid symbols and lines indicate the results of the current study, and open symbols denote the results of Wicker et al.(217). The dashed lines indicate the regression of the combined data.

Summary

Critical power can be conceived as the highest metabolic rate in which a steady state can be achieved in VO_2 (163), lactate (163, 166), and phosphocreateine (35, 99). It has been proposed that this maximal steady state metabolic rate is dictated by a balance between glycolytic flux and oxidative phosphorylation (35). There is some data indicating that the pyruvate dehydrogenase complex (155) and cytochrome c oxidase of the electron transport chain (23) are sensitive to redox status. It is possible that attenuating the inhibition of these enzymes

essential for energy production with NAC allowed the maximum steady state (as CP) to occur at a higher metabolic rate, and thus a higher power output. Besides metabolic processes, NAC may have affected excitation-contraction coupling through the variety of redox sensitive proteins located within the myocyte. Consistent with this, our EMG data suggests less of a change in motor unit recruitment patterns associated with the fatiguing process with NAC. However, discrimination of the mechanisms responsible for the increase in critical power seen with NAC is beyond the scope of this study.

Significance

An improvement in critical power of 6 W, which was the average in this study, may appear to be inconsequential. The average values for CP were 226 W for the placebo condition and 232 W after NAC pretreatment, and the corresponding values for *W'* were 16.4 and 15.5 kJ. The effect of NAC is most pronounced at power outputs only slightly higher than critical power. For example, an individual exercising at 235 W with the average values listed above would be predicted to fatigue in approximately 29 minutes in a normal condition. However, pretreatment with NAC would increase this prediction to about 78 minutes, a difference of 49 minutes. The discrepancy narrows as power output increases until the predicted time to fatigue values are virtually identical.

This degree of improvement in CP and thus on time to fatigue could have dramatic effects in athletic competition. Even more important could be the effects in diseased populations. A person with a chronic disease such as congestive heart failure or COPD would be expected to have lower aerobic fitness levels and thus a lower absolute critical power. However, the work required to perform a particular activity remains constant, so an increase in critical power has the potential to improve a patientôs functional abilities.

Limitations

There are some limitations that should be noted as they apply to this study. First, our intent was to measure reduced glutathione in addition to total, but this was not possible due to technical failure. This would have verified that the observed increase in total glutathione was due to an increase in reduced glutathione, which is the active antioxidant form. Also, this would allow for the calculation of oxidized glutathione, which should make up a larger proportion of the total following exercise, and the increase at fatigue would be predicted to be greater in the placebo condition than in NAC. This would have allowed us to conclude that NAC attenuated fatigue due to a reduction in oxidative stress rather than some other mechanism. However, based on the studies of Medved et al. (132) and Ferreira et al.(personal communication), we believe that the increase in total glutathione was due to an increase in the reduced form and that the concentrations of oxidized glutathione were elevated at fatigue. However, no conclusions can be made about the relative proportion of reduced and oxidized glutathione in both conditions and how these proportions may have changed in an intensity-dependent manner. Second, the sample size used in this study was small, which may have reduced the statistical power. A randomized, double-blind crossover study was used to reduce the impact of this limitation. Finally, the available pharmacokinetic data using an oral administration of NAC used smaller doses than were used in this study (20, 21, 41, 153). Using that data to plot the dose versus the time-to-peak concentration (t_{max}) yields a very strong linear relationship. Extrapolation of this relationship to the dosage used in this study resulted in a predicted t_{max} of approximately 3 hours. For pragmatic reasons we did not wait until this predicted t_{max} was achieved, nonetheless after one hour, our dosing did result in a significant increase in the concentration of total glutathione in red blood cells. However, it is possible that waiting longer would have produced greater changes.

Future Directions

There are several potential avenues of research stemming from these findings. First, the effects of differing doses of oral NAC are still unclear, especially when the model proposed by Reid (169) of the effects of cellular redox state is considered. Is this why some subjects increased critical power with NAC while others did not? How can basal and optimal redox states be measured to determine the proper dosage for each individual? Secondly, *W'* is thought to consist of stored oxygen, a source of high-energy phosphates (primarily PCr), and the energy produced from anaerobic glycolysis. Why would administration of NAC decrease this energy source, and are similar results seen with other antioxidants? What effect does mathematical modeling have on *W'*? Finally, this study evaluated healthy, young, active males only. Would parallel effects also be seen with females and aged individuals? More importantly, many chronic diseases exhibit marked oxidative stress, so would NAC affect fatigue development in these conditions as well?

Conclusions

In conclusion, this study provides support for the apparent intensity-dependent effectiveness of NAC on time to fatigue. Specifically, a 70 mg/kg dose of NAC resulted in a significant increase in critical power with no significant changes in *W'*. Specific mechanisms leading to these results are still unclear, though NAC prevented significant changes in EMG measurements in some instances were significant changes were seen with placebo. Further research is warranted to understand the interactions of ROS and antioxidants in fatigue development.

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