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The effect of N-acetylcysteine supplementation on recovery of strength following eccentric muscle injury

Ryan C. Luke

ACCEPTANCE

This dissertation, THE EFFECT OF N-ACETYLCYSTEINE SUPPLEMENTATION ON RECOVERY OF STRENGTH FOLLOWING ECCENTRIC MUSCLE INJURY, by RYAN C. LUKE, was prepared under the direction of the candidate's Dissertation Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Education, Georgia State University.

The Dissertation Advisory Committee and the student's Department Chair, as representatives of the faculty, certify that this dissertation has met all standards of excellence and scholarship as determined by the faculty. The Dean of the College of Education concurs.

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ABSTRACT

THE EFFECT OF N-ACETYLCYSTEINE SUPPLEMENTATION ON
RECOVERY OF STRENGTH FOLLOWING
ECCENTRIC MUSCLE INJURY
by
Ryan C. Luke

This study determined the effect of N-acetylcysteine (NAC) supplementation on recovery of strength following eccentric muscle injury. Female subjects (n = 21, age = 20.7 ± .10 yr, weight = 68.05 ± 10.3 kg, height = 1.69 ± .07 m) performed one bout of eccentric exercise involving the forearm flexor muscles. Subjects were given a placebo (food-grade cellulose; n = 10) or NAC supplement (10 $mg \cdot kg^{-1}$ $bw \cdot d^{-1}$; n = 11) for 7D prior to and 14D following the exercise bout. Maximal Voluntary Contraction (MVC) torque, muscle soreness, range of motion (ROM), and arm circumference were measured at pre-exercise, immediately post-exercise, and at 1D, 3D, 7D and 10D post-exercise. In addition, serum interleukin-6 (IL-6), serum creatine kinase (CK), and serum glutathione were measured. Subjects also completed a food frequency questionnaire to determine the antioxidant content of their diet. There was no difference in the loss and subsequent recovery of muscular strength between the placebo and NAC group immediately post-exercise (26.93 ± 6.4 vs. 24.95 ±9.4 Nm), 1D (27.83 ± 5.7 vs. 26.9 ± 8.5 Nm), 3D (38.35 ± 6.7 vs. 34.69 ± 10.2 Nm), 7D (46.9 ± 8.8 vs. 42.5 ± 11.8 Nm), or 10D (57.83 ± 11.7 vs. 52.92 ± 14.3 Nm) post-exercise (p = .274). In addition, there was no difference in muscle soreness (p = .752), arm circumference (p = .535), ROM (p = .539), serum CK (p = .449), serum glutathione (p = .967), or serum IL-6 (p = .360) at any time point. Scores on the food frequency questionnaire demonstrated that dietary antioxidant intake was not different between groups (41.04 ± 8.04 vs. 33.01 ± 12.6; p = .054). In conclusion, a bout of

eccentric forearm flexor exercise resulted in muscle injury and a significant decrease in subjects' ability to produce force. Supplementation with NAC had no effect on recovery of strength, arm circumference, ROM, serum CK, serum IL-6, or serum glutathione at any time point following the exercise bout. These results demonstrate that NAC has no effect on recovery of strength following eccentric muscle injury.

THE EFFECT OF N-ACETYLCYSTEINE SUPPLEMENTATION ON RECOVERY OF STRENGTH FOLLOWING ECCENTRIC MUSCLE INJURY

by

Ryan Charles Luke

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ABBREVIATIONS

CK	Creatine Kinase
DOMS	Delayed Onset Muscle Soreness
IL-6	Interleukin-6
kg	Kilogram
$mg \cdot kg^{-1}$ $bw \cdot d^{-1}$	Milligrams per kilogram body weight per day
MVC	Maximal Voluntary Contraction
$pg \cdot mL^{-1}$	Picograms per milliliter
μM	Micromolar
mm	Millimeter
$mg \cdot d^{-1}$	Milligrams per day
NAC	N-acetylcysteine
Nm	Newton meter
NSAIDs	Non-steroidal anti-inflammatory drugs
$U \cdot L^{-1}$	Units per liter
VAS	Visual Analog Scale

CHAPTER 1

THE EFFECT OF N-ACETYLCYSTEINE SUPPLEMENTATION ON RECOVERY OF STRENGTH FOLLOWING ECCENTRIC MUSCLE INJURY

Introduction

Exercise induced muscle damage is regularly observed in individuals following novel or unaccustomed exercise involving eccentric muscle contractions (1). This type of muscle damage is associated with increased muscle soreness and swelling, increased intramuscular proteins in the bloodstream, and significant (but temporary) decrements in MVC torque (2). The decrease in MVC torque can persist for time periods up to (and in some cases, exceeding) one month (3).

Recovery and regeneration following eccentric muscle injury adheres to a fairly predictable timeline. The entire process is best characterized by a model describing four different stages of injury and subsequent recovery and regeneration; (1) intial, (2) autogenic, (3) phagocytic, and (4) regenerative (4).

The muscle injury process is augmented or magnified by the production of reactive oxygen species (ROS). Overproduction of ROS may overwhelm the body's endogenous antioxidant defense mechanisms and lead to oxidant stress. This oxidant stress may result in further (e.g., "secondary") damage to the muscle following the initial mechanical insult. Antioxidant supplementation has been purported to increase the body's ability to scavenge free radicals and combat production of ROS following exercise-induced muscle injury.

The desire to speed the process of recovery from muscle injury or reduce the symptoms of soreness, swelling, and/or decrements in MVC torque have led some researchers to investigate the effects of supplementation with antioxidants on symptoms of exercise-induced muscle damage (5, 6). However the results of these studies have been equivocal (5, 7).

Exercise-Induced Muscle Damage

Causes

Exercise induced muscle damage is a common occurrence and can be the consequence of a broad range of activities (8-10). This type of muscle damage is particularly associated with events requiring the performance of eccentric muscle contractions (1, 11). Eccentric muscle contractions occur when a contracting muscle is forcibly lengthened. These types of contractions take place when an individual endeavors to stop or slow their body or a segment of their body when it is already in motion (e.g., lowering a barbell during a barbell biceps curl). In contrast, a concentric contraction occurs when a contracting muscle shortens while developing tension and an isometric contraction occurs when a muscle remains at the same length while developing tension which can cause injury (12). All of these muscle actions are components of daily movement. However, previous studies have demonstrated that when compared with repetitive execution of concentric and isometric contractions, repetitive execution of eccentric muscle contractions will result in significantly greater muscle damage (11, 13-15).

The high forces associated with eccentric muscle contractions make them particularly injurious. Force production during eccentric contractions can be 80-100% higher than forces produced during isometric or concentric muscle contractions (3). This type of muscle action is associated with higher forces because the external force applied can overcome the ability of the muscle to resist that force (16). As a result, the contracting muscle is forced to lengthen. This lengthening occurs while additional tension is produced. When a muscle is forced to lengthen at a slow velocity, tension can reach 125% of maximal isometric tetanic force (16). Although these contractions are associated with much higher forces, it is interesting to note that energy demands are lower and fewer motor units are recruited during eccentric muscle contractions (17). Therefore, during an eccentric muscle contraction, a smaller number of motor units (when compared to those active during concentric and isometric contractions) are exposed to significantly greater forces.

Symptoms

A decrease in force generating capability, as measured by MVC torque, is thought to the most accurate and reliable indicator of eccentric contraction-induced muscle damage because a reduction in MVC torque persists until the muscle returns to an undamaged state (18, 19). The magnitude of MVC torque loss following the injury protocol is dependant on the workload encountered during the injurious bout of exercise. If the load encountered during the eccentric contraction protocol is adequate, the immediate post-injury MVC torque deficits can be significant. In humans, performance of two sets of 35 maximal eccentric contractions of the forearm flexors has been shown to result in immediate, post-injury isometric force deficits greater than 50%. However, in

the same study a protocol of 24 maximal eccentric contractions was performed with the opposite limb. This injury protocol resulted in much smaller changes in values for immediate post-injury MVC torque and MVC torque had fully recovered by 2 days post-injury while the 70-contraction protocol experienced significantly greater reductions in MVC torque and had not completely recovered at 5 days post-injury (10). A large number of additional studies (human and animal) utilizing effective eccentric injury protocols have also demonstrated that performance of eccentric contraction protocols can result in post-injury isometric force deficits in excess of 40-50% when compared to pre-injury isometric force measurements (14, 20-22). If the workload during the eccentric contraction protocol is great enough, the impaired function (e.g., MVC torque, isometric tetanic force) may persist for some time. Depending on the severity of the injury, the time required for recovery to pre-injury MVC torque or isometric tetanic force levels can range from 10-14 days post-injury to 30 days post-injury for both animals and humans (3, 14, 23).

The muscle soreness experienced following eccentric muscle injury is termed Delayed Onset Muscle Soreness (DOMS). This condition is the result of a grade one muscle strain injury resulting from small micro-tears in the muscle (24). As its name would suggest, DOMS does not occur immediately following eccentric muscle injury. The temporal sequence of DOMS usually begins 6-12 hours post-exercise when the injury presents as tender or aching muscles that are apparent during palpation or movement (25). Peak pain usually occurs between 48-72 hours post-injury and usually subsides 8-10 days post-injury (4, 14, 26). There is a great deal of research examining DOMS but there is not a clear single mechanism that can be attributed to the onset and

persistence of pain following eccentric muscle injury (24). Proposed mechanisms have included lactate accumulation (27), involuntary muscle spasms (28), connective tissue damage (29), muscle tissue damage (30), inflammation (31), and increased muscle temperature (32). A common factor among these proposed mechanisms is the increased production of free radicals (24). This commonality points to a possible reason for the attenuation of DOMS that has been associated with supplementation of antioxidants following eccentric muscle injury (33). However, the precise nature of the relationship between free radical production and DOMS has not been determined.

It has been suggested that swelling and edema are another possible cause of DOMS because of the potential for pain caused by increased muscular pressure. However, studies investigating this connection have found that the greatest swelling occurs when soreness is subsiding (14). In addition, Newham & Jones (1987) did not find an increase in muscular pressure following eccentric muscle injury when DOMS was most apparent (34). Measurement of muscular swelling is accomplished by comparing girth measurements of the injured muscle at different time points. Human studies involving injury of the forearm flexors have found that swelling gradually develops in the days following eccentric muscle injury and peaks at five days post-injury (14).

As previously stated, eccentric muscle injury results in an increase in intramuscular proteins in the bloodstream (35). For the purposes of this review, the focus will be on Creatine Kinase (CK) and Interleukin-6 (IL-6). Previous studies have demonstrated that these proteins are following an acute bout of eccentric exercise (36-38).

The most commonly studied of these proteins is CK. Normal resting CK levels are typically between 50 and 250 U/L (34). Muscle injury (especially eccentric contraction-induced muscle injury) can result in a dramatic rise in serum CK levels. Eccentric exercise of the forearm flexors has resulted in average post-injury CK levels of 6,000 U/L (38); a 2,380-11,900% rise above baseline levels. The CK response to eccentric exercise is clearly significant. However, the magnitude of changes in CK are very poorly correlated with the magnitude of decreases in MVC torque. Serum CK levels normally show a delayed response to eccentric muscle injury, peaking at 2-4 days post-injury (14), while MVC torque (or isometric tetanic strength) values are immediately depressed. In one study (n = 109) utilizing eccentric contraction-induced injury of the forearm flexors, CK levels peaked at 4 days post-injury when post-injury MVC torque was reduced by >50% (14). In addition, Warren et al., (1999) calculated that the percent variance in MVC torque accounted for by variance in serum CK was only moderate (18). Further support for the dissociation between the variables of CK levels and MVC torque comes from studies examining the repeated bout effect. These studies demonstrate an elimination of the increase in CK levels following eccentric muscle injury while the immediate decrements in MVC torque were nearly identical (34, 39).

There is a large amount of inter-subject variability in the CK response (18). The previously mentioned study by Nosaka et al., (1992) saw an average rise in CK levels from 60 U/L to 6,000 U/L. However, the variability displayed between subjects was quite large with one subject presenting a CK level of 25,000 U/L. This variability, when combined with the small sample sizes utilized by many studies, contributes to the relative unpredictability of the magnitude of the CK response following eccentric exercise for

individual subjects (14). These findings suggest that caution should be used when interpreting results associated with serum CK response to eccentric contraction-induced muscle injury (18).

Interleukin 6 was the first cytokine identified as a "myokine"; a cytokine produced from muscle (40). It is primarily a pro-inflammatory cytokine that possesses some anti-inflammatory properties. Interleukin 6 functions to increase muscle protein breakdown by reducing circulating levels of insulin-like growth factor 1 (IGF-1) while inducing myoblast proliferation and differentiation (41, 42). It is significantly elevated with eccentric contraction-induced muscle injury, and precedes the appearance of other cytokines in the circulation (43). In fact, IL-6 seems to be the cytokine most consistently elevated in response to trauma. During exercise, it is released into the circulation (can increase up to 100 fold) to promote liver glycogenolysis (44). The size of the workload and the muscle mass involved during eccentric contraction-induced muscle injury seem to play a role in post-injury serum IL-6 levels. In support of this assertion, Nosaka and Clarkson, (1996) had male subjects perform 24 maximal eccentric contractions of the biceps brachii and found no changes in serum IL-6 levels (45). In contrast, Smith et al., (2000) had male subjects perform 4 sets (12 repetitions per set) of eccentric bench press and leg curl exercises at an intensity equivalent to 100% 1RM and found significant increases in IL-6 at 12, 24, and 72 hours after exercise (46). Other works have clearly demonstrated the elevation of serum IL-6 following eccentric contraction-induced muscle injury. However, when compared to prolonged exercise, eccentric contraction-induced muscle injury results in a delayed peak and a slower decrease of serum IL-6 during recovery (47). It is also important to note that contracting skeletal muscle is the primary

source of serum IL-6 (48). Therefore, it is not surprising that exercise involving a limited muscle mass (e.g., eccentric contraction-induced muscle injury of the forearm flexors) may be insufficient to increase plasma IL-6 above pre-exercise levels (45, 47).

Mechanism

It is commonly accepted that the higher forces associated with eccentric muscle contractions result in muscle damage. However, there is disagreement concerning the primary mechanism behind immediate post-injury signs of muscle damage (i.e., decreased contractile force). The presence of disrupted sarcomeres indicates that damage to force generating and force bearing structures may be responsible for the loss of force production (3, 49). However, strength losses have been shown to be 2.5-7.6 times greater than histopathology results would suggest (3). Therefore, immediate post-injury force deficits cannot be completely or primarily responsible for initial force deficits. It is most likely that initial decreases in force are primarily due to a disruption in excitation-contraction (E-C) coupling, resulting in an inability to activate contractile proteins in the muscle tissue (3, 50). The loss of force generating and force bearing structures accounts for a greater portion of the deficits in isometric strength as the recovery process progresses. Strength deficits can be completely attributed to contractile protein loss by 14 days post-injury (21, 51, 52).

Although there is not complete agreement regarding the exact mechanism behind initial force deficits following eccentric muscle injury, it is commonly accepted that mechanical injury is the primary initiating event causing the immediate post-injury decline in force (26). It is apparent that the degree of injury in muscle tissue is associated

with the tension produced during eccentric contractions, thus supporting the role of mechanical factors in the initial stages of muscle damage (4, 26, 53). It also has been argued that the initiating event in eccentric muscle damage is metabolic in nature. However, the possibility of a metabolic contribution to the injury process during eccentric contractions seems unlikely due to the smaller energy demands of these muscle actions (17).

The immediate/initial trauma associated with eccentric muscle contractions has been termed "microinjury". This term was adopted because the initial sites of injury are primarily sub-cellular and affect a relatively small percentage of the total number of fibers in the muscle (4). However, during the time period immediately following muscle injury, further myofibrillar damage occurs. This damage is thought to be the result of a disruption in intracellular calcium homeostasis (1, 54-56). A two-part model gives a simple picture of the phases of muscle damage; (1) the initial phase involving the major muscle damage that occurs during the exercise bout and (2) the secondary damage that occurs through processes associated with disruptions in calcium homeostasis and the ensuing inflammatory response (57).

However, a different model of the overall response to eccentric muscle injury provides a more complete picture of muscle damage and subsequent recovery. This model involves four distinct stages; the initial stage (1) includes the trauma-inducing event that activates the degenerative and regenerative phases in the injury and recovery processes. The autogenic stage (2) lasts for 4-6 hours and immediately follows the injury-inducing event. This stage is associated with an increase in activity of the indigenous proteolytic and lipolytic systems, resulting in an increase in degradation of

cellular structures within the muscle fiber. The phagocytic stage (3) occurs from 4-6 hours after injury and lasts for 2-4 days following injury. It is marked by the manifestation of a distinctive inflammatory response in the muscle. There is no distinct delineation between the degenerative processes (stages 1-3) and the regenerative stage (4), but there is clear evidence of myofiber regeneration by 4-6 days post-injury and by 10-14 days most of the injured muscle fibers appear normal (4).

To induce eccentric muscle injury in a laboratory setting, researchers have utilized various activities such as forearm extension, knee flexion, knee extension, eccentric stepping, or downhill running (23, 58-61). However, due to their lower frequency of use, the forearm flexors and extensors are more susceptible to damage than the knee flexors and extensors (61). Regardless of the muscle or muscle group that is injured, the damage manifests itself in the form of local pain/soreness, swelling, an increase in intramuscular proteins in the blood, inflammation, and a temporary decrement in function of the injured muscle (i.e., decreased force or torque production) (3, 14). These symptoms will be discussed below.

Skeletal Muscle Recovery from Eccentric Injury

Skeletal muscle repair is a synchronized process involving the activation of various cellular responses. Following the initial (1) stage of muscle injury (actual injurious event), the autogenic (2) phase of muscle injury is marked by indigenous cellular systems that begin to degrade damaged tissue. This stage is followed by the phagocytic stage (3) identified by the “typical” inflammatory response of neutrophils and

macrophages. This stage precedes and overlaps with the regenerative (4) stage where muscle repair, regeneration, and growth are evident (4).

Initial Stage of Muscle Injury

The initial stage of muscle injury involves the traumatic mechanical event that triggers the various degenerative and regenerative phases involved in the injury process. The immediate events or symptoms associated with this stage are most likely the result of mechanical disruption in the sarcolemma, sarcoplasmic reticulum (SR), connective tissue, and contractile and E-C coupling proteins in the muscle fiber (4). The high specific tensions associated with eccentric contractions result in mechanical disruption of these tissues. This mechanical disruption may disrupt calcium (Ca^{2+}) homoestasis and permit phospholipase A_2 to come into contact with phospholipid substrates in the cell membrane resulting in lysis of structural components in the sarcolemma (62). Phospholipase A_2 possesses a Ca^{2+} binding site and its activity increases as a function of Ca^{2+} concentration (63). Increased activity of this enzyme can have several detrimental effects on the cell, including production of detergent fatty acids and lysophospholipids (64).

Regardless of the mechanism and exact focal point, it is apparent that muscle fibers are acutely injured during eccentric exercise. Histological data support this assertion. Ogilvie et al., (1988) have identified three types of lesions muscles damaged by eccentric contractions: (1) focal disruptions of the A-band, (2) Z-line dissolutions, and (3) clotted fibers (65). In addition, data from other laboratories indicates damage to E-C coupling proteins immediately following eccentric contraction-induced muscle injury (66). The combination of these factors and the processes involved in the

"secondary" injury phases results in an immediate and prolonged decrease in MVC torque, increased muscle soreness, muscle swelling, and intramuscular proteins in the bloodstream (10, 67).

Autogenic and Phagocytic Stages of Muscle Injury

The inflammatory process following the initial stage of muscle injury (autogenic and phagocytic stages) is predictable. It has been suggested that the relationship between inflammation and muscle repair, regeneration, and growth is mechanistic in nature (24). Some of these processes disrupt muscle homeostasis and others promote muscle repair and regeneration (68). Following modified muscle use and/or injury, neutrophils and macrophages rapidly invade the injured tissue.

Neutrophils invade muscle tissue within one hour after eccentric muscle injury (69), and concentrations can remain elevated for up to five days (70). Studies utilizing injury resulting from ischemia and reperfusion have yielded results suggesting that neutrophils are partly responsible for damage in healthy muscle (71). This damage may be phagocytic in nature (21). It may also result from the release of proteases that degrade cellular debris, and/or the release of cytolytic and cytotoxic molecules that can damage muscle (72). Neutrophils may also cause further post-injury muscle damage via neutrophil-derived ROS (73). This is significant because it has been purported that ROS play a role in the initiation and progression of muscle fiber injury after the initial mechanical insult (74). Regarding this theory, it is important to note that the extent to which neutrophils damage muscle is governed by the redox status of the muscle (75). Eccentric muscle contraction causes increased release of superoxide and hydroxyl

radicals that can react with other circulating compounds to form more cytotoxic agents (76).

The exact role that neutrophils play in the regenerative process has not been determined. However, recent research suggests that neutrophils may cooperate in the repair and remodeling process through oxidative or proteolytic modification of damaged tissue. This modification serves to allow subsequent phagocytosis of debris by neutrophils or macrophages (67). In support of this postulated role, studies examining myofiber regeneration in animals depleted of neutrophils show more tissue debris in muscles following recovery from injury; suggesting the possibility that the impaired capacity to oxidatively modify and remove tissue debris (via ROS-mediated damage and subsequent phagocytosis) could slow the regenerative process (77). In addition, other animal experiments have shown that reduced neutrophil invasion into injured muscle or reduced neutrophil production of free radical oxidants results in reduced membrane lysis during inflammation (73). Considering these data, it is possible that neutrophil-mediated damage to cellular structures is a necessary feature of muscle growth and repair.

In injured muscle, the invasion of neutrophils is followed by the appearance of macrophages (78). There is currently a limited understanding of the specific role that macrophages play in muscle following eccentric exercise-induced injury. However, it has been demonstrated that macrophages play a major role in promoting muscle damage (in vitro and in vivo) and that their cytolytic capacity is increased by the presence of neutrophils (79). The first macrophages to enter injured skeletal muscle fibers are of a phagocytic phenotype. Their concentration is highest at damaged sites in injured muscle and their numbers decline at the end of the phagocytic stage of muscle injury (80).

Macrophages may also play a role in muscle repair and remodeling by increasing the rate of proliferation of myoblasts and/or by increasing the number of MyoD-expressing cells (81). Another possible connection between macrophage invasion and muscle repair and regeneration comes through studies investigating the effect of NSAID ingestion on the rate of muscle regeneration. NSAIDs inhibit COX-2 formation; which has been shown (in vitro and in vivo) to reduce the rate of muscle repair following injury. This reduced rate of muscle repair coincides with a reduction in the number of macrophages in the injured muscle during regeneration (82-84).

The first three stages of muscle injury have been discussed; the following portion of this review will focus on the regenerative (4) stage of muscle injury and its reliance on satellite cell activation, proliferation, and differentiation.

Regenerative Stage of Muscle Injury

The processes involved in the regenerative (4) stage of muscle repair, regeneration, and growth involve the activation, proliferation and eventual differentiation of myogenic cells known as satellite cells. These cells are the main source for myofiber repair in adults and are an extremely effective local cellular repair system (85). They are also responsible for replenishment of the satellite cell pool following eccentric muscle injury (86). Therefore, the activation, proliferation, and differentiation of satellite cells is an important part of this process. This sequence is controlled by a number of signals.

It has long been apparent that healthy adult skeletal muscle has an extensive capacity to regenerate following exercise induced injury. This capacity is mainly due to skeletal muscle satellite cells. These cells were first described by Alexander Mauro in

1961 (87). Satellite cells are normally quiescent and are located in a space between the basal lamina and the sarcolemma of a muscle fiber (88). Activation can be the result of signals originating from their associated muscle fiber or from the microenvironment. These signals include injury (89), degenerative disease (68), extrinsic mechanical stretch to the muscle fiber (86), secreted growth factors (90), or an increase in Nitric Oxide (NO) levels (91). Regardless of the initiating signal, it is clear that activation is essential to initiating muscle growth and repair (88). If activation does not occur, strength declines, muscles atrophy, and disability arises (92).

Following activation, satellite cells can divide into self-renewing cells and/or committed skeletal myoblasts (93). To do this, satellite cells will leave their quiescent area and migrate outside of the basal lamina in order to proliferate, fuse, and differentiate to generate new muscle fibers or repair damaged ones (94) Most committed cells (cycling myoblasts) become activated, leave the space between the basal lamina and the sarcolemma of the muscle fiber and proliferate (85). Therefore, a majority of these myoblasts differentiate and contribute to new fiber formation. However, some activated myoblasts can return to quiescence, thus replenishing the satellite cell pool (86). In a healthy, disease free individual, satellite cells are only activated following mechanical activity or muscle trauma (95).

It is possible to inhibit or attenuate satellite cell activation, proliferation, or differentiation in healthy individuals following exercise-induced muscle injury. Previous research suggests that treatment of muscle injury symptoms via NSAID ingestion results in attenuated satellite cell activation and protein synthesis (96, 97). This attenuation of satellite cell activity has been attributed to inhibition of cycoloxygenase (COX) activity,

which governs prostaglandin synthesis. In regards to NSAID treatment following muscle damage, it is clear that altering the inflammatory response via pharamacoligical intervention is contraindicated.

The effect of NSAID treatment following exercise-induced muscle injury is well documented. However, muscle regeneration also depends on other cellular processes (besides prostaglandin synthesis) involving non-muscle cells. One of the earliest of these processes is the inflammatory response. The inflammatory response facilitates myogenesis via phagocytosis of cellular debris and the release of chemoattractants and growth factors. Many aspects of muscle regeneration remain unclear and most likely involve molecules that have yet to be defined.

Antioxidants

Antioxidants can be classified as enzymatic and non-enzymatic. Enyzmatic antioxidants comprise the body's natural defense system against oxidants (e.g., ROS). Non-enzymatic antioxidants are obtained from food or other dietary sources (e.g., antioxidant or multi-vitamin supplements). Enzymatic antioxidants combat oxidants (e.g., ROS) in a very specific manner while non-enzymatic antioxidants are less specific (98). Antioxidants function in complex synergy in order to protect cells against ROS-induced damage. Both types of antioxidants have intracellular and extracellular sites of action. However, the sites of action for enzymatic antioxidants are primarily intracellular (99). Typically, antioxidants protect against ROS by converting ROS into less reactive molecules (known as scavenging) or by preventing the transformation of less reactive ROS into more highly reactive forms (100). This review will focus on antioxidants most

often used in research examining the effect of supplementation on symptoms of exercise-induced muscle injury.

Vitamin C

Vitamin C exists in two forms; (1) as L-ascorbic acid or in its oxidized form L-dehydro-ascorbic acid (101). Ascorbic acid is a water-soluble antioxidant vitamin that readily donates electrons to neutralize ROS (102). It has two primary functions as an antioxidant; (1) to directly scavenge specific ROS and lipid hydro-peroxides and (2) helping recycle vitamin E from its radical form (100). Vitamin C also recycles uric acid, glutathione, and β-carotene from their radical forms (103).

As a water-soluble vitamin, ascorbic acid is easily absorbed but it is not stored in the body (104). It is transported in the plasma and located in the cytosolic compartment of neutrophils, monocytes, and lymphocytes. Very small amounts have also been found in the cytosol of skeletal muscle fibers (101). Vitamin C depletion-repletion studies have indicated that 100 mg/day of ascorbic acid results in saturation of neutrophils, monocytes, and lymphocytes. Plasma concentrations of ascorbic acid continue to increase with doses up to 2500 mg/day. However, plasma concentrations reach levels nearing saturation at a dose of 400 mg/day (105).

Vitamin E

Vitamin E is a lipid-soluble antioxidant. It is absorbed in the small intestine and exists in eight different natural forms. The most biologically active form is α-tocopherol

(101). This is the form of vitamin E that is most often used in studies examining the effect of supplementation on symptoms of eccentric contraction-induced muscle injury.

Vitamin E is transported in plasma lipoproteins and, as a fat-soluble vitamin, is able to be stored in the body (106). Intense exercise has been shown to increase plasma α-tocopherol levels; suggesting that it is mobilized from other tissues in response to exercise (107).

Vitamin E functions as a chain-breaking antioxidant by stopping progression of the lipid peroxidation chain reaction. It also acts as a scavenger of superoxide, hydroxyl and lipid peroxyl radicals (100). This scavenging activity results in formation of a radical form of vitamin E that can be regenerated into a non-radical form by vitamin C (103).

N-Acetylcysteine (NAC)

N-Acetylcysteine (NAC) is a contributor of antioxidant thiols (108). A thiol is an organo-sulfur compound that contains a carbon-bonded sulfhydryl group (109). N-acetylcysteine is used in clinical practice to facilitate glutathione biosynthesis, thereby improving the enzymatic antioxidant defense system and possibly decreasing the damaging effects of ROS (108). It acts as a scavenger of ROS including hypo-cholorous acid, hydroxyl radical, and hydrogen peroxide (110). Furthermore, NAC has been shown to decrease free radical production and oxidative stress at rest and during prolonged exercise (111). Therefore, it is possible that NAC supplementation may reduce the oxidative stress and muscle soreness induced by eccentric exercise.

Previous studies examining the effect of NAC supplementation on symptoms of exercise-induced muscle injury have utilized a NAC dosages of 1,800 mg/day (112), and

10 mg/kg of bodyweight (113, 114). The dosage of NAC utilized in clinical practice is commonly between 600-1500 mg/day (115-117). At dosages of 1,200 mg twice daily or lower, NAC is well tolerated. However, doses higher that this are associated with a much higher rate of unpleasant side effects such as nausea, cramps, and diarrhea (118).

Effect of Pharmacological Intervention on Muscle Injury

The role of ROS in secondary damage following eccentric contraction-induced muscle injury points to a potential justification for pharmacological intervention via antioxidant supplementation or ingestion of NSAIDs. However, treatmen A reduction in ROS-induced muscle damage would possibly serve to reduce symptoms associated with eccentric contraction-induced muscle injury.

Reduction in Free Radical Formation

In an attempt to examine the effect that reduction of free radical formation has on the secondary injury process, Nguyen et al., (2003) found that a decrease in superoxide production was associated with a reduction in mouse muscle membrane damage; revealing a possibly causal relationship (119). These findings seem to agree with those of Smith et al., (1989) who found that administration of the superoxide combatant superoxide dismutase (SOD) was associated with a decrease in muscle damage following exercise-induced muscle injury (120). In an investigation examining the role of ROS in muscle damage and inflammation, Aoi et al., (2004), concluded that delayed-onset muscle damage induced by prolonged exercise is partly related to inflammation via phagocyte infiltration caused by ROS. When these researchers administered doses of α-tocopherol (an antioxidant), they found that this substance had the ability to attenuate

inflammatory changes normally associated with muscle injury (121). Weinheimer et al., also demonstrated that inhibition or attenuation of the inflammatory response resulted from NSAID ingestion. In this case (and as previously stated), it was concluded that the drugs inhibit the formation of prostaglandins; which are important mediators of the inflammatory response (122). These data suggest that alterations in the inflammatory response via antioxidant supplementation or treatment with NSAIDs may attenuate the inflammatory process following eccentric contraction-induced muscle injury.

Non-Steroidal Anti-Inflammatory Drugs

In many situations, attenuation of the inflammatory response would seem to be a positive outcome and studies such as this would seem to offer support for use of antioxidant supplements or NSAIDs during recovery periods. Attenuation of this response could potentially reduce symptoms such as swelling, soreness, and stiffness. A reduction in these symptoms could result in a faster return to training and/or competition.

Promoting the acceleration and improvement in recovery has been the aim of many researchers, coaches, athletes, and casual exercisers. In their own way, each of these groups has attempted to determine if the healing and recovery process can be altered (through pharmacological agents, supplementation, additional rest, and/or additional exercise) in order to improve performance by accelerating and/or aiding recovery from exercise induced muscle injury (57). The previously mentioned studies demonstrate that NSAID treatment and/or antioxidant supplementation may reduce the inflammatory response following exercise induced muscle injury. However, what impact

does this attenuation have on recovery of muscle function following exercise-induced muscle injury?

Studies examining treatment of the symptoms of exercise-induced muscle injury via ingestion of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) suggest that the inflammatory process (e.g., the initial stages of necrosis and phagocytosis of injured myofibers) plays an important role in the recovery process (121, 123-125). As previously stated, the initial stages of the inflammatory process involve an increase in the production of ROS (121). In order to determine the effect of a reduced inflammatory response on satellite cell activation, Mackey et al., (2007), and Weinheimer et al., (2007) examined the effect that NSAID ingestion has on satellite cell activation in trained athletes. Both of these previously mentioned studies found that ingestion of anti-inflammatory agents was associated with a significant decrease in satellite cell activation and protein synthesis following endurance exercise or exercise-induced muscle injury (124, 125). Therefore, these studies suggest that a reduction in the inflammatory response may inhibit/reduce satellite cell activation following muscle injury.

Considering the previously mentioned research it is reasonable to conclude that attenuation of the inflammatory response would lead to an initial acceleration in the recovery process followed by a reduced rate and volume of muscle repair and regeneration in injured skeletal muscle (95). The previously mentioned findings lead to the conclusion that ingestion of NSAIDs in an attempt to reduce inflammation following exercise induced muscle injury may lead to an attenuation in recovery of muscle function.

As previously stated, antioxidants have shown some promise as ergogenic aids because of their ability to neutralize muscle derived oxidants, such as ROS. Reactive

oxygen species may play a causal role in muscle fatigue. This role has been termed "redox braking" (126). However, the observed inhibition of redox braking has possible negative side effects. This "braking" has been proposed as a possible protective mechanism in exercising muscle. Redox braking is thought to promote muscle fatigue in order to limit the amount of muscle damage due to continued use. If this phenomenon is a protective mechanism in working muscle, then overriding this mechanism would most likely lead to increased muscle injury and increased recovery time (126). In this case, the advantages of prolonged muscular activity and reduced fatigue would seem to be outweighed by the disadvantages of increased muscle injury.

Antioxidants

Attempting to attenuate or inhibit the production of free radicals during recovery from exercise induced muscle injury may present a similar situation. Conclusions on this topic are difficult as research dealing with the effect of antioxidant supplementation on muscle repair and regeneration following eccentric contraction-induced muscle injury is equivocal in nature and the factors involved in the muscular healing process and their connection to each other is still a new research area in laboratories around the world (123).

As previously stated, the autogenic (2) and phagocytic (3) stages of eccentric muscular injury are associated with an inflammatory response in which phagocytosis and neutrophil respiratory burst result in the production of ROS (4). Production of ROS imposes an oxidative stress upon the tissue (35). Therefore, ROS have been implicated in the "secondary" damage that follows the initial mechanical insult. The exact nature of

the relationship between ROS production, eccentric contraction-induced muscle injury, and muscle soreness is unclear (24). However, recent data suggest that attenuation of neutrophil-mediated ROS production and secondary damage in injured muscle may have negative consequences (77).

Non-enzymatic antioxidants have been proposed to reduce ROS production and thereby reduce the secondary damage following eccentric contraction-induced muscle injury that is associated with ROS production (24). Most of the research to date has focused on the effects of the antioxidant vitamins C (ascorbic acid) and E (tocopherol) (74). Very few studies have investigated the effects of NAC on recovery of MVC torque, soreness, swelling, and inflammation following eccentric contraction-induced muscle injury.

Animal Studies

There are many notable animal studies that have examined the effect of antioxidant supplementation on protection and recovery from exercise-induced muscle damage. These studies usually involve supplementation for the period prior to an exercise bout with no post-exercise supplementation. These studies usually involve vitamin E exclusively because many animals can (unlike humans) synthesize vitamin C (127). Therefore, investigators do not normally provide vitamin C to animals. Vitamin E has been purported to reduce intramuscular protein diffusion by improving membrane integrity (74).

Many of these animal studies have utilized downhill walking, running or endurance exercise protocols to induce muscle injury. Warren et al., (1992) investigated

the effect that five weeks of pre-injury vitamin E supplementation in rats had on indices of muscle damage following 150 minutes of downhill walking. Injury in soleus muscle was determined by reductions in in-situ maximal tetanic force (~20%), number of intact fibers per square millimeter and/or elevations in muscle glucose 6-phosphate dehydrogenase activity and plasma CK activity. Measurements were taken either immediately or 2 days following the exercise intervention. Although solues muscle vitamin E levels were significantly higher in the supplementation group, they showed no attenuation of muscle damage in any of the markers of muscle injury when compared to the control group (128). These results suggest that pre-injury vitamin E supplementation does not exert a protective effect on muscle tissue and has little effect on the immediate and early (2d) symptoms of exercise-induced muscle injury. This study did not include measurements at later time points, therefore it is not possible to form conclusions about the effect of vitamin E supplementation on the later stages of muscle recovery and regeneration.

Unfortunately, many of the exercise interventions utilizing downhill walking, running, or other forms of endurance exercise do not incur significant muscle injury in animals as a result of the prescribed exercise. Instead, these studies focus on the contractile properties and/or the oxidative status of muscle immediately following fatiguing or endurance exercise. Coombes et al., (2001) attempted to determine the effects of vitamin E and α-lipoic acid on muscle contractile properties. Rats were supplemented with either vitamin E or α-lipoic acid for 8 weeks before measurement of maximum specific tension before and after an in-situ (tibialis anterior) fatigue (did not induce significant muscle injury) protocol. No differences were found between the

groups in maximum specific tension before and after the fatigue protocol. However, in two non-exercise control groups it was found that maximal twitch tension and tetanic force production at low stimulation frequencies (≤ 40 Hz) were significantly lower in antioxidant supplemented animals compared with control animals. In order to determine which antioxidant was associated with depressed force production, a second experiment was conducted using an in vitro rat diaphragm preparation. Results from these experiments indicated that vitamin E was associated with a decrease in skeletal muscle force production at low stimulation frequencies (129). However, in a review of this study, McGinley et al., (2010) noted that the dose of vitamin E administered in this experiment was extremely (i.e., well above physiological levels) high which may have contributed to the depressed force production in treatment animals and the in vitro preparations.

As previously mentioned, it has been proposed that oxidative stress may result in fatigue and further (secondary) muscle damage following exercise (111). Therefore, other animal studies have utilized endurance exercise protocols in an attempt to determine the effect of antioxidant supplementation on indices of oxidative stress before and after exercise. In a study examining rat muscle before, during, and after 90 minutes downhill treadmill running (at a speed of 16 m/min at -16° grade) sixty-six male Sprague-Dawley rats were pretreated with a normal rat diet or diet + antioxidants (2,000 mg vitamin C and 1,000 IU vitamin E/kg) for 2 weeks. Rats were sacrificed either at rest, immediately, 2 hrs, or 48 hrs post-exercise. Malondialdehyde (MDA) and protein carbonyl (PC) concentrations and glutathione status in blood, vastus lateralis, vastus intermedius, and soleus muscles were determined. You et al., (2005) found that a

combination of vitamin E and vitamin C supplementation resulted in reduced protein carbonyl (formed when amino acids are oxidized), (130) content in the vastus intermedius and soleus muscles of the treatment group. This difference was significant at all time points. However, this same effect was not present in the vastus lateralis muscle of the treatment group (131). These results suggest that a protective effect may be realized through antioxidant supplementation and that protective effect may be muscle-specific. These results would suggest a role for antioxidants to provide protection against the initial insult experienced during the bout of injurious exercise. However, it is difficult to transfer conclusions from studies utilizing aerobic exercise to studies involving eccentric contraction-induced muscle injury.

It has been established that chronic ingestion of NSAIDs results in reduced satellite cell activity and protein synthesis in aerobic athletes (97). However, very few studies have examined the effect of antioxidant supplementation on muscle adaptation to aerobic exercise. An interesting study by Strobel et al., (2010) addressed this question. This group examined the effect of vitamin E supplementation in male Wistar rats following a 14-week aerobic exercise program. The rats were divided into four groups: (1) sedentary control, (2) sedentary vitamin E, (3) exercise control, and (4) exercise vitamin E. The animals ran on a treadmill 4 days/week at 70% VO_{2max} for up to 90 minutes/day. The training program continued for 14 weeks. Following training, there was a significant increase in markers of skeletal muscle mitochondrial biogenesis and antioxidant defenses. However, antioxidant supplementation significantly reduced these markers (PGC-1α mRNA, PGC-1α and COX IV protein and citrate synthase) activity in both supplementation groups when compared to the non-supplementation groups (132).

These results indicate that vitamin E supplementation may suppress skeletal muscle mitochondrial biogenesis regardless of aerobic training status. These results suggest that antioxidant supplementation may be contraindicated for endurance athletes. The exercise involved in this study did not result in significant muscle injury so caution should be taken when transferring these results to populations experiencing significant (e.g., eccentric contraction-induced) muscle injury. However, these results (i.e., reduced cyclo-oxygenase activity) suggest that antioxidant supplementation may have a negative impact on the regenerative capacity of muscle following modified use.

Other animal studies have utilized isolated eccentric contraction exercise interventions to induce muscle injury. Van Der Meulen et al., (1997) investigated the effects that 5-8 days of vitamin E infusion had on markers of muscle injury following in-situ performance of 225 eccentric contractions of the extensor digitorum longus muscle in rats. The infusion significantly increased (3-fold) the muscle content of vitamin E. However, despite the increased muscle vitamin E content, the only significant difference between controls and rats in the treatment group was decreased serum CK response to injury. There were no differences in other indices of muscle damage (133). These results indicate that although vitamin E did not reduce initial muscle injury or attenuate secondary muscle injury, supplementation may prevent enzyme loss or membrane lysis following muscle damage. Unfortunately, it was not possible to extend the data collection period of this study until baseline strength levels were recovered to determine if the differences at 3d had a significant impact on complete recovery of strength following eccentric contraction-induced injury.

Numerous human studies have attempted to investigate the effect of antioxidant

supplementation on symptoms of exercise-induced muscle injury. This section divides those studies based on the antioxidant used in the supplementation protocol and the effect (if any) of antioxidant supplementation on symptoms of fatigue or muscle injury.

Studies Utilizing Vitamin E

Many human studies have utilized downhill running to induce eccentric muscle injury. Sacheck et al., (2003) examined the effect of 12 weeks 1000 IU/d vitamin E supplementation vs. a placebo on antioxidant status, creatine kinase, lipid peroxidation, and DNA damage at 6, 24, and 72 hours following 45 minutes of downhill running. The males in the supplementation group displayed reduced serum CK values at 24 hours post-exercise and decreased MDA levels immediately post-exercise when compared to the placebo condition (134). However, one possible issue with this study is the possibility of a repeated bout effect. Subjects performed a "baseline" version of the test at the pre-supplementation session and performed another downhill running trial at 12 weeks post-supplementation. In addition, no measure of muscle function (i.e., MVC torque) was included in the post-injury assessment. Therefore, the exercise intervention employed in this study may not have resulted in significant muscle injury as a result of the protective effect incurred following the baseline downhill running trial.

This same issue (repeated bout effect) is seen in a two-part study examining the effect of 48 days vitamin E supplementation on eccentric muscle damage. Cannon et al., (1990) and Cannon et al., (1991), utilized a double-blind, crossover design. Subjects received either placebo or α-tocopherol until the day before a 45-minute downhill running protocol. Supplementation was then resumed at 3 days post-exercise. In each

portion of this study it was found that vitamin E had no effect on levels of circulating leukocytes, superoxide release from neutrophils, lipid peroxidation, or serum CK (135, 136). Unfortunately, the authors did not include any measures of muscle function (e.g., MVC torque) before or after the exercise intervention. In addition, the crossover design of this study did not or could not account for the existence of a repeated bout effect, making the second trial (regardless of supplementation condition) different from the first in regards to the muscle injury response to the bout of downhill running. Therefore, it is not possible to form conclusions regarding the effect of vitamin E supplementation on exercise-induced muscle injury because the degree of injury imparted by the exercise intervention is unknown.

The previously mentioned studies highlight a common problem when examining the research associated with antioxidant supplementation and exercise-induced muscle injury. Many exercise interventions do not result in significant muscle injury (as defined by significant deficits in MVC torque) and many studies do not include any measures of muscle function (e.g., MVC torque) in their pre- and post-injury analyses. This problem is illustrated in a study by McBride et al., (1998) who supplemented males with 1200 IU/day of vitamin E for 2 weeks before they engaged in a heavy resistance exercise protocol. This protocol involved previously trained individuals performing 4 sets and 10 repetitions (16 sets total) of squat, bent over row, bench press, and military press (137). Following the exercise session, the experimental group displayed significantly lower plasma malondialdehyde (MDA) and CK values (138). These results suggest that vitamin E supplementation may decrease muscle membrane disruption following exercise-induced muscle injury. However, it is important to note that the researchers

primary intent was to examine free radical production following resistance training exercise and as a result, they did not include a measure of muscle function in their analysis. In addition, subjects were experienced with strength training so the injury response may have been attenuated in this sample when compared to a sample of non-exercising subjects. Therefore, it is not possible to decipher the magnitude of injury experienced as a result of the exercise intervention.

Despite these shortcoming, Avery et al., (2003) conducted a study using the same exercise intervention to determine the effect of vitamin E supplementation on recovery from repeated bouts of exercise. The only difference was that subjects performed repeated bouts of the exercise intervention. Following 21 days of vitamin E supplementation (1200 IU/day) or placebo, subjects performed the first exercise bout. Subjects then performed the same exercise protocol twice more with two days separating each exercise session. Reductions in strength and power were not significantly different between the two groups. In addition, there were no significant differences in muscle soreness, MDA, or CK between the two groups. These results indicated that vitamin E supplementation had no effect on recovery from repeated bouts of resistance exercise (139). The reductions in MVC torque, although statistically significant, were not of the magnitude normally seen following heavy eccentric contraction-induced muscle injury. Reductions in maximal squat strength and maximal bench press strength were -11% and -13% respectively. Therefore, the magnitude of the injury experienced as a result of the exercise intervention may not have been significant enough to trigger the previously discussed response of muscle injury, inflammation, and regeneration.

Many studies have attempted to utilize exercise interventions involving eccentric injury of the quadriceps muscle group. Due to frequency of use, this muscle group is difficult to injure and many exercise interventions have not resulted in significant decrements in MVC torque.

However, Beaton et al., (2002) employed an exercise intervention that resulted in a significant initial deficit in isometric torque. This group found that vitamin E supplementation (1200 UI/day for 30 days pre-exercise) had no effect on the symptoms of eccentric contraction-induced muscle injury of the knee extensors. Following the exercise intervention, MVC torque was decreased by approximately 50% in both groups. Muscle biopsies taken 24 hours post-exercise revealed Z-band streaming but no damage to the structural proteins desmin and dystrophin. Supplementation with vitamin E had no effect on MVC torque (at 2, 4, and 7 days post-injury), macrophage infiltration and serum CK levels following eccentric injury of the knee extensors (140). However, the rapid return (isometric torque was not significantly different from pre-exercise levels at 4d post-exercise) to baseline levels of strength and the absence of edema in the subjects during the recovery period suggests that the injury response may not have been significant following the exercise intervention. Considering the high number of contractions that were performed by the subjects, it is possible that fatigue may have been responsible for a portion of the immediate post-exercise strength deficits.

The previously mentioned study also points to another potential problem with exercise interventions focusing on the quadriceps muscle group; it takes a large volume of work to injure this muscle group and the recovery is extremely rapid when compared to other muscle groups that are not used as frequently. As an alternative, other

researchers have utilized forearm flexor exercise interventions to induce muscle injury. This muscle group is used less frequently than the quadriceps muscle group and is more easily injured. The injury response following exercise is also much greater when compared to the quadriceps muscle group. However, this muscle group is much smaller in size than the quadriceps so injury of this muscle group may not result in large-scale changes in blood-borne markers of inflammation when compared to exercise interventions such as downhill running. In addition, muscle biopsies of the forearm flexors are difficult and risky to obtain when compared to biopsies of the vastus lateralis so researchers utilizing forearm flexor injury as their exercise intervention are normally forced to rely on blood-borne markers of inflammation to represent muscular events.

Despite these previously mentioned limitations, injury of the forearm flexors has been utilized in one study examining the effect of vitamin E supplementation on recovery from eccentric contraction-induced muscle injury. Phillips et al., (2003) used an exercise intervention involving eccentric contraction-induced muscle injury of the forearm flexors in 18-35 year-old males to examine the effect of vitamin E supplementation on symptoms of muscle injury. They supplemented the experimental group with mixed tocopherols (vitamin E), flavonoids, and docosahexaenoate and the placebo group was given rice powder in capsules. The supplements were taken 7 days before and for 7 days following an acute bout of eccentric exercise. Following exercise, IL-6 levels were significantly lower in the experimental group three days following the exercise intervention (141). Unfortunately, the researchers did not include a measure of muscle function in their analysis and they did not follow the subjects for a period of time exceeding 3d post-

exercise. Therefore, it is difficult to determine the extent of muscle injury that was induced by the eccentric exercise.

Studies Utilizing Vitamin C

Due to its water-soluble nature, vitamin C is not stored in the body in significant amounts. The majority of vitamin C is transported in the plasma. Therefore, it is plausible to predict that an acute dose of vitamin C (provided at the appropriate time) may impart protection against increased ROS production during injurious exercise by increasing plasma antioxidant levels.

Considering this, it is no surprise that a number of studies have utilized an acute dose of vitamin C immediately before or after a bout of injurious exercise. Thompson et al., (2001) administered an acute dose of vitamin C to subjects 2 hours before 90 minutes of intermittent shuttle running exercise. Following exercise, the treatment group displayed significantly higher plasma and lymphocyte vitamin C levels. However, there were no significant differences in serum CK, serum aspartate aminotransferase, or DOMS between the groups (142). It is important to note that, considering the modest CK response and DOMS following exercise, this exercise protocol most likely did not result in significant muscle damage in either group. Furthermore, the researchers did not include any other measures of muscle damage following the exercise. As a result, it is difficult to make any conclusions based on this data.

In another acute dose supplementation study Ashton et al., (1999) had subjects ingest 1000 mg of L-ascorbic acid 2 hours before performance of an incremental maximal exercise test on a cycle ergometer. Pre- and post-exercise measurements of

electron spin resonance (ESR) signal of α-phenyl-*tert*-butylnitrone (PBN) adduct, lipid hydroperoxides, and malonlydialdehyde (MDA) were compared. Supplementation resulted in significant differences in these measures with individuals in the treatment group displaying lower post-exercise values in these free-radical related parameters, suggesting that vitamin C supplementation provided a protective effect against ROS production (143). However, the methodology in this study suffers from a familiar flaw; researchers did not assess any indices of muscle damage following exercise.

A few studies have utilized an exclusive post-injury dose of vitamin C instead of a pre-injury/exercise supplementation regimen. Since vitamin C is primarily located in plasma and not stored in great quantities, raising plasma levels of vitamin C to correspond with increased ROS production may offer increased protection at a time of increased oxidant production.

Another study by Thompson et al., (2003) found that 400 mg/day of vitamin C for 3 days following 90 minutes of intermittent shuttle running (the same protocol as the previously mentioned study) had no effect on muscle soreness, plasma IL-6, serum CK, malondialdehyde (MDA) or MVC torque (144). Due to the shared methodology (e.g., insufficient muscle injury protocol), this study shares the same limitations as the previously mentioned study by Thompson et al., (2001).

Due to their synergistic relationshiop, many studies have chosen to combine vitamin E and vitamin C supplementation. However, in a unique study by Childs et al., (2001) researchers administered a combination of NAC (10mg/kg of body weight for 7 days following exercise) and vitamin C (12 mg/kg for 7 days following exercise) to

subjects who had performed 30 eccentric contractions of the elbow flexors. In this case, the supplementation regimen was associated with increases in serum CK and lactate dehydrogenase (LDH) indicating reduced cell membrane integrity (114). However, MVC torque was not tested following injury so it is difficult to draw any definitive conclusions about the possible effect of the supplementation regimen on recovery of muscle function.

The majority of vitamin C studies have used a pre-exercise supplementation strategy or the combination of a pre- and post-injury supplementation strategy. A number of studies have demonstrated attenuated muscle damage and soreness when high does of vitamin C are administered prophylactically.

In one of the first studies to examine the effect of vitamin C supplementation on symptoms of eccentric contraction-induced muscle injury, Kaminsky and Boal (1992) produced results demonstrating a reduction in DOMS following exercise-induced muscle injury. They found that 3000 mg/day of vitamin C for 3 days prior to injury and 4 days post-injury decreased muscle soreness following 15 minutes of cyclic plantar flexion and extension (145).

These results were encouraging and established a foundation for future research in this area. In support of these findings, Bryer et al., (2006) also found that supplementation with 3000 mg of vitamin C each day for 14 days prior to 70 eccentric contractions of the forearm flexors and for 4 days post-injury resulted in a significant reduction in muscle soreness for the first 24 hours post-injury and at 96 hours post-injury. This study also found a significant attenuation in the glutathione ratio (which is used as a

marker of oxidative stress) for the first 24 hours post-injury. However, despite the changes in soreness and markers of inflammation, the vitamin C supplementation had no effect on MVC torque (33). This study points to a common theme in the research examining the effect of antioxidant supplementation on symptoms of muscle injury; in many cases inflammatory markers or markers of muscle damage are significantly lower in treatment groups but these reductions in inflammatory markers are not associated with significant differences in muscle function. For example, Thompson et al., (2001) gave subjects vitamin C or a placebo for 2 weeks prior to the aforementioned shuttle running test. Serum IL-6 increased immediately after exercise in both groups. However, values in the treatment group were lower than in the placebo group 2 hours after exercise. The treatment group also experienced a more rapid return to baseline IL-6 levels. Although differences did not reach statistical significance, the treatment group also exhibited reductions in muscle soreness, MVC torque, and plasma concentrations of MDA. These results suggest that prolonged vitamin C supplementation may have some modest beneficial effects on recovery from unaccustomed exercise (146). These reductions (although non-significant) suggest that vitamin C supplementation may protect against secondary muscle damage following exercise-induced muscle injury. However, Davison et al., (2006) used the same time period of supplementation with a higher dosage (1000 mg/day) to investigate the effect of 2 weeks of supplementation with vitamin C on cortisol, adrenocorticotrophic hormone, interleukin-6, oxidative stress and neutrophil responses to 2.5 hours of cycling exercise at 60% VO_2max. In contrast to the previously mentioned study, the results yielded no significant differences between the experimental group and the placebo group (147).

Other results involving a pre-exercise supplementation regimen have suggested that vitamin C supplementation may improve the response of enzymatic antioxidants. Khassaf et al., (2003) attempted to determine the effects of vitamin C supplementation on the ability of lymphocytes to express protective enzymes and heat shock proteins (HSPs) following a bout of single leg cycling exercise. Following the supplementation regimen and exercise bout, it was found that baseline activity of superoxide dismutase (SOD) and catalase were elevated in the treatment group when compared to the placebo group (148). These results suggest that vitamin C supplementation may result in up-regulation of protective enzymes with antioxidant properties. This up-regulation may, in turn, provide protection against initial and secondary exercise-induced muscle damage.

Tauler et al., (2003) reported similar results when they examined the effect of high amounts of vitamin C intake prior to a duathlon competition. The researchers divided the subjects into two separate groups (n=16); a high dietary vitamin C intake group and a low dietary vitamin C intake group. The high group was noted as having a vitamin C intake 7 times greater than the low group. Blood samples were obtained at baseline, immediately post-race, and 1 hour post-race. The low intake group displayed significantly higher plasma LDH level following the race (149). These results suggest that the vitamin C supplementation may have resulted in a lower degree of cell lysis in the high intake group and that a higher vitamin C intake may have contributed to this lower degree of cell lysis. Therefore, vitamin C supplementation may offer protection against the symptoms of exercise-induced muscle damage. However, it is important to note that the exercise interventions used in these studies were biased towards endurance activities and these results may not be transferable to studies involving higher intensity

activities leading to greater muscle damage (e.g., eccentric contraction-induced muscle injury).

Although the results of these studies provide some evidence that pre- and post-injury vitamin C supplementation may impart a protective effect during and after injurious activity there is also a great deal of evidence to suggest that vitamin C supplementation has no effect on the symptoms of exercise-induced muscle injury.

In a recent study, Connolly et al., (2006) attempted to determine the effect of vitamin C supplementation on symptoms of eccentric contraction-induced muscle injury. Subjects ingested 3000 mg/day of vitamin C for 3 days prior to and 5 days following eccentric contraction-induced injury of the forearm flexors. The supplementation had no effect on strength loss, point tenderness (DOMS), elbow flexor ROM, or subjective pain (150). However, this study did not describe the training status of the participants and it included both males and females in the sample with unequal distribution between the groups. As a result, there was a great deal of variability in the loss of strength following eccentric muscle injury. Therefore, it is difficult to form conclusions regarding the effect of vitamin C on the symptoms of eccentric contraction-induced muscle injury based on these results.

In another study examining the effects of a pre- and post-supplementation strategy, Thompson et al., (2004) used a 30 minute downhill (-18%) running protocol to induce eccentric muscle injury. This same group had previously reported that vitamin C supplementation decreased the levels of plasma IL-6 following an unaccustomed bout of demanding exercise (shuttle running) (146). Fourteen male subjects ingested 400 mg of vitamin C each day for 14 days prior to and 3 days following exercise. Plasma vitamin C

concentrations increased in the supplementation group but there were no significant differences in circulating markers of muscle damage (CK, myoglobin, plasma IL-6), muscle function (MVC torque), or muscle soreness. This study did not report the data associated with muscle function following injury. However, the authors did state that near-complete recovery had occurred 48 hours following the exercise bout. In addition, the average VO_2max for subjects included in this study was 54 ml/kg/min for the supplementation group and 56 ml/kg/min for the control group. These values do not place the subjects in an "elite" category of fitness, but the authors stated that subjects "regularly took part in a variety of activities but were unfamiliar with the exercise protocol used in the present investigation" (151). This statement suggests that subjects were physically active and the mild injury response to the activity suggests that either the subjects were at least moderately trained or the protocol was not sufficient to induce significant muscle injury. Therefore, it would be unwise to rely on the results of this study to make any conclusions regarding the effect of vitamin C supplementation on symptoms following exercise-induced muscle injury.

Other studies have suggested that pre- and post-injury vitamin C supplementation following eccentric contraction-induced muscle injury may be contraindicated. Close et al., conducted a study during which subjects were given vitamin C (1000 mg/day) or a placebo 2 hours before and 14 days following downhill running exercise. The supplementation group in this study displayed delays in recovery of MVC torque in spite of a smaller increase in MDA (152).

Studies Utilizing Vitamin E & Vitamin C

Vitamin C and vitamin E work synergistically. Therefore, it seems reasonable to examine the effect of combined vitamin E and vitamin C supplementation on indices of damage following exercise-induced muscle injury.

As a result of this logic, numerous studies have examined the effect of simultaneous vitamin E and vitamin C supplementation on the symptoms of eccentric contraction-induced muscle injury. Shafat et al., (2004) found that vitamin C (500 mg/day) and vitamin E (1200 IU/day) taken for 37 days attenuated the decline in torque during 300 eccentric contractions of the knee extensors and attenuated the decline in muscle function for 2 days following the injury protocol when compared with a placebo group (153). Similar results were seen from another study; this one involving eccentric contraction-induced muscle injury of the forearm flexors. During the supplementation protocol, females were instructed to ingest a daily dose of vitamin C (1000 mg) and vitamin E (400 IU) for 14 days before and 2 days after injury. The females in the treatment group displayed decreased blood protein carbonyls and MDA; results that were indicative of reduced oxidative stress and damage (74). Considering the equivocal nature of the research involving exclusive vitamin C supplementation or exclusive vitamin E supplementation, these results provide evidence of a possible effect of antioxidant supplementation on symptoms of exercise-induced muscle injury. However, there are contradictory findings from other studies examining the effect of simultaneous vitamin C and vitamin E supplementation on the symptoms of eccentric contraction-induced muscle injury.

Mastaloudis et al., (2004a, 2004b, and 2006)) conducted a series of studies

examining the effects of concurrent vitamin C and vitamin E supplementation on symptoms of exercise-induced muscle injury following ultramarathon running. Numerous measurements were taken between three studies. Subjects ingested vitamin C (1000 mg/day) and vitamin E (300 mg/day) for 6 weeks before participating in a 50-km ultramarathon. The supplementation resulted in reduced lipid peroxidation and attenuated the exercise-induced increase in DNA damage in women, but not in men. However, antioxidant supplementation did not reduce inflammation and had no effect on muscle function (hamstring and quadriceps) or muscle protein levels in the blood following ultramarathon participation (154-156). As with previous studies involving endurance exercise, caution should be taken when using these results to determine or predict the effect of antioxidant supplementation on symptoms of eccentric contraction-induced muscle injury.

In another study examining concurrent supplementation of vitamin C and vitamin E Petersen et al., (2001) utilized a downhill running protocol (90 minutes, -5% grade at 75% VO_2max) to examine the effect of combined vitamin C (500 mg/day) and vitamin E (400 mg/day) supplementation on protection from exercise-induced muscle injury and the post-injury response. Researchers utilized a randomized, double-blind, placebo-controlled design and had subjects ingest the supplements or a placebo for 14 days before and 7 days after exercise. Plasma vitamin concentration showed a significant increase in the supplementation group. However, following exercise, the groups exhibited identical exercise-induced changes in cytokine, muscle enzyme, and lymphocyte subpopulations (157). However, this study suffered from methodological flaws similar to the previously mentioned study by Thompson et al., (2004) in that this study also included subjects that

were quite fit. The average VO_2max of the subject population was 63.8 ml/kg/min. As a result, the active population included in this study would be difficult to injure. In addition, there was no measurement of muscle function in this study so it is not possible to conclude that significant injury occurred as a result of the exercise protocol.

As previously stated, it has been suggested that antioxidant supplementation may provide a protective effect during fatiguing or injurious exercise. To investigate this possibility, Fisher et al., (2004) observed the effect of combined vitamin C and vitamin E supplementation on release of IL-6 from contracting human skeletal muscle. Fourteen male subjects completed 3 hours of knee extensor (leg extension) exercise at a rate of 60 extensions per minute. Resistance was set at 50% of the highest intensity that subjects were able to maintain for 1 minute during a previous familiarization trial. Net release of IL-6 during exercise was completely blunted in the treatment group when compared to the control group. The post-exercise arterial IL-6 level was also significantly lower in the treatment group. In addition, the control group displayed higher levels of Plasma 8-iso-prostaglandin F (a marker of lipid peroxidation) when compared to the treatment group. The control group also realized increases in C-reactive protein and cortisol levels while the treatment group saw no changes in these levels (158). These results suggest that simultaneous vitamin C and vitamin E supplementation may exert a protective effect on contracting skeletal muscle during exercise. However, the low intensity of the exercise protocol utilized in this study makes it difficult to transfer these findings to individuals performing high-intensity, injurious exercise (e.g., eccentric muscle contractions).

The body of research examining the effect of concurrent supplementation with vitamin C and vitamin E on symptoms of exercise-induced muscle injury or fatiguing

exercise is similar to the previously reviewed body of research examining exclusive vitamin E and vitamin C supplementation. The available research involves distinct differences in exercise interventions, measures of muscle damage, and subject populations; making it difficult to form a uniform conclusion regarding the effect of antioxidant supplementation on symptoms of injurious or fatiguing exercise.

In addition, there are few studies that have directly compared the possible effects of vitamin C or vitamin E supplementation on symptoms of exercise-induced muscle injury. Jakeman et al., (1993) compared the effect of 21 days of pre-injury and 7 days of post-injury vitamin C (400 mg/day) or vitamin E (400 mg/day) supplementation on muscle damage following 60 minutes of box-stepping exercise. The only difference of significance associated with vitamin C supplementation was a significant reduction in the decline in Low Frequency Force (LFF) at the 20:50 Hz ratio following exercise. No effects were found for vitamin E (159). The same group conducted a similar study (again involving one hour of box-stepping exercise) using the same supplementation protocol. Prior to exercise eight subjects received 400 mg of vitamin C daily for three weeks before and one week after exercise, eight received 400 mg of vitamin E for the same period, and another group of eight subjects received a placebo. Although plasma levels of each antioxidant rose in their respective groups, there were no significant differences between the groups in any blood borne measures of muscle damage or inflammation (160).

All of the previously mentioned studies supplemented subjects with pharmacological supplements (ascorbic acid, NAC, or vitamin E). Connolly et al., (2006) chose to examine the effect of antioxidant-rich tart cherry juice on the symptoms of eccentric contraction-induced muscle injury. Subjects received a daily dose of .682 L

of tart cherry juice or placebo of equal caloric content for 4 days prior to and 4 days following eccentric contraction-induced injury of the elbow flexors. Subjects in the group receiving the tart cherry juice experienced a reduction in muscle soreness and an attenuation of the loss in MVC torque when compared to the placebo group (161). The results of this study demonstrated a clear effect of antioxidant supplementation (smaller decrement in MVC torque) previous to and following eccentric muscle injury. In fact, strength in the treatment group had recovered to above-baseline levels at 96h post-injury while strength in the placebo group had only recovered to approximately 85% of baseline strength. Unfortunately, this study is the only work to examine the effect that consumption of an antioxidant rich food with anti-inflammatory properties on the symptoms of exercise-induced muscle injury when compared to an iso-caloric placebo.

Studies Utilizing NAC

To date, numerous studies have examined the effect of NAC supplementation on symptoms of fatiguing contractions, skeletal muscle inflammation, and eccentric contraction-induced muscle injury. The previously mentioned Childs et al., study and other works have led to differing conclusions regarding the effects of NAC supplementation on symptoms of exercise-induced muscle injury. A majority of these studies have utilized animal models. In addition, many of these studies have been ischemia reperfusion studies so care should be taken when translating the results of these studies to models utilizing eccentric contraction-induced muscle injury. However, these studies do offer evidence suggesting that NAC supplementation imparts a protective effect on muscle tissue during and after injurious or fatiguing exercise.

Kearns et al., (2010) investigated the effects of NAC in a model of compartment syndrome (essentially a ischemia and reperfusion model of injury). Using rats, the authors investigated the effects of NAC supplementation on twitch and tetanic contractile function, tissue myeloperoxidase activity, and neutrophil respiratory burst activity at 1 hour, 24 hours, and 7 days following decompression. NAC supplementation had a very clear benefit for the rats. Treatment preserved twitch and tetanic contractility, reduced neutrophil infiltration at 24 hours post-decompression, and reduced neutrophil respiratory burst activity. Therefore, the authors concluded that NAC supplementation alleviated the symptoms of simulated compartment syndrome. In these rats, treatment preserved muscle contractility, possibly by attenuating neutrophil activation and the resultant oxidant injury (162). These results suggest that NAC supplementation exerts a protective effect on muscle tissue during the initial injury and possibly during the secondary injury process. However, using these results, it is not possible to make conclusions regarding the longer-term effects of antioxidant supplementation on symptoms of muscle injury because no measurements were taken past 24 hours post-decompression.

In a similar study, Bolcal et al., (2007) also used an ischemia reperfusion model to investigate the effects of NAC supplementation on the symptoms following muscle injury. Forty-four New Zealand white rabbits were used in this study. One group served as a control, another group served as an α-placebo (co-enzyme Q_{10}) group, a third group served as a β-placebo group (β-glucan), and a fourth group served as the treatment group; receiving supplementation of NAC. Following baseline measurements the common iliac artery was clamped and after 60 minutes of ischemia the limb was perfused for 180 minutes. Following perfusion, a biopsy was taken from the adductor magnus muscle and

blood samples were taken. The control group displayed significant increases in malonyldialdehyde (MDA) while the NAC and placebo groups saw no significant increases in this variable. The NAC supplemented group and placebo groups also saw increases in glutathione peroxidase and superoxide dismutase. Levels of nitric oxide (NO) increased in all groups except the control group (163). These results suggest that antioxidant supplementation (in various forms) may attenuate the initial injury response during and immediately after ischemia reperfusion. However, no measurements were taken past the immediate post-perfusion time point; making it difficult to form conclusions from these studies regarding the effect of antioxidant supplementation on the secondary injury response following reperfusion injury.

Koksal et al., (2003) also utilized ischemia reperfusion model to investigate the effects of NAC supplementation on symptoms of muscle in jury in rats. Ischemia was induced via 4 hours vascular clamping of the hind limb and muscle injury was evaluated following 1 hour reperfusion in three groups; (1) a saline (control) group, (2) a DMSO group, and (3) a NAC group. Plasma levels of CK, LDH, thiobarbituric acid reactive substances (TBARS), and blood HCO3, as well as muscle tissue TBARS, were measured at the end of reperfusion. NAC and DMSO groups showed a significant attenuation of plasma CK, plasma TBARS, and muscle tissue TBARS when compared to the control group. In addition, neutrophil infiltration in the DMSO and NAC groups was significantly lower than the control group (164). These results agree with the previously mentioned studies; suggesting that NAC can improve the symptoms of ischemia reperfusion injury in an animal model.

As previously stated, one should take care when using the results of perfusion/reperfusion injury studies to make conclusions regarding the effects of NAC supplementation on symptoms of eccentric contraction-induced muscle injury. However, results of other studies support the conclusions of the previously mentioned works.

In another study utilizing New Zealand White Rabbits, Jiang et al., (2001) examined the role of NAC in preventing diaphragm injury produced by inspiratory reserve loading (IRL). The rabbits were divided into three groups: (1) control with no exercise, (2) exercise with no supplementation, and (3) exercise with supplementation. Free radical scavengers (superoxide dismutase, NAC, and mannitol) were given intravenously to the treatment group before and after the IRL intervention. The rabbits were killed on day 3 to obtain the diaphragm muscles. Hematoxylin and eosin staining indicated that abnormal muscle was more prominent in the exercise with no supplementation group when compared to the treatment group. In addition, diaphragmatic contractility was found to be better maintained in the treatment group when compared to the exercise with no treatment group (165). These results indicate that free radical scavengers may prevent the development of diaphragm injury during IRL.

While these results offer evidence supporting the efficacy of NAC supplementation during periods of modified muscle use, it is important to note that these studies involved perfusion/reperfusion injury and diaphragm muscle injury. The use of these exercise/injury interventions and/or muscle groups makes it difficult to transfer these results to different populations experiencing different sources of mechanical muscle injury.

However, work has been done examining the effect of NAC supplementation in exercising subjects or animals. Pinheiro et al., (2010) used 24 male Wistar rats to investigate the effects of NAC supplementation on fatigue and muscle damage following 60 minutes of electrically stimulated contractile activity. Rats were divided into placebo and NAC groups before receiving treatment and being exposed to the exercise intervention. In addition, tetanic force and muscle fatigue were assessed during, immediately following and 1 hour after the 60 minutes of contractions. Plasma CK, plasma LDH, muscle content of ROS, thiobarbituric acid-reactive substances (TBARS) and myeloperoxidase (MPO) activity were measured 1 hour following the exercise intervention. NAC supplementation resulted in an attenuation of the loss of force following the fatiguing protocol. The NAC group saw force decreases of 38.22 ± 7.4% of the pre-exercise force values while force values for the placebo group decreased by 70.8 ± 4.5%. In addition, NAC treatment decreased plasma CK, plasma LDH, ROS, TBARS and MPO when compared to the placebo group (166). These results suggest that NAC supplementation may result in attenuation of force loss during fatiguing exercise and muscle damage following this type of exercise intervention.

A limited number of human studies have also examined the effect of NAC supplementation on symptoms of eccentric contraction-induced muscle injury. Silva et al., (2008) had 29 participants ingest NAC (10 mg/kg/day) or a placebo for 14 days before and 7 days following eccentric contraction-induced injury of the forearm flexor muscles. The NAC group displayed a significantly higher concentration of IL-10 (an anti-inflammatory cytokine) at day 7 when compared to the placebo group. The presence of IL-10 inhibits pro-inflammatory cytokine production by activated monocytes and

macrophages. Based on these results, the authors concluded that NAC supplementation played an important role in improving the endogenous anti-inflammatory response following eccentric contraction-induced muscle injury (113). However, it is important to note that there were no other differences in other indices of muscle damage between the two groups. In addition, these authors did not include MVC torque (or any other measure of muscle function) as a measurement of muscle damage. Therefore, there is little justification for this conclusion based on the information obtained in this study.

Other groups have attempted to induce eccentric muscle injury of the knee extensors to study the effect of NAC supplementation on the resulting symptoms. Kersick et al., (2010) supplemented subjects with a placebo, epigallocatechin gallate (EGCG) (1800 mg/day), or a high dose of NAC (1800 mg/day) for 14 days prior to eccentric exercise of the knee extensors. The authors measured muscle soreness, serum markers of muscle damage, peak isometric torque, serum markers of oxidative stress and serum markers of inflammation at baseline and 6, 24, 48, 72 hours post-injury. Of these measures, only soreness at 24 hours post-injury was attenuated in the treatment groups (112). However, the magnitude of injury induced by the protocol utilized in this study was not comparable to similar studies examining eccentric contraction-induced muscle injury. The baseline values for peak isometric torque were not reported, but peak isometric torque was not significantly different from baseline at 72 hours following the eccentric injury protocol. Therefore, when compared to previous works examining recovery from eccentric muscle injury, the stimulus in this study was not sufficient to induce injury of a magnitude required for a valid examination of recovery.

Conclusion

The literature examining the effect of antioxidant supplementation on the symptoms of eccentric contraction-induced muscle injury is equivocal. Many studies have identified a protective role for vitamin E, vitamin C, or NAC (alone or in combination) against fatigue, oxidative stress, muscle damage and/or inflammation. However, each study providing evidence for a positive effect is countered by another study providing equally convincing evidence for either no effect or, occasionally, a negative effect of antioxidant supplementation. Definitive conclusions are complicated by numerous factors. Studies vary greatly in the timing and dosage of antioxidants, subject population, and exercise intervention. In addition, there is a great deal of variation in the magnitude of muscle damage that has been induced by different exercise interventions. The metabolic demands of exercise interventions have varied from low (e.g., single limb eccentric contractions) to high (e.g., exhausting endurance exercise). It is clear that the intensity and duration of the exercise intervention has a significant effect on ROS production. For example, aerobic exercise results in a larger oxygen turnover and increased mitochondrial ROS production but eccentric exercise results in greater mechanical damage and greater ROS-mediated secondary damage.

Previous research has determined that NAC supplementation aids or improves the body's natural (enzymatic) antioxidant defense system. In addition, research examining the effect of NAC on maintenance of muscle force during fatiguing exercise suggests that NAC plays a role in reducing ROS production in muscle tissue during exercise. These results also suggest that NAC may reduce further (secondary) post-injury muscle damage by inhibition or attenuation of ROS production.

Many animal studies have also demonstrated reduced inflammation following exercise-induced muscle injury in NAC-supplemented animals and seem to offer support for a protective effect of NAC during and immediately following a bout of injurious or fatiguing exercise. These studies have also demonstrated a preservation of twitch characteristics following a fatiguing exercise protocol. However, few studies have examined the long-term effects of NAC supplementation on recovery of strength following eccentric contraction-induced muscle injury in humans. The studies that have attempted to examine this have either not included a sufficient exercise intervention (resulting in minimal muscle damage and short recovery) or have not included a measure of muscle function (e.g., MVC torque) following eccentric contraction-induced muscle injury.

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CHAPTER 2

THE EFFECT OF N-ACETYLCYSTEINE SUPPLEMENTATION ON RECOVERY OF STRENGTH FOLLOWING ECCENTRIC MUSCLE INJURY

Introduction

Many individuals experience the symptoms of exercise-induced muscle injury following novel or unfamiliar physical activity (1). The list of these symptoms includes delayed-onset muscle soreness (DOMS) and swelling (2), release of muscle proteins into the bloodstream (3), ultra-structural damage (4), and most notably, initial and continued decrements in muscle function (i.e., decreased ability to produce force) (5, 6). Treatment of these symptoms through administration of antioxidant supplements or Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) may attenuate muscle soreness and/or improve initial post-exercise recovery of strength (7-10).

Antioxidant supplementation may reduce post-exercise (i.e., secondary) muscle damage through inhibition of Reactive Oxygen Species (ROS) production and a subsequent reduction in oxidative stress in human and animal tissues (11-14). Results of studies examining the effect of antioxidant supplementation on muscle injury and regeneration are equivocal; some have found it to be contraindicated (15, 16), some have found no effect (17), and others have shown a beneficial effect (18, 19).

Many studies have reported that antioxidant supplementation decreases the initial inflammatory response and/or is associated with attenuated strength deficits in the initial and/or short-term post-exercise time period (immediately post-exercise to three days

post-exercise). N-acetylcysteine (NAC) supplementation has demonstrated a clear anti-inflammatory effect in human and animal disease models (19-21). However, few studies have examined the longer-term effect of supplementation (past three days post-exercise) on the recovery of muscular strength following exercise-induced muscle injury requiring recovery periods greater than three days (9, 10, 13, 22, 23). It has been demonstrated that NAC supplementation attenuates inflammatory changes immediately following and up to three days after injurious exercise in animal models. This attenuation is partly related to a reduction in neutrophil activation and/or phagocyte infiltration (9, 14).

Reduction of the inflammatory response following muscle injury may be contraindicated as the ROS-mediated secondary muscle damage (in combination with neutrophil and phagocyte activation and infiltration) associated with the inflammatory response may be necessary for complete recovery of muscle function following exercise-induced muscle injury (24-26). Considering these findings, it seems possible that treating the initial symptoms of exercise-induced muscle injury may mean trading short-term gains for long-term negative consequences when individuals attempt to speed their recovery via pharmacological intervention.

Supplements containing antioxidants are widely available and many individuals use them on a daily basis (27). Numerous animal studies indicate that the commercially available amino acid & antioxidant nutritional supplement N-acetyl cysteine (NAC) has an anti-inflammatory effect and/or increases glutathione levels which may impart a protective effect on muscle tissue during injurious exercise and decrease the loss of muscular strength and/or the magnitude of the inflammatory response (and other symptoms) immediately following exercise-induced muscle injury (9, 10, 19, 22, 23, 28).

However, there is research suggesting that NAC supplementation has no effect (28, 29) or that it may have a pro-oxidative effect following a bout of injurious exercise (15). Therefore, an initial positive benefit (e.g., smaller decrement in isometric torque production, reduced inflammation and secondary damage) may be followed by a negative long-term consequence; as seen with other treatments following exercise-induced muscle injury.

The long-term effect of other treatments resulting in alteration of the inflammatory response can be seen in studies examining the effect of NSAIDs on recovery following exercise-induced muscle injury. Over-the-counter doses of NSAIDs may result in supressed tissue protein synthesis following high-intensity eccentric exercise (30), or high volume endurance exercise (31), and there is evidence of inhibited recovery of muscular strength and hypertrophy following NSAID administration in animal injury models (32, 33).

Considering the results of studies in this area and lack of data concerning the long-term effect of NAC supplementation on recovery of muscular strength following exercise-induced muscle injury, the effect of NAC supplementation on complete recovery of muscle function follwing exercise-induced muscle injury is not known. Therefore, the purpose of this study was to determine the effect of pre- (7D) and post-exercise (10D) NAC supplementation on recovery of muscular strength following eccentric contraction induced muscle injury of the forearm flexors.

Current research examining the effect of NAC supplementation before and following a bout of injurious exercise suggests that in the short-term, supplementation

may provide a benefit and reduce initial strength losses and inflammation following exercise-induced muscle injury (9, 10, 22, 23). However, other studies have suggested that the initial protective effect (reduced inflammation and secondary damage and/or attenuated deficits in muscular strength) may result in long-term consequences (e.g., incomplete recovery of muscular strength) (15). In addition, there is evidence suggesting that, in models of muscle injury, NAC supplementation may result in increased oxidative stress and enhancement of the post-exercise inflammatory response, resulting in inhibited or incomplete recovery of muscular strength following a bout of injurious exercise (30, 34, 35).

Specifically, it was hypothesized that NAC supplementation before (7D) and after (10D) a bout of eccentric exercise would result in a greater deficit in muscular strength at 10D post-exercise in the NAC supplemented group when compared to the placebo group.

Methods

Subjects

Twenty-one non-smoking, "low risk", females (as specified by guidelines of the American College of Sports Medicine) (36), from Georgia State University were recruited to participate in this study (See mean characteristics in Table 1, see individual descriptive characteristics in Appendix A). Each subject was informed of the purpose and potential risks of participation before signing a written informed consent document approved by the Georgia State University Institutional Review Board (See Appendix B). During the screening process, each subject was asked to describe their current and past exercise habits to determine if they met the criteria for inclusion. Subjects were excluded if they had participated in resistance training within the last six months, had recent biceps brachii or elbow injury, and/or had used anti-inflammatory medication or vitamin/mineral supplements within the six weeks prior to their participation. Following an initial evaluation (see Appendix C), subjects meeting the criteria for inclusion in this study were then asked to avoid changing their current exercise habits (e.g., beginning a strength training program or a weight-loss exercise program) for the duration of the protocol.

Placebo (n = 10)				NAC (n = 11)			
Subject	Age (yrs)	Weight (kg)	Height (m)	Subject	Age (yrs)	Weight (kg)	Height (m)
Mean	20.50	68.91	1.70	Mean	21.00	67.27	1.68
SEM	0.97	11.87	0.06	SEM	1.00	9.19	0.09

Table 1. *Subject descriptive characteristics (n = 21).*

To increase the probability of subject compliance with the protocol, each subject was urged to take the provided supplement on a daily basis and was reminded of this directive during each visit to the laboratory. Subjects were asked to bring their pill boxes to each laboratory visit; allowing for a pill count to determine if the supplement was being taken as directed. In addition, each subject was instructed to abstain from the consumption of alcohol and caffeine for 12 hours prior to all testing. Subjects were also directed to refrain from treating symptoms of exercise-induced muscle injury imparted from the exercise protocol (e.g., non-steroidal anti-inflammatory drugs, massage, ice, etc.).

Experimental Design

This study was a 17-day, randomized, parallel design involving a dietary supplement versus a placebo. Subjects reported to the Applied Physiology Laboratory at Georgia State University and were randomly assigned to a treatment (n = 11) or a placebo (n = 10) group for seven days before and ten days following an acute bout of eccentric exercise.

Subjects visited the laboratory a total of eight times during the 17-day duration of the study. During their first visit and second visits, each subject received their NAC supplement (Sigma Aldrich©, 10 $mg \cdot kg^{-1}$ $bw \cdot d^{-1}$) or placebo (food-grade cellulose) and completed two maximal voluntary contraction (MVC) torque familiarization trials involving the forearm flexor muscles of the non-dominant arm. Each familiarization trial consisted of three attempts each at 90°,100°, and 110° of forearm flexion.

Following the familiarization trials, subjects reported to the lab on day 7 of the study. A baseline venous blood sample was taken from the dominant arm and additional blood samples were collected at 1D, 3D, 7D, and 10D post-exercise. Each subject also completed a baseline subjective evaluation of muscle soreness using a visual analog scale (1-100). Additional evaluations were made immediately post-exercise and at 1D, 3D, 7D, and 10D post-exercise. MVC torque, circumference of the upper arm and range of motion (ROM) of the elbow joint were also measured at these time points. Following baseline measurements, each subject completed the exercise protocol designed to elicit eccentric muscle injury of the forearm flexors. Measurements quantifying the symptoms of eccentric contraction-induced muscle injury were taken at the previously mentioned time points.

Analysis of bloodborne inflammatory markers was done at different time points. These time-points were selected based on previous work demonstrating that serum CK peaks between two and four days post-exercise and returns to normal/baseline levels at ten days post-exercise (37-40). In addition, Childs et al., (2001) found that serum IL-6 levels peak at three days following a bout of eccentric forearm flexor exercise (15). Therefore, measurements of serum IL-6 were performed on pre-exercise and 3D serum

samples. N-acetylcysteine supplementation has been purported to increase serum glutathione levels under normal (e.g., uninjured) conditions (41). There is a large degree of inter-subject variability in regards to oxidative stress and reduced serum glutathione levels following eccentric exercise-induced muscle damage (6). Therefore, pre-exercise serum glutathione levels were compared between the groups to determine if supplementation had an effect on serum glutathione under normal conditions.

Supplementation

During their first visit, subjects were given capsules containing either 10 $mg \cdot kg^{-1}$ $bw \cdot d^{-1}$ of N-acetylcysteine (Sigma-Aldrich©) or a placebo (food-grade cellulose) divided into two doses per day. Subjects were instructed to take the capsules two times per day (one with breakfast and one with their lunch) for the duration of the study (7D pre-exercise and 10D post-exercise).

Muscular Strength

Muscular strength was represented by isometric maximal voluntary contraction (MVC) torque. Due to the voluntary nature (subjects were asked to exert maximal effort but level of effort was still voluntary) of the muscular strength testing, MVC torque was defined as the peak isometric torque recorded during a four-second isometric contraction (procedure described below).

Subjects performed all muscular strength (e.g., MVC torque) testing on a modified arm curl machine (see Figure 1) partnered with a load cell (Transducer Techniques® MLP 150, Transducer Techniques, Temecula, CA) and a signal conditioner

(Transducer Techniques® TMO-2, Transducer Techniques, Temecula CA). One end of the load cell was attached to a bar on the arm curl machine and the opposite end was bound to the subjects arm with a wrist strap. The signal conditioner was interfaced with a desktop computer running TestPoint® (Measurement Computing Corporation ©, Norton, MA) software.

Clarkson et al., (1992) found that MVC torque is immediately reduced by approximately 50% immediately following performance of a bout of exercise similar to the bout employed in this study. In addition, they found that MVC torque values gradually recovered and, for most subjects, was complete by ten days post-exercise. Therefore, MVC torque was measured on eight different occasions (two familiarization trials, pre-exercise, immediately post-exercise, and 1D, 3D, 7D, and 10D post-exercise). The first and second familiarization trials were conducted to acquaint the subject with the equipment and procedures in order to ensure that a valid maximal baseline peak MVC torque measurement was achieved.

After a brief warm-up, subjects were asked to attempt three maximal voluntary contractions of the non-dominant arm forearm flexor muscles for a four second duration at 90°, 100°, and 110° of elbow flexion (measured with a goniometer) for a total of nine MVC torque attempts (three attempts x three different angles) during each data collection session. Kasprisin et al., (2000), demonstrated that the isometric torque developed by the biceps brachii was significantly affected by joint angle during maximum voluntary contractions (42). Therefore, measurements were taken at three different elbow joint angles. To accomplish this, the seat and arm pad of the machine was adjusted to each subject's height and each subject's arm was securely placed in the testing equipment with

two straps; orienting the elbow in the same location before each trial and preventing any extraneous movement of the arm during data collection. The subject's elbow joint angle was then determined (via goniometer) and secured in that position. To accomplish isolation of the biceps brachii muscle, each subject was required to elevate their feet off of the ground (resting on a step stool) during MVC torque trials. In addition, a lab technician placed a hand on the subject's back and shoulder of the non-dominant arm to ensure that the subject did not lean back during the testing procedure and that back and shoulder muscles were not involved during MVC torque trials.

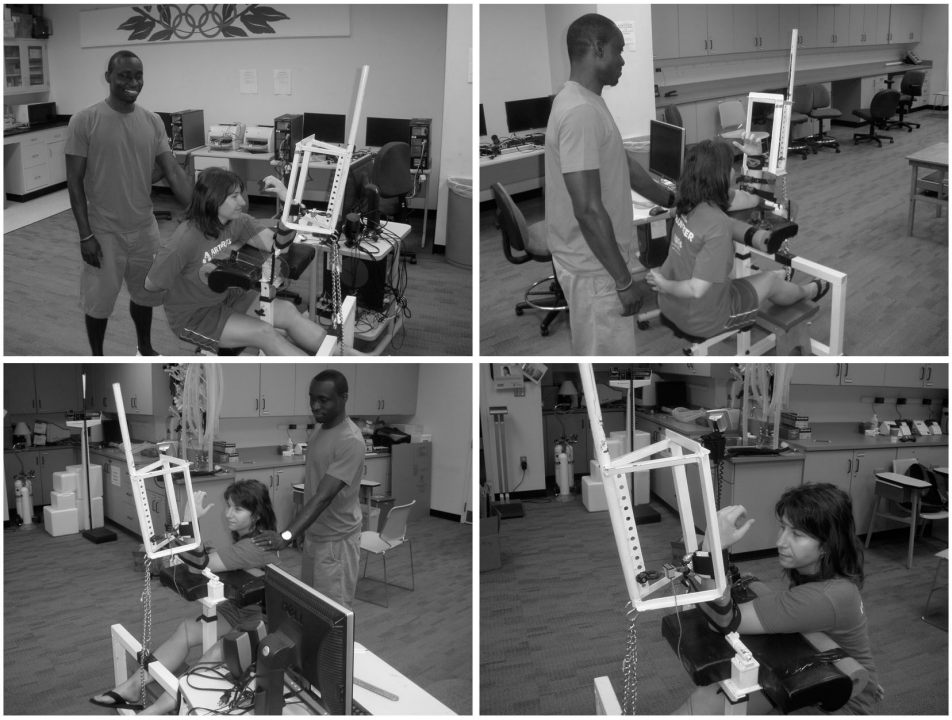

Figure 1. Modified arm curl machine.

Standard instructions to exert as much force as possible against the lever arm were given to each subject before an attempt. For motivational purposes, verbal encouragement was provided during each four-second MVC torque trial. Peak torque measured during the three attempts at each angle of elbow flexion was recorded for analysis. A 60-second rest was given between each attempt. The mean of all 9 attempts was used to represent mean peak torque at each time point.

Injury Protocol and Peak Eccentric Torque

Subjects performed the eccentric exercise protocol on the same modified arm curl machine (see Figure 1) utilized during MVC torque testing sessions. The eccentric exercise protocol consisted of 50 eccentric contractions (2 sets of 25 repetitions). The eccentric contractions were performed using a resistance equal to 80% of the subject's average peak MVC torque at 90°, 100°, and 110° of elbow flexion.

Subjects were instructed to perform each contraction over a period of four seconds. Peak eccentric torque was recorded during each eccentric contraction effort. Following each eccentric contraction, the resistance arm on the machine was raised back to the starting position by a lab technician; allowing the subject to avoid the concentric portion of the lift and rest between eccentric contractions. Subjects were given 30 seconds of rest between repetitions and five minutes of rest between sets in order to prevent forearm flexor muscle fatigue. Verbal encouragement was given during performance of the eccentric contractions.

Range of Motion

Range of motion (ROM) was measured at pre-exercise, immediately post-exercise, and 1D, 3D, 7D, and 10D post-exercise. Subjects stood with their non-dominant arm in a completely relaxed position while looking away from the researcher performing the measurement. This position was used to mark maximal elbow extension. Following achievement of this position, subjects were asked to flex their non-dominant arm until maximal voluntary flexion had been achieved. This position was used to mark maximal elbow flexion. The difference between these two points was calculated to

represent total ROM. The mean of three attempts was taken to determine mean ROM at each time point.

Rating of Muscle Soreness

Each subject rated the soreness of the non-dominant forearm flexor muscle group pre-exercise, immediately post-exercise, 1D, 3D, 7D, and 10D post-exercise. Standard instructions to rate muscle soreness only (i.e., ignoring feelings of fatigue or weakness) in the elbow flexors were given to each subject prior to the subjective soreness rating at each time point. Subjects rated their level of muscle soreness after doing an arm curl with a 2.5 lb. dumbbell. Starting with their arm in a relaxed position at their side, each subject performed maximal flexion of their non-dominant (injured) arm a total of three times. After each attempt, subjects indicated their subjective rating of muscle soreness by placing a pencil mark on a 100 mm visual analogue scale (See Appendix E) (43). A score of "0" corresponded to a perception of "no soreness", while a score of "100" indicated a feeling of "very, very sore." The mean of the three soreness ratings was calculated and used to represent subjective muscle soreness at each time point.

Arm Circumference

Non-dominant upper arm circumference was assessed pre-exercise, immediately post-exercise, and at 1D, 3D, 7D, 10D post-exercise. Upper arm circumference was measured at a point 2/3 of the distance between the acromion process and the lateral epicondyle of the humerus. Three measurement marks were placed around the circumference of each subject's arm with indelible ink before the first measurement and the same point was used for all successive measurements. Measurements were taken

using an original standard Gulick tape measure (Richardson Products, Inc. Frankfort, IL) while the subject allowed their arm to hang down by their side in a relaxed position. The mean value of three measurements at each time point was used for analysis.

Blood Analysis

Venous blood samples (approximately 3.0 mL) were collected during each visit into tubes containing a polymer gel and clot activator (Vacutainer, Beckton Dickinson, Franklin Lakes, NJ) for collection of serum. Serum was analyzed for Creatine Kinase (CK) activity at pre-exercise, 1D, 3D, 7D, and 10D post-exercise. Blood samples were allowed to clot at room temperature for 30 min before being centrifuged between 2,000 and 3,000 g for 15 minutes at 4 ± 2°C. The separated serum was then transferred to a freezable tube and stored at -20°C until analyzed for serum CK activity, serum IL-6, and serum glutathione.

Serum CK activity was measured in duplicate with a spectrophotometer (Beckman DU 650, Beckman Coulter, Inc. Fullerton, CA) using a CK reagent set (Pointe Scientific, Inc. Lincoln Park, MI) (See Appendix F). Serum IL-6 levels were measured in duplicate with a microplate reader (Opsys MR, Dynex Technologies, Chantilly, VA) using human IL-6 ELISA kits (Ray Biotech, Norcross, GA) (see Appendix F). Serum glutathione levels were measured in duplicate with a microplate reader (Opsys MR, Dynex Technologies, Chantilly, VA) using a QuantiChrom™ Glutathione Assay Kit (Gentaur Molecular Products, Kampenhout, Belgium) (See Appendix F).

Food Frequency Questionnaire

Subjects completed a food frequency questionnaire at the conclusion of the study (See Appendix E). This instrument was designed to determine subject's consumption of antioxidant-rich foods for the duration of the study (7D pre-exercise and 10D post-exercise). There were five categories listed on the questionnaire with each category representing a group of high-antioxidant foods. Subjects were asked to record how many servings of each food they consumed during the seventeen-day period of the study. Each category was weighted based on the antioxidant content of the food group. Calculations for the categories were based on data obtained from Wu et al., (2004) (44).

Statistical Analyses

Data were analyzed using a group (NAC, Placebo) by time ANOVA with repeated measures on time-point measures of MVC torque, non-dominant forearm flexor muscle soreness, non-dominant upper arm circumference, serum CK, and serum IL-6 activity. Differences between MVC torque values at the three angles measured was determined through use of an arm angle (90°, 100°, 110° of elbow flexion) by time (pre-exercise, immediately post-exercise, 1D, 3D, 7D, and 10D post-exercise) repeated measures ANOVA with repeated measures on time. Percent decline in peak eccentric torque, pre-exercise glutathione levels, and results from the food frequency questionnaire were compared between groups using an independent samples t-test. Statistical significance for all analyses was determined at an alpha level of $p < 0.05$. Dependent samples t-tests were performed upon observation of a significant F-ratio in order to determine the location of any mean differences. To reduce the risk of Type 1 errors,

Bonferroni adjustments were applied to all post-hoc means comparisons tests. All statistical analyses were performed using SPSS 12.0 (SPPS, Chicago, IL) data analysis software.

Results

Peak Eccentric Torque

Mean decline in peak eccentric torque during the eccentric exercise protocol was not significantly different between the placebo and NAC groups (39.3% vs. 38.1% respectively; $p = .81$). Mean maximum peak eccentric torque, taken as the mean of the first ten repetitions performed, was significantly higher than mean minimum peak eccentric torque (represented by the mean of the last ten repetitions performed) in all subjects (46.9 Nm vs. 28.9 Nm; $p < .01$) (see data in appendix F).

Muscular Strength

There were no statistically significant differences in percent decline in force between the two groups at any time point following performance of the injury protocol ($p = .386$). Additional analysis of percent decline in torque at each specific arm angle also demonstrated that there were no significant differences between groups at 90°, 100°, or 110° of elbow flexion at any time point ($p = .333$, $p = .287$, and $p = .494$, respectively).

Analysis of MVC torque absolute data (in Nm) revealed no statistically significant differences between the NAC and placebo groups at any time point ($p = .240$). There was a main effect for time ($p < .01$), and post-hoc analyses revealed that, following Bonferroni correction of the predetermined alpha level of .05, absolute MVC torque values were significantly different from pre-exercise immediately post-exercise, and at 1D, 3D, and 7D post-exercise ($p < .001$). Following Bonferroni corrections, it was observed that absolute MVC torque values at 10D post-exercise were not significantly

different than pre-exercise values (56.66 ± 13.50 vs. 52.72 ± 11.66; p = .007) (see Figure 3).

MVC torque values between angles were not significantly different at any time point (p = .971). Thus, comparisons at each time point are based on a torque value calculated as a mean of all nine trials (three trials per angle) at the three different angles (90°, 100°, and 110° of elbow flexion) measured per time point.

Subjects were familiarized with the MVC torque testing procedure after the second familiarization trial, as evidenced by the absence of significant differences between MVC torque measurements during the second familiarization trial and that measured immediately before the acute bout of eccentric exercise (p = 0.134) and an intra-class correlation coefficient of 0.99 (p < 0.01) between torque values at both time points (see all MVC torque data in Appendix G).

In order to normalize values and account for subject size differences and differences in baseline MVC torque, results were expressed as percent decline in MVC torque from pre-exercise (i.e., baseline) values. MVC torque declined significantly in both groups following the acute bout of eccentric exercise. There was a main effect for time (p < .01), and values for percent decline in MVC torque (compared to pre-exercise values) were significant at all angles of elbow flexion immediately following (p < .001), and at 1D (p < .001), 3D (p = < .001), and 7D (p = < .001) following the bout of injurious exercise. Post-hoc tests (using Bonferroni corrections) revealed that values for percent decline in MVC torque were not statistically different from pre-exercise at 10D post-exercise (0% vs 6.43 ± 6.82%; p = .007) (see Figure 2).

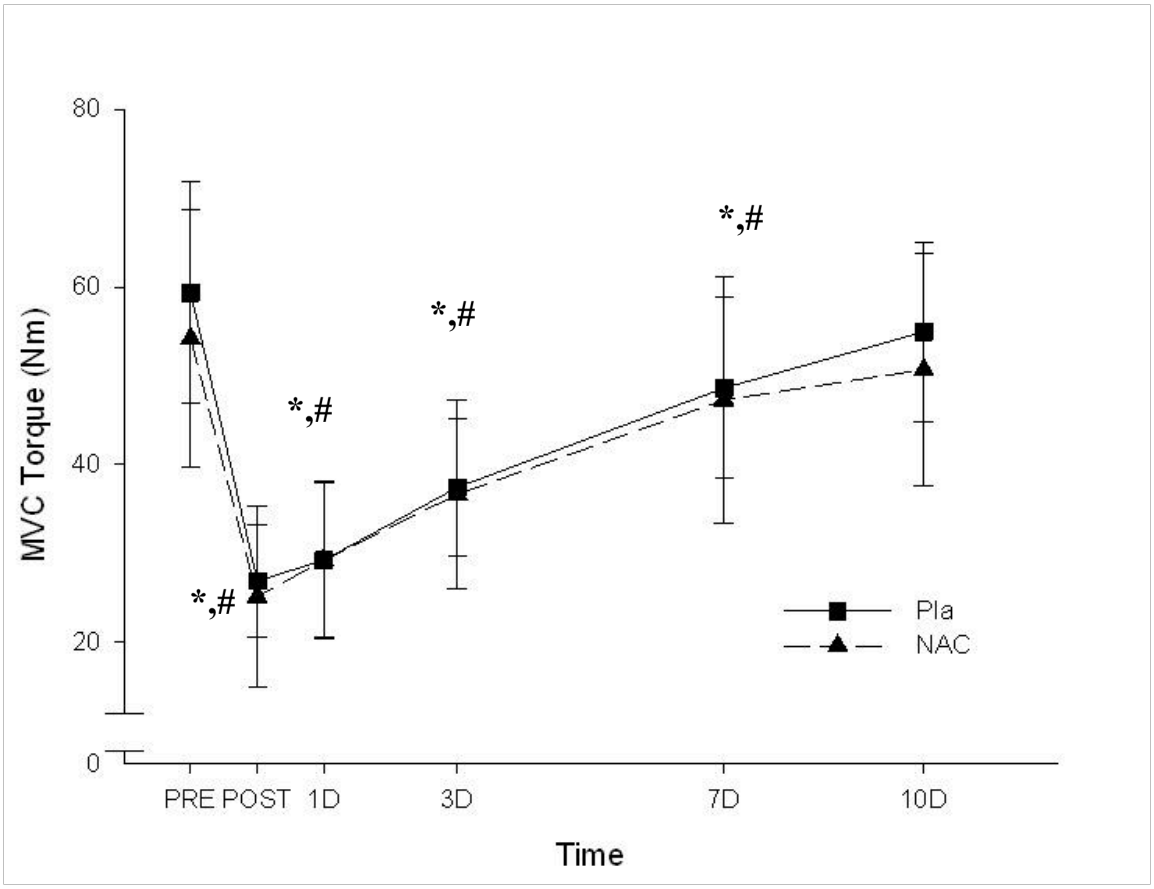

Figure 2. MVC torque (Nm) at baseline (PRE), immediately post-exercise (POST), 1D, 3D, 7D, and 10D post-exercise.

* indicates values significantly different from pre-exercise (PRE) for placebo group.
# indicates values significantly different from pre-exercise (PRE) for NAC group.

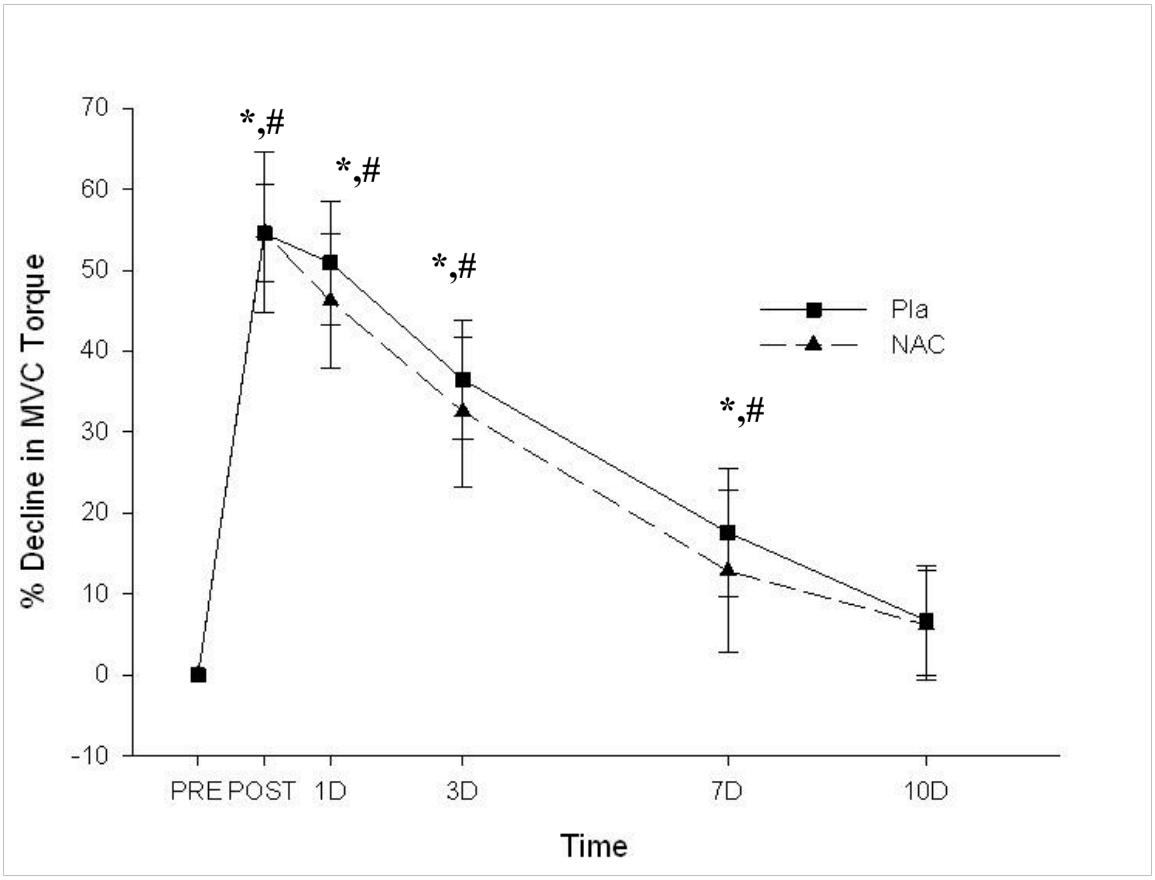

Figure 3. Percent decline in MVC torque measured at baseline (PRE), immediately post-exercise (POST), 1D, 3D, 7D, and 10D post-exercise.

* indicates values significantly different from pre-exercise (PRE) for placebo group.
# indicates values significantly different from pre-exercise (PRE) for NAC group.

Range of Motion

There was a significant decrease in ROM for both groups following the bout of injurious exercise (see data in Appendix H). There was a main effect for time ($p < .01$) and mean values for all subjects' ROM were significantly different from baseline (pre-exercise) immediately post-exercise (97.7°, $p < .001$) and at 1D (107.8°, $p < .001$), and 3D (110.5°, $p < .001$) post-exercise. Recovery to pre-exercise ROM values were

complete by 7D post-exercise for all subjects. There were no significant differences in ROM of the injured arm between the placebo and NAC group at any time point ($p = .539$) following performance of the eccentric exercise protocol (see Figure 4).

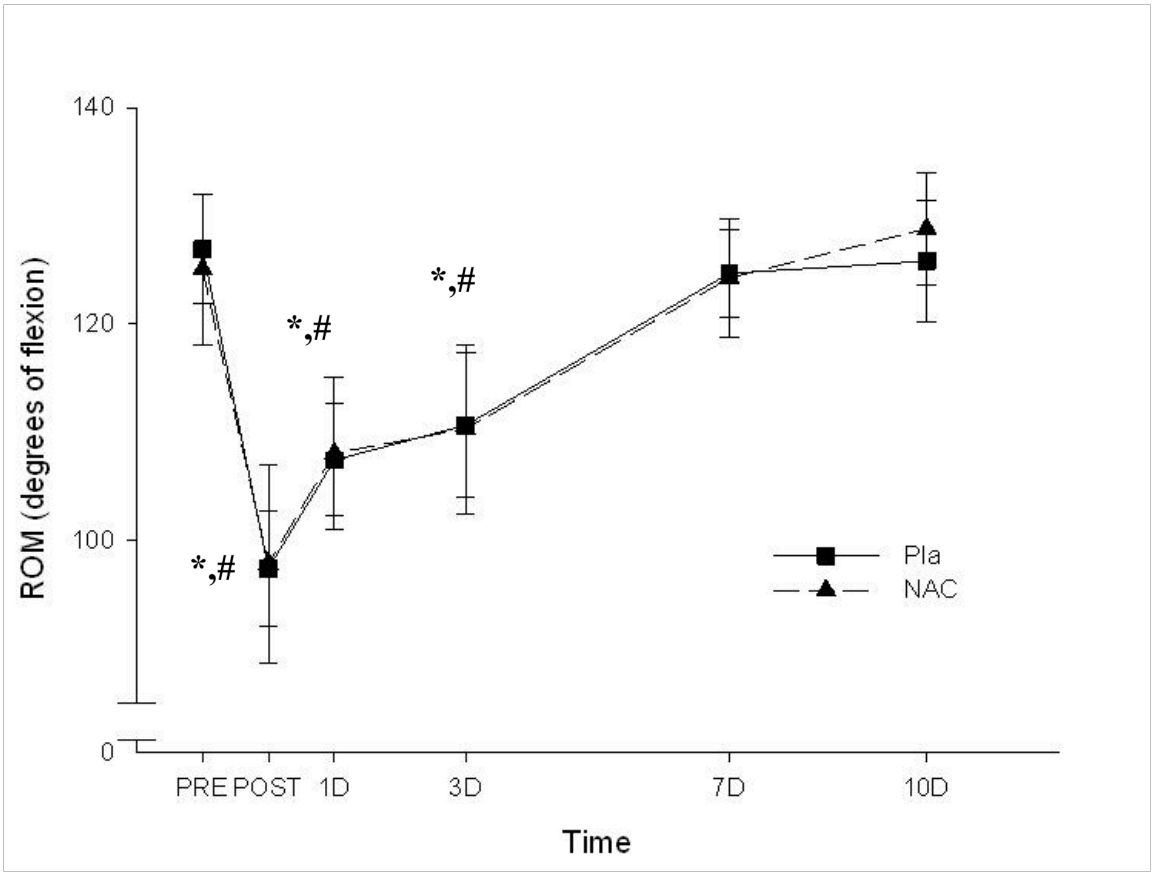

Figure 4. Non-dominant elbow joint range of motion (degrees of flexion) at baseline, immediately, 1D, 3D, 7D, and 10D post-exercise.

* indicates values significantly different from pre-exercise (Pre) for placebo group.
# indicates values significantly different from pre-exercise (Pre) for NAC group.

Rating of Muscle Soreness

Each subject rated soreness of the injured (non-dominant) forearm flexor muscle group pre-exercise, immediately post-exercise, 1D, 3D, 7D, and 10D post-exercise using a visual analog scale (see data in Appendix I). There was a main effect for time ($p < .01$) and mean soreness ratings for all subjects were significantly higher than pre-exercise ratings (.62 ± 1.05 mm) at 1D (29.41 ± 15.3 mm, $p < .001$), 3D (73.75 ± 8.7 mm, $p < .001$), and 7D (13.49 ± 8.91 mm, $p < .001$) post-exercise. All subjects had returned to

baseline levels of muscle soreness at 10D post-exercise. There were no statistically significant differences between the placebo and NAC group's mean soreness ratings at any time point ($p = .752$) (see Figure 5).

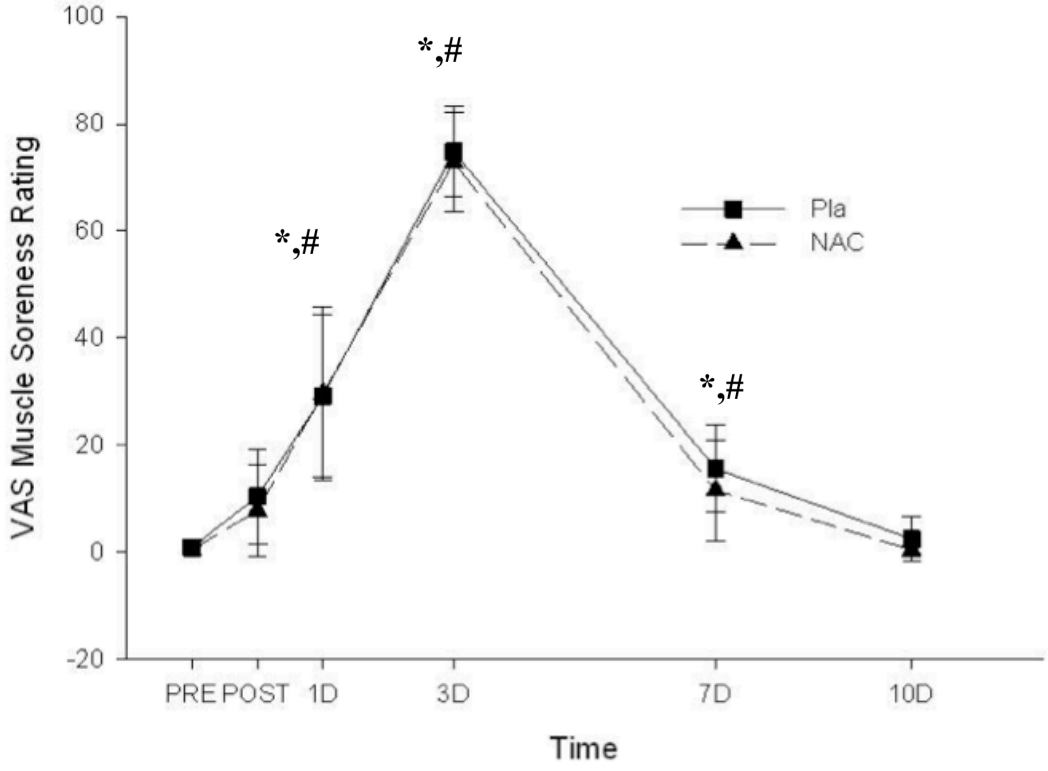

Figure 5. Visual analog scale muscle soreness rating (mm) at baseline, immediately, 1D, 3D, 7D, and 10D post-exercise.

* indicates values significantly different from pre-exercise (Pre) for placebo group.
# indicates values significantly different from pre-exercise (Pre) for NAC group.

Arm Circumference

Circumference of the injured arm was assessed pre-exercise, immediately post-exercise, and at 1D, 3D, 7D, 10D post-exercise. The mean value of three measurements at each time point was used for analysis. Values for circumference were converted to change from baseline (pre-exercise) values in order to account for differences in subject's arm circumference (see data in Appendix J).

There was a main effect for time ($p < .001$) following exercise-induced muscle injury and values were significantly higher than baseline (0mm) immediately post-exercise (0mm vs. .11 ± .01mm; $p < .001$) and at 1D (0mm vs. .30 ± .21 mm; $p < .001$), 3D (0mm vs. .52 ± .24 mm; $p < .001$), and 7D (0mm vs. .85 ± .25 mm; $p < .001$) post-exercise. Circumference values at 10D post-exercise were not statistically significant from pre-exercise values (0 mm vs. .13 ± .10 mm; $p > .05$). There was no difference in circumference measurements (change from baseline) between the placebo and NAC group at any time point ($p = .535$) (see Figure 6).

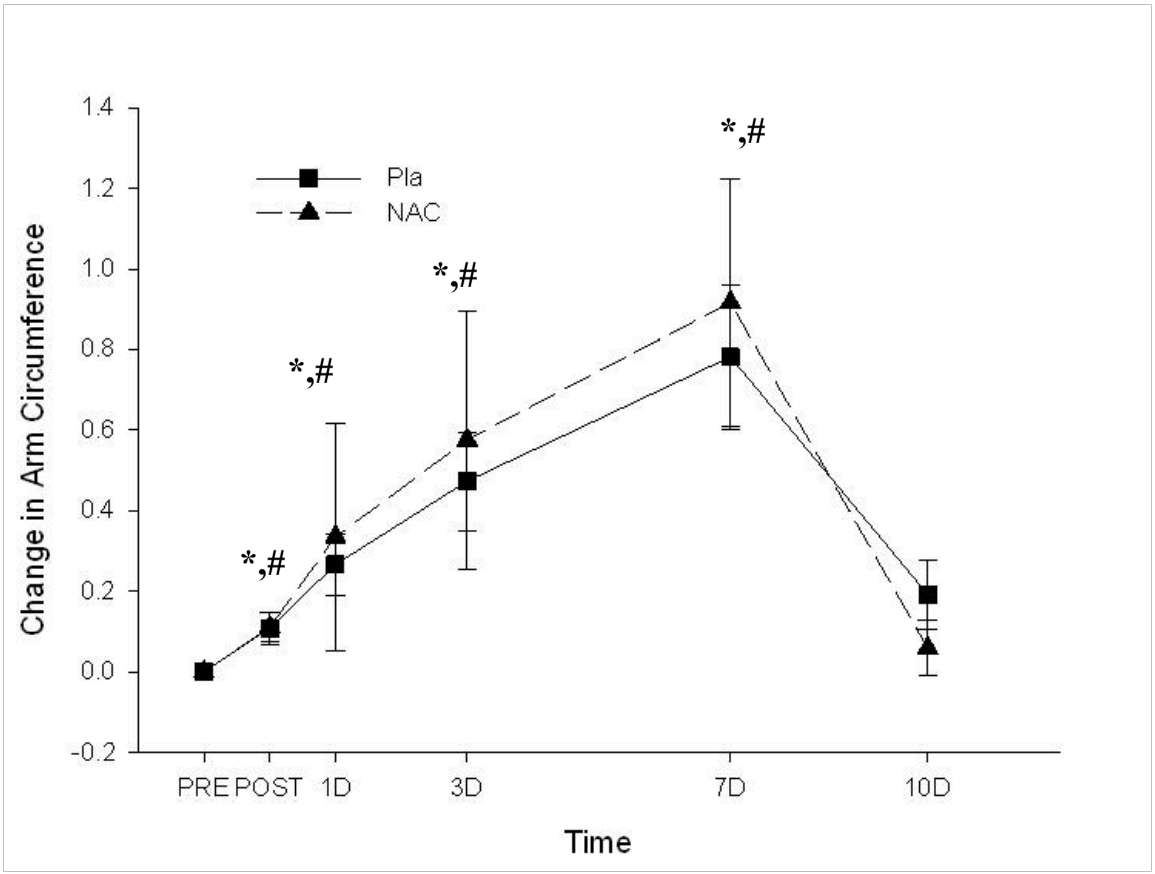

Figure 6. Change in non-dominant arm circumference (mm) at baseline (PRE), immediately post-exercise (POST), 1D, 3D, 7D, and 10D post-exercise.

* indicates values significantly different from pre-exercise (Pre) for placebo group.
# indicates values significantly different from pre-exercise (Pre) for NAC group.

Blood Analysis

Serum CK levels peaked at 3D post-exercise (750 ± 468.92 U·L) for both groups and were significantly higher when compared to pre-exercise (154 U·L), 1D (225 U·L), 7D (175.39 U·L), and 10D (50.12 U·L) post-exercise ($p < .001$) (see Figure 7).

The coefficient of variation between duplicate samples at each time point was less than 5% (± 3.24%). There was not a significant group x time interaction ($p = .449$) (see

data in Appendix K). Therefore, the treatment had no effect on CK response to exercise-induced muscle injury.

Analysis of IL-6 data indicated there was no significant difference between groups at either time point ($p = .360$) and there was no significant effect for time between pre-exercise and 3D serum IL-6 levels (22.09 ± 5.59 pg·mL vs. 22.21 ± 5.22 pg·mL; $p = .973$); indicating that the eccentric injury protocol had no effect on serum IL-6 levels following performance of the eccentric exercise protocol at this time point (see Figure 8).

The coefficient of variation between glutathione samples was less than 5% (± 3.5%). There was no significant difference in pre-exercise serum glutathione levels between the placebo and NAC groups (3.18 ± .88 μM vs. 3.3 μM ± .61 respectively; $p = .967$) (see Figure 9) (see data in Appendix M).

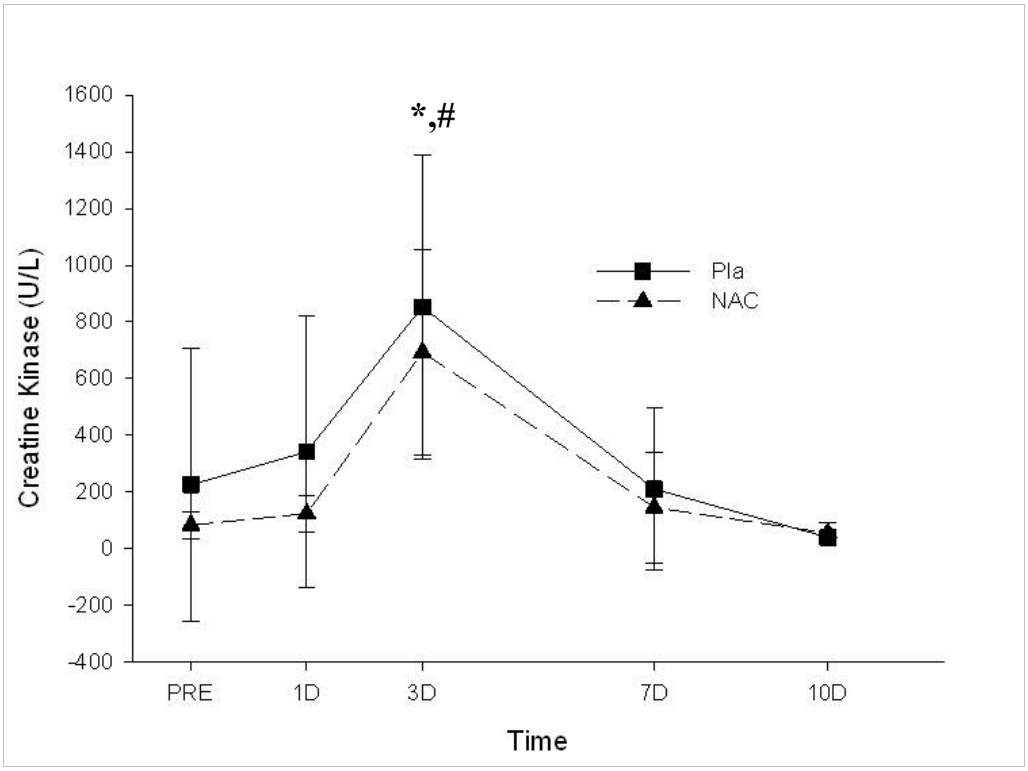

Figure 7. Serum creatine kinase (U·L) at baseline (PRE), 1D, 3D, 7D, and 10D post-exercise.

* indicates values significantly different from pre-exercise (Pre) for placebo group.
# indicates values significantly different from pre-exercise (Pre) for NAC group.

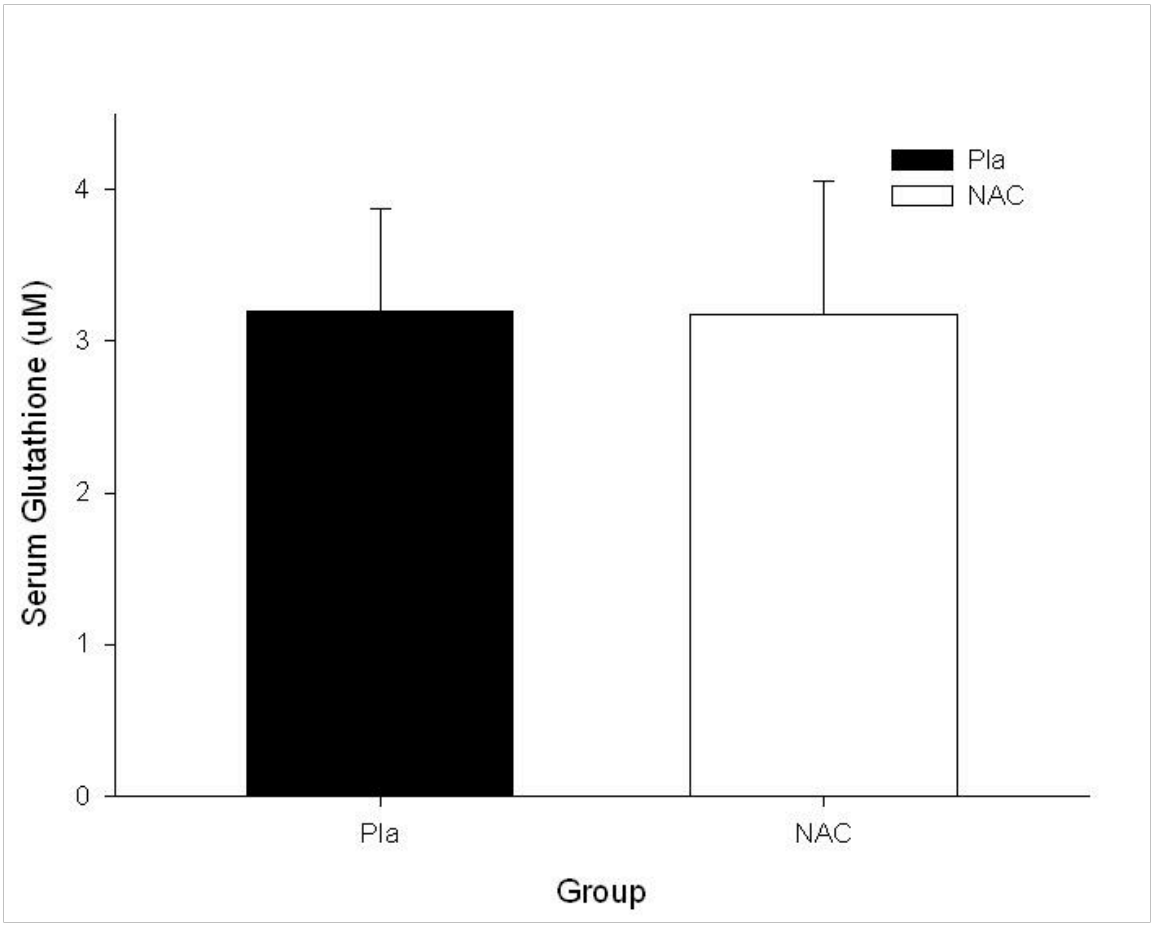

Figure 8. Serum glutathione (μM) at baseline. There was no significant difference between groups (p = .967).

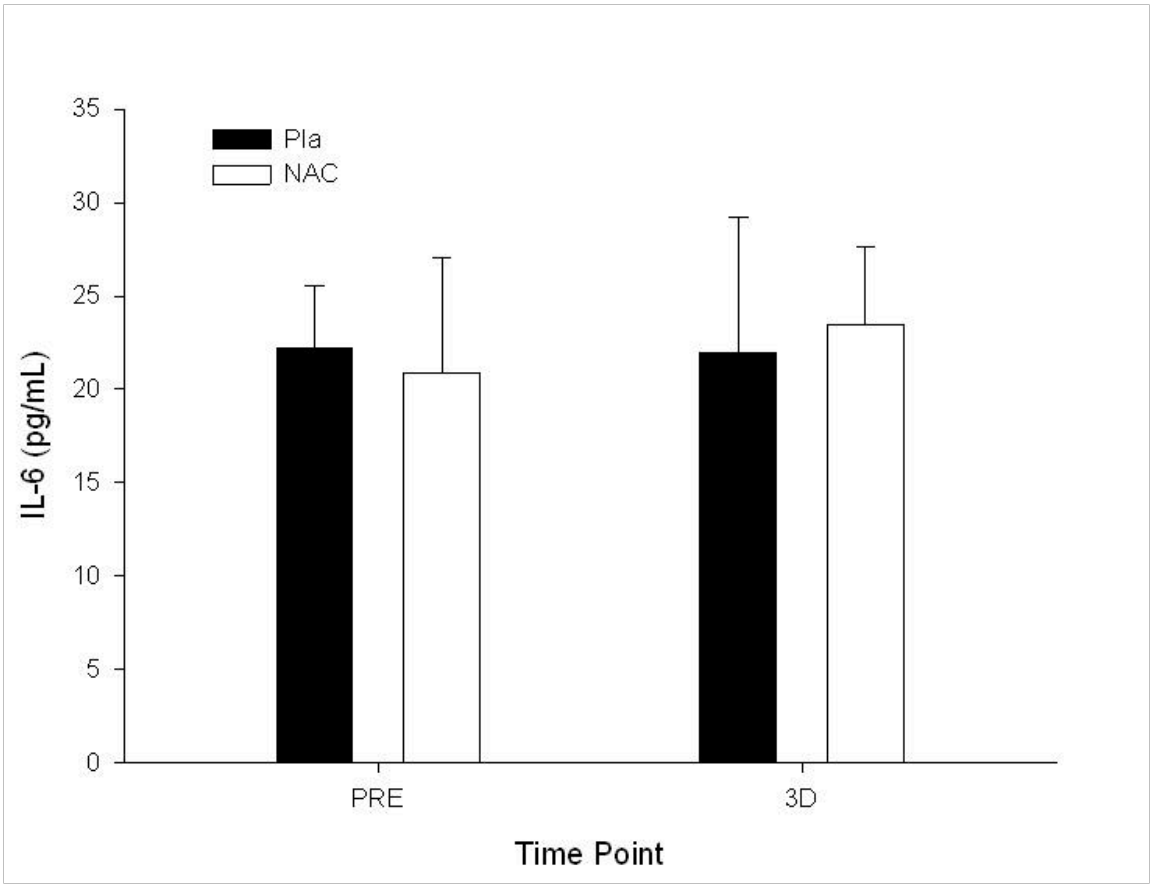

Figure 9. Serum IL-6 at baseline (PRE) and 3D post-exercise. There were no significant differences between groups (p = .973).

Food Frequency Questionnaire

Scoring of the food frequency questionnaire revealed that there was not a statistically significant difference between the weighted scores (based on antioxidant content of the food group consumed) for the placebo (41.04 ± 8.04) or NAC (30.74 ± 14.89) group in regards to consumption of high-antioxidant foods for the duration of the study (p = .057) (see data in Appendix N).

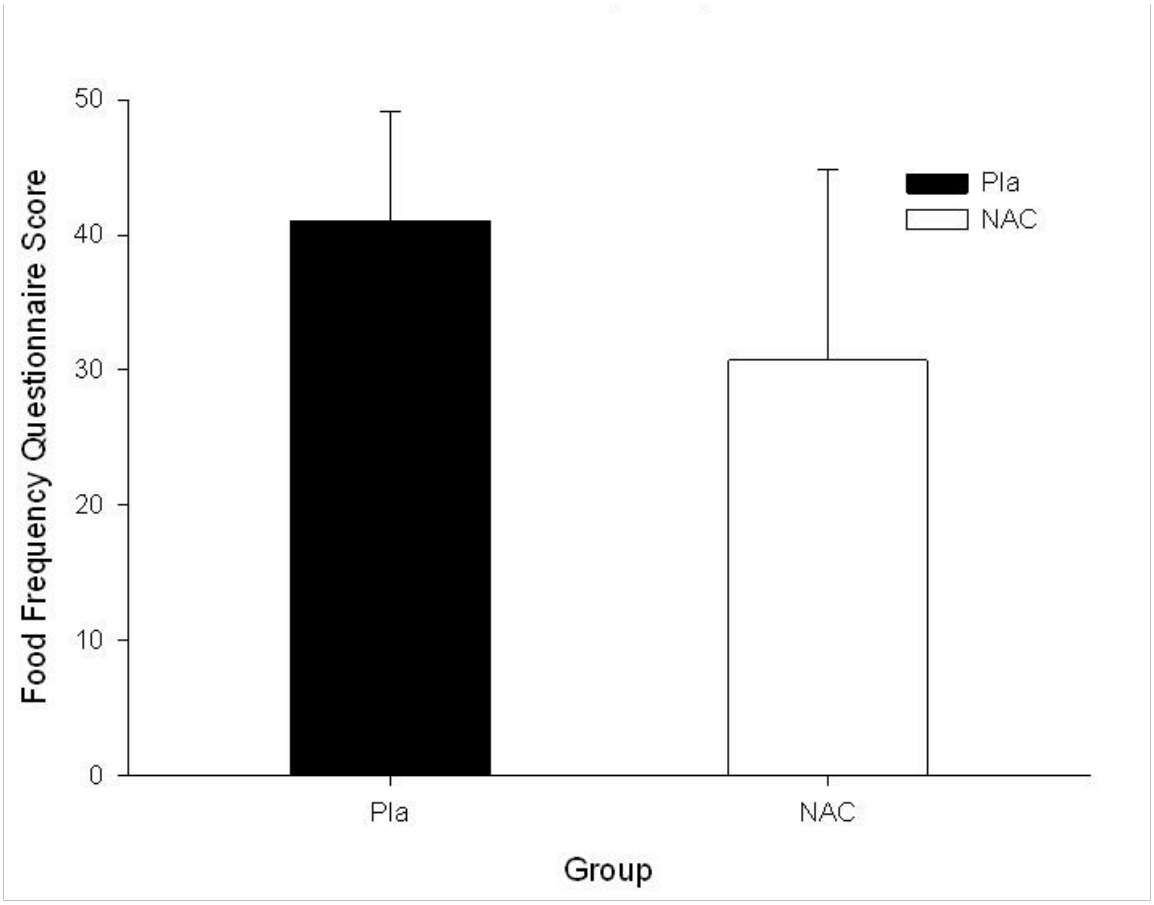

Figure 10. Food frequency questionnaire scores. There was no significant difference between groups ($p = .054$).

Discussion

The primary objective of this study was to determine the effect of the dietary supplement N-acetylcysteine (NAC) on recovery of muscular strength (MVC torque) following eccentric-contraction induced muscle injury. Following performance of an injury-inducing eccentric exercise protocol, we found that there was no difference in the loss and subsequent recovery of muscular strength between the placebo and NAC supplement group at any time point (immediately post-exercise, 1D, 3D, 7D, or 10D post-exercise). Therefore, the results of this study did not support our hypothesis. In addition, NAC supplementation at this dosage had no effect on any of the other markers of muscle injury.

The response of skeletal muscle to an acute bout of eccentrically biased exercise has been well established. The primary variable measured in this study was the decline and subsequent recovery of the ability to generate muscular force with the injured muscle group (represented as MVC torque). Prior studies have found that force deficits are maximal immediately following a bout of eccentric exercise. Depending on the injury model and the magnitude of injury, recovery begins within the first 24-48 hours and is usually (though not always) complete by three days to fourteen days post-exercise (45). Subjects in this study experienced a significant reduction in the ability to produce isometric force with the injured muscle group; MVC torque was reduced by 54.89% immediately post-exercise and most subjects exhibited a complete recovery of MVC torque (mean deficit of 2.78% at 10D post-exercise) in the subsequent ten-day post-exercise period.

Previous works have also demonstrated that delayed-onset muscle soreness (DOMS) is noticeable twenty-four to forty-eight hours after the bout of injurious exercise and reaches a peak between two to four days following that exercise. The localized muscle soreness and pain are gradually reduced following this peak and soreness is typically not evident by ten days post-exercise (46). Subjects in this study also experienced the expected pattern of DOMS, rating a statistically significant increase in soreness (mean of 73.75 on a 100mm Visual Analog Scale) at 3D post-exercise and reporting little to no pain (mean 1.33 rating) at 10D post-exercise.

Serum CK displays a delayed response to exercise-induced muscle injury; peaking two to five days following exercise before returning to baseline within approximately seven days (47). Serum CK levels of subjects in this study demonstrated a statistically significant peak at 3D post-exercise (750.01 U·L), representing an approximate 5-fold increase from baseline, with a return to normal/baseline levels at 10D post-exercise (50.12 U·L). The magnitude of this increase would have been greater with the exclusion of one subject who displayed an elevated pre-exercise serum CK level of 1586.7 U·L. The reason for this subject's elevated pre-exercise CK is unknown. Exclusion of this subject would have reduced the mean pre-exercise (baseline) serum CK level to 78.69 U·L and reduced the mean 3D peak to 682.4 U·L; representing an approximate 8-fold increase in serum CK levels following the bout of eccentric exercise.

Changes in bloodborne muscle protein levels (such as serum CK) are poorly correlated with decreases in MVC torque and there is a great deal of inter-subject variability in these measures when compared with MVC torque (37-40). Therefore,

measurement of these proteins is not recommended as the sole representative measure of muscle injury.

One study has documented an increase in IL-6 activity following eccentric-contraction induced injury of the forearm flexors (48). However, other studies have not reported a significant increase in IL-6 following similar forearm flexor eccentric exercise (2, 3). Subjects in this study did not demonstrate an increase in serum IL-6 levels at 3D post-exercise. Therefore, it seems likely that the relatively small muscle mass injured in this study was not sufficient to increase serum IL-6 levels found in serum following the bout of eccentric exercise or it is possible that serum IL-6 peaked at a different time point than those that were measured.

Earlier works have also demonstrated that exercise-induced muscle injury of the forearm flexors is associated with a decrease in ROM of the injured arm (45). Following a bout of eccentric exercise, range of motion is significantly reduced immediately after the exercise bout. Total ROM remains significantly lower than pre-exercise levels until five to seven days post-exercise when values return to pre-exercise levels (5). Results of this study clearly demonstrate that subjects' injured arm ROM followed a predictable pattern after the bout of injurious exercise. Mean ROM was significantly reduced immediately post-exercise (97.7°), at 1D (107.8°), and at 3D (110.5°) post-exercise. Values for ROM returned to baseline, pre-exercise values by 7D post-exercise.

Eccentric exercise-induced muscle injury also results in swelling of the injured muscle. Other groups have used arm circumference to represent muscle swelling following injurious exercise. These groups have reported that arm circumferences are

slightly elevated immediately post-exercise, peak between four and six days post-exercise, and return to baseline levels by ten days post-exercise (45). The changes in upper arm circumference of the injured arm also followed previously observed patterns. Subjects experienced a statistically significant increase in arm circumference of .30mm .53mm and .86mm at 1D, 3D and 7D post-exercise, respectively.

Considering these well-established findings, it is apparent that the bout of eccentrically biased exercise performed by subjects participating in this study resulted in a pattern of exercise-induced muscle injury and subsequent recovery that is consistent with previous findings.

NAC supplementation is purported to increase enzymatic antioxidant levels. In order to determine the effectiveness of the NAC supplementation on serum glutathione levels, pre-exercise serum glutathione levels were compared between groups. Following the analysis, we found that the supplement dosage administered did not have a significant effect on serum glutathione levels when compared to a placebo group following a seven-day supplementation period. The dosage used in this study was consistent with that used in previous studies and was chosen to limit the possibility of side effects (GI distress, etc.). Previous works had demonstrated a significant effect on glutathione levels (and other markers of inflammation or antioxidant content) using similar doses (49-51).

Vats et al., (2002) demonstrated that a dose of 400 $mg \cdot d^{-1}$ of NAC was sufficient to offset changes in the glutathione ratio in response to high-altitude stress and Silva et al., (2008) observed an augmented anti-inflammatory response (increased IL-10 in the treatment group) associated with a dosage of 10 $mg \cdot kg^{-1}$ $bw \cdot d^{-1}$ of NAC (19, 49).

Kerksick et al., (2009) also demonstrated that subjects ingesting 10 $mg \cdot kg^{-1}$ $bw \cdot d^{-1}$ of NAC had significantly higher total blood antioxidant status following a seven-day loading period (28). However, the possibility exists that the chosen dose was insufficient to have a significant effect on serum glutathione levels.

Higher doses of NAC have been utilized in a number of studies; a dose of 7,200 mg of NAC (four doses of 1,800 mg over thirty-six hours) was sufficient to increase whole-blood glutathione levels following treatment (52). Similarly, a dose of 140 $mg \cdot kg^{-1}$ $bw \cdot d^{-1}$ has also been associated with a significant increase in plasma glutathione levels (53). However, these high doses were accompanied by a significant increase in side effects. In addition, higher doses have also been associated with a cytotoxic effect; resulting in production of additional ROS (e.g., superoxide, hydrogen peroxide, and/or hydroxyl radical) (54, 55). This supplement is supposed to have an antioxidant effect. However, under high doses and/or certain conditions (e.g., exercise-induced muscle injury) supplementation with NAC can have a pro-oxidant effect (15).

It has been observed that normal levels of glutathione exert a feedback inhibition on the enzyme gamma-glutamyl-cysteine synthetase (56). This may partly explain the non-significant increase in glutathione levels in healthy subjects with normal pre-supplementation serum glutathione levels; normal pre-exercise levels in healthy individuals would most likely result in glutathione exerting a feedback inhibition on gamma-glutamyl-cysteine synthetase thereby by preventing or minimizing any changes following supplementation with NAC.

In addition to the possibility of a lower dose being insufficient, differences in serum reduced glutathione (or the ratio of reduced glutathione to oxidized glutathione) may have been found later in the study when oxidative stress was likely higher and subjects were experiencing the symptoms of exercise-induced muscle injury. Therefore, future studies should include analysis of serum glutathione levels at different time points following injurious exercise.

All subjects were repeatedly reminded and encouraged to take their supplements as prescribed. Each subject was told to bring their pill boxes to each data collection session to allow for a pill count to determine if they were taking the supplement as directed. In addition, subjects were questioned about their compliance with the supplementation regimen. Using this method, the investigators have a high degree of confidence that subjects had taken the supplement as prescribed. However, the possibility exists that subject adherence to the supplementation protocol was not 100%. Non-compliance by subjects in the NAC group may have significantly impacted their results (when compared to non-compliance by placebo group subjects) by reducing the frequency and dosage of NAC supplementation during the duration of the study. However, because of the blinded nature of the protocol, non-compliance could have affected each group equally.

The arm curl machine that was designed for this project was based on a similar device used by Clarkson et al., (1992) to investigate the response to an acute bout of high-intensity eccentric exercise (45). The device used in this study was constructed by attaching hinges and a custom made arm to a pre-fabricated arm curl bench (Yukon Fitness, Cleveland, OH). The custom made arm of the machine was designed to allow

attachment of a load cell (Transducer Techniques® MLP 150, Transducer Techniques, Temecula, CA). The opposite end of the load cell was attached to a wrist strap.

Following modification of the arm curl machine, the load cell was tested for calibration in tension. This was accomplished by hanging weights from one end of the load cell while the other was attached to an overhead steel beam. The results of this calibration procedure indicated that the coefficient of variation was less than 1% between three trials per load each day and less than 5% between days during a seven-day period (See data in Appendix N). The load cell was also calibrated in concert with the arm of the custom-built arm-curl machine. The coefficient of variation between trials (3 trials per day) and days for this form of calibration was also less than 5% (See data in Appendix O).

The exercise protocol employed in this study is similar to that used in a substantial body of published research (2, 3, 5, 45, 47, 57-60). It was not unexpected therefore, that all subjects experienced significant symptoms of exercise-induced muscle injury following performance of this exercise intervention (i.e., decline in isometric force production, muscle soreness, increased serum CK, decreased ROM, and increased circumference of the injured arm).

Animal studies utilizing infusion of NAC have demonstrated that supplementation provides protection from the initial deficits in muscular strength associated with exercise-induced muscle injury (9, 22, 23). However, these findings have not been confirmed in previous human studies investigating the effect of NAC supplementation on the symptoms of exercise-induced muscle injury (15, 19, 28) and were not confirmed in this

study. It has been well documented that NAC supplementation or infusion results in delayed fatigue during exhaustive exercise (61-63). Therefore, it is possible that eccentric contraction induced muscle injury in humans, similar to that induced by the exercise protocol in this study, is a unique situation that is unaffected by NAC supplementation at this dosage and/or increased glutathione levels.

In conclusion, a novel bout of eccentrically biased forearm flexor exercise resulted in muscle injury and a significant decrease in the ability to produce force for ten days. N-acetylcysteine supplementation had no effect on recovery of strength at any time point following the bout of injurious exercise. These results suggest that NAC supplementation may not provide a benefit to athletes and casual exercisers attempting to treat the symptoms of exercise-induced muscle injury. However, these results also indicate that supplementation with NAC in this dosage is not harmful under the specific conditions encountered in this study.

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APPENDIXES

APPENDIX A

PHYSICAL CHARACTERISTICS OF SUBJECTS

Placebo			
Subject	Age (yrs)	Weight (kg)	Height (m)
1	22	70.45	1.69
2	21	53.18	1.71
3	19	57.27	1.64
4	19	78.64	1.73
5	20	71.36	1.71
6	21	74.09	1.83
7	21	94.09	1.74
8	21	67.27	1.64
9	21	60.00	1.61
10	20	62.73	1.71
Mean	20.50	68.91	1.70
SEM	0.97	11.87	0.06

NAC			
Subject	Age (yrs)	Weight (kg)	Height (m)
11	22	79.55	1.78
12	21	59.09	1.73
13	20	56.82	1.69
14	20	61.36	1.68
15	20	59.09	1.54
16	21	77.73	1.57
17	21	68.18	1.60
18	21	57.73	1.63
19	23	81.36	1.79
20	22	71.36	1.75
21	20	67.73	1.74
Mean	21.00	67.27	1.68
SEM	1.00	9.19	0.09

APPENDIX B

INFORMED CONSENT FORM

Georgia State University
Department of Kinesiology and Health
Informed Consent

Title: The effect of N-acetyl cysteine supplementation on recovery of strength following eccentric contraction induced muscle injury.

Principal Investigator: Dr. J. Andrew Doyle
Student Principal Investigator: Ryan Luke

I. Purpose:

You are invited to participate in a research study. The purpose of the study is to find out how an amino acid diet supplement affects strength after muscle injury. You are invited to participate because you are an 18 to 40 year-old healthy female who does not currently lift weights. A total of 20 people will take part in this study. This study will require about seven hours of your time over seventeen (17) days.

II. Procedures:

If you decide to participate, you will be asked to do the following things:
1. Come to Sports Arena room G18 on six different days.
2. Talk to Dr. J. Andrew Doyle, Dr. Chris Ingalls, Dr. Jeffrey Rupp, or Ryan Luke.
3. Take an amino acid diet supplement once a day for 17 days.
4. Do arm curl exercises on an arm curl machine.
5. Rate your level of muscle soreness on a rating scale.
6. Give six blood samples. Each sample will be around 4 ml of blood and the total amount taken will be 26 ml or .9 ounce of blood.
7. Complete a survey asking how often you eat certain foods.

III. Risks:

Being a part of this study may cause biceps muscle soreness. There are slight risks involved with drawing blood such as; excessive bleeding, fainting or dizziness, bruising, and infection. In very rare and extreme cases, a condition called exertional rhabdomyolysis may result from the exercises you will be doing. If you experience too much muscle soreness or pain, bruising, swelling, and/or a darkening of the urine, seek immediate medical care.

IV. Benefits:

Participation in this study may not benefit you personally. We hope to see how a diet supplement can affect strength after biceps muscle injury. Compensation will be provided in the form of a $20.00 Visa gift card. Failing to participate in the entire study will not exempt you from compensation. The amount of compensation received will be prorated to reflect the duration of your participation in the study.

V. Voluntary Participation and Withdrawal:

Consent Form Approved by Georgia State University IRB May 27, 2011 - May 19, 2012

You are a volunteer. You do not have to be in this study. If you decide to be in the study and change your mind, you have the right to drop out at any time. You may skip sessions or stop participating at any time. Whatever you decide, you will not lose any benefits to which you are otherwise entitled.

VI. Confidentiality:

We will keep your records private to the extent allowed by law. We will use a code rather than your name on study records. Only Dr. JA Doyle, Dr. Chris Ingalls, Dr. Jeffrey Rupp, and Ryan Luke will have access to the information you provide. Information will be stored in a locked file cabinet in a locked room. The code identifying you will be stored separately from your personal data. A code sheet identifying all research participants will be destroyed at the end of the study. Your name and other personal facts will not appear when we present this study or publish its results. The findings will be summarized and reported in group form. You will not be identified personally.

Your privacy will be protected to the extent allowed by law. To make sure that this research is being carried out in the proper way, the Georgia State University IRB may review study records. The Office of Human Research Protections and/or the Food and Drug Administration may also look over study records during required reviews.

VII. Contact Persons:

Contact Dr. JA Doyle at 404-413-8478, or Ryan Luke at 404-413-8050 if you have questions about this study. If you have questions or concerns about your rights as a participant in this research study, you may contact Susan Vogtner in the Office of Research Integrity at 404-413-3513 or svogtner1@gsu.edu.

VIII. Disclaimer

If you have any question about this study, or believe you have suffered any injury because of participation in the study, you may contact Dr. JA Doyle at 404-413-8478 or Ryan Luke at 404-413-8050. Georgia State University, however, has not set aside funds if something should occur.

VIII. Copy of Consent Form to Subject:

We will give you a copy of this consent form to keep.

If you are willing to volunteer for this research, please sign below.

_____	_____
Participant	Date
_____	_____
Principal Investigator or Researcher Obtaining Consent	Date

APPENDIX C

HEALTH HISTORY FORM

Applied Physiology Laboratory
Department of Kinesiology and Health
Georgia State University

Health History

All information given is personal and confidential. The information will enable us to better understand you and your health and fitness habits.

Subject Number_____ Date_____
(granted by researcher)
Address_____ Home Phone_____

City/State_____ Zip Code_____

E-mail_____ Bus Phone_____

DOB_____Gender_____Height_____Weight_____Race_____

Signs and Symptoms

Have you ever experienced any of the following:
(please circle yes or no)

yes no 1. Pain, discomfort, tightness or numbness in the chest, neck, jaw or arms.

yes no 2. Shortness of breath at rest or with mild exertion.

yes no 3. Dizziness or fainting.

yes no 4. Difficult, labored or painful breathing during the day or at night.

yes no 5. Ankle swelling.

yes no 6. Rapid pulse or heart rate.

yes no 7. Intermittent cramping.

yes no 8. Known heart murmur.

yes no 9. Unusual shortness of breath or fatigue with usual activities.

If you answered yes to any of the above:

How often do you experience the symptom?_____

Have you ever discussed the symptom with a doctor?_____

Explain the symptom in more detail:_____

_____

Major Risk Factors

yes no 1. Do you have a body mass index ≥ 30 or a waist girth >100 cm?

yes no 2. Have you had a fasting glucose of ≥ 110 mg/dl confirmed by measurements on at least 2 separate occasions.

yes no 3. Has your father or brother experienced a heart attack before the age of 55? Or has your mother or sister experienced a heart attack before the age of 65?

yes no 4. Do you currently smoke or quit within the past 6 months?

yes no 5. Has your doctor ever told you that you have high blood pressure?

yes no 6. Do you have high cholesterol?
Total cholesterol:_____HDL:_____Date tested:_____

yes no 7. Do you have a sedentary lifestyle? (sitting most of the day in your job with no regular physical activity)

Medical Diagnoses

Have you ever had any of the following? Circle all that apply:

Asthma *(lung disease causing difficult breathing)*	coronary artery disease	heart surgery
Angioplasty *(surgical opening of heart artery)*	angina *(chest pain)*	hypertension
Heart clicks *(abnormal heart sounds)*	heart murmur	heart attack
Emphysema *(lung disease)*	bronchitis *(lung inflammation)*	stroke
Emboli *(abnormal blood pressure)*	phlebitis *(inflammation of vein)*	anemia
Cancer	emotional disorders	eating disorders
Osteoporosis *(decreased bone mass/density)*		

Any special problems not listed above:_____

If any of the above are circled, please give details and explain_____

General

yes no 1. Are you pregnant?

yes no 2. Do you have arthritis or any bone or joint problem?
If yes, please explain:_____

_____

yes no 3. Do you currently exercise?
If yes, how long have you been exercising?_____

What do you do and how often?_____

yes no 4. Are you taking any medication, vitamins or supplements?
Name them and their dosage (list both prescribed & over-the-counter medications)
Drug name & dosage / purpose of drug / prescribed or over-the-counter

_____

_____

_____

My signature certifies that all of the above is true, to the best of my knowledge.

Signature:_____ Date:_____

****

STAFF USE ONLY

****

Comments:_____

Stratification(circle one): Low Risk Moderate Risk High Risk

Resting blood pressure:_____Resting heart rate:_____

yes no Do meds affect BP or HR?

Date:_____Initials:_____

APPENDIX D

IRB PERMISSION LETTER

INSTITUTIONAL REVIEW BOARD

Mail: P.O. Box 3999
Atlanta, Georgia 30302-3999

In Person: Alumni Hall
30 Courtland St, Suite 217

Phone: 404/413-3500
Fax: 404/413-3504

October 6, 2010

Principal Investigator: Doyle, James Andrew

Student Principal Investigator: Luke, Ryan

Protocol Department: Kinesiology & Health

Protocol Title: The effect of N-acetyl cysteine supplementation on recovery of strength after eccentric muscle damage.

Funding Agency:

Submission Type: Protocol H10478

Review Type: Full Board Review

Approval Date: May 20, 2010

Expiration Date: May 19, 2011

The Georgia State University Institutional Review Board (IRB) reviewed and approved the above referenced study and enclosed Informed Consent Document(s) in accordance with the Department of Health and Human Services. The approval period is listed above.

Federal regulations require researchers to follow specific procedures in a timely manner. For the protection of all concerned, the IRB calls your attention to the following obligations that you have as Principal Investigator of this study.

1. When the study is completed, a Study Closure Report must be submitted to the IRB.

2. For any research that is conducted beyond the one-year approval period, you must submit a Renewal Application 30 days prior to the approval period expiration. As a courtesy, an email reminder is sent to the Principal Investigator approximately two months prior to the expiration of the study. However, failure to receive an email reminder does not negate your responsibility to submit a Renewal Application. In addition, failure to return the Renewal Application by its due date must result in an automatic termination of this study. Reinstatement can only be granted following resubmission of the study to the IRB.

3. Any adverse event or problem occurring as a result of participation in this study must be reported immediately to the IRB using the Adverse Event Form.

4. Principal investigators are responsible for ensuring that informed consent is obtained and that no human subject will be involved in the research prior to obtaining informed consent. Ensure that each person giving consent is provided with a copy of the Informed Consent Form (ICF). The ICF used must be the one reviewed and approved by the IRB; the approval dates of the IRB review are stamped on each page of the ICF. Copy and use the stamped ICF for the coming year. Maintain a single copy of the approved ICF in your files for this study. However, a waiver to obtain informed consent may be granted by the IRB as outlined in 45CFR46.116(d).

All of the above referenced forms are available online at https://irbwise.gsu.edu. Please do not hesitate to contact Susan Vogtner in the Office of Research Integrity (404-413-3500) if you have any questions or concerns.

Sincerely,

Susan K. Laury, IRB Chair

Federal Wide Assurance Number: 00000129

APPENDIX E

DATA COLLECTION FORMS

Peak Eccentric Torque

Subj #_____

Seat 1 2 3 4 5

Arm Lever 1 2 3 4 5

Arm Pad 1 2 3 4 5

Arm Injured L R

Rep #	Set 1	Rep #	Set 2
1		26	
2		27	
3		28	
4		29	
5		30	
6		31	
7		32	
8		33	
9		34	
10		35	
11		36	
12		37	
13		38	
14		39	
15		40	
16		41	
17		42	
18		43	
19		44	
20		45	
21		46	
22		47	
23		48	
24		49	
25		50	

Subj #_____

Seat 1 2 3 4 5

Arm Lever 1 2 3 4 5

Arm Pad 1 2 3 4 5

Arm Tested L R

MVC Torque

	90	100	110		
				Fam1	
Average				Average	
StDev				StDev	
CV				CV	
	90	100	110		
				Fam2	
Average				Average	
StDev				StDev	
CV				CV	
	90	100	110		
				Pre-Injury	
Average				Average	
	90	100	110		
				Post-Injury	
Average				Average	
% Decline				% Decline	
	90	100	110		
				1d	
Average				Average	
% Decline				% Decline	
	90	100	110		
				3d	
Average				Average	
% Decline				% Decline	
	90	100	110		
				7d	
Average				Average	
% Decline				% Decline	
	90	100	110		
				10d	
Average				Average	
% Decline				% Decline	

Rating of Exercise Induced – Muscle Soreness
Visual Analogue Scale: 100 mm

Subject #	
Date	
Time	
Trial (circle one)	Pre Post 1d 3d 7d 10d

Soreness_____

0 No Soreness — **100** Very, Very Sore

Circumference_____

ROM _____

Soreness_____

0 No Soreness — **100** Very, Very Sore

Circumference_____

ROM _____

Soreness_____

0 No Soreness — **100** Very, Very Sore

Circumference_____

ROM _____

Subject Number _____

During an average week, how many servings of each food group do you consume?

	# of Servings (1 serving = 1 cup)						
1. Beans/Legumes	0-1	2-4	4-6	6-8	8-10	11+	**x 3**
e.g., Red Beans, Red Kidney Beans, Pinto Beans, Black Beans						**Score**	
	# of Servings (1 serving = 1 piece or 1 cup)						
2. Fruits	0-1	2-4	4-6	6-8	8-10	11+	**x 1.5**
e.g., Blueberries, Cranberries, Blackberries, Raspberries, Strawberries, Apples, Cherries, Plums						**Score**	
	# of Servings (1 serving = 1 cup)						
3. Dark Green Vegetables	0-1	2-4	4-6	6-8	8-10	11+	**x 1.25**
e.g., Spinach, Broccoli, Artichoke Hearts, Brussels Sprouts						**Score**	
	# of Servings (1 serving = 1/4 cup)						
4. Nuts	0-1	2-4	4-6	6-8	8-10	11+	**x 1.15**
e.g., Pecans, Almonds, Walnuts						**Score**	
	# of Servings (1 serving = 1 potato)						
5. Potatoes	0-1	2-4	4-6	6-8	8-10	11+	**x 1.1**
e.g., White Russet Potatoes, Yukon Gold Potatoes						**Score**	

Total Score

APPENDIX F

ASSAY INSTRUCTIONS AND PROCEDURES

GLUTATHIONE ASSAY KIT INSTRUCTIONS

QuantiChrom™ Glutathione Assay Kit (DIGT-250)

Colorimetric Determination of Reduced Glutathione at 412nm

DESCRIPTION

Glutathione is a tripeptide of glycine, glutamic acid and cysteine. In the red blood cell, the reduced form of glutathione is vital in maintaining hemoglobin in a reduced state and hence protecting the cells from oxidative damage. Glutathione is involved in detoxification of hydrogen peroxide through glutathione oxidase. Low levels of glutathione are found in deficiencies of key enzymes involved in glutathione metabolism, such as glucose-6-phosphate dehydrogenase, glutathione synthase and glutathione reductase.

Simple, direct and automation-ready procedures for measuring reduced glutathione are becoming popular in Research and Drug Discovery. BioAssay Systems' QuantiChrom™ Glutathione Assay Kit is designed to accurately measure reduced glutathione in biological samples. The improved 5,5'-dithiobis(2-nitrobenzoic acid (DTNB) method combines deproteination and detection into one reagent. DTNB reacts with reduced glutathione to form a yellow product. The optical density, measured at 412 nm, is directly proportional to glutathione concentration in the sample. The optimized formulation has a long shelf life and is completely free of interference due to sample turbidity.

KEY FEATURES

Sensitive and accurate. Linear detection range 0.4 - 100 µM in 96-well plate.
Simple and convenient. The procedure involves mixing the DTNB Reagent with sample, removing protein precipitates for proteinaceous samples, adding a second Reagent and reading the optical density.
Low interference. Amino acids and common buffers do not interfere.

APPLICATIONS:

Direct Assays: reduced glutathione in whole blood, plasma, serum, urine, tissue and cell extracts.
Drug Discovery/Pharmacology: effects of drugs on glutathione metabolism.

KIT CONTENTS (250 tests in 96-well plates)

Reagent A: 30 mL Reagent B: 30 mL
Standard: 10 mL (= 100 µM glutathione)

Storage conditions. Store Reagent A and Reagent B at 4°C. The glutathione standard should be stored at -20°C. Shelf life: 12 months.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Important: equilibrate Reagents to room temperature before use.

Procedure using 96-well plate:

1. *Blank and Standard*. Transfer 100 µL water and 100 µL Standard into wells of a clear-bottom 96-well plate. Pipette 200 µL water into the Blank and Standard wells.
2. *Samples*. Whole blood samples should be diluted 20-fold with water prior to the assay (*n* = 20). Deproteination is required for blood, serum, plasma and other proteinaceous samples. Mix 120 µL sample with 120 µL Reagent A in 1.5-mL centrifuge tubes. Vortex to mix well. Pellet 2 min at 14,000 rpm in a table centrifuge. If solution remains clear, no centrifugation was necessary.
3. Transfer 200 µL sample/Reagent A mixture into wells of the 96-well plate. Add 100 µL Reagent B. Tap plate lightly to mix.
4. Incubate 20 to 25 min at room temperature. Read $OD_{400\text{-}450nm}$ (peak 412 nm). Signal is stable for at least 60 min.

Procedure using Cuvet:

Mix 400 µL sample with 400 µL Reagent A, centrifuge sample tubes if precipitation occurs. Transfer 600 µL supernatant and mix with 400 µL Reagent B. Incubate 25 min at room temperature. Measure OD_{412nm} against water. Transfer 400 µL Standard and 800 µL Water into a clean cuvet and measure OD_{412nm} against water.

CALCULATION

Subtract blank OD (water) from the Standard and Sample OD values. The glutathione concentration of Sample is calculated as

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STD} - OD_{BLANK}} \times 100 \times n \ (\mu M)$$

OD_{SAMPLE}, OD_{STD} and OD_{BLANK} are optical density values of the sample, Standard and sample "Blank" (water or buffer in which the sample was dissolved). *n* is the dilution factor (20 for blood samples).
Conversions: 1 mg/dL glutathione equals 32.5 µM, 0.001% or 10 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tube and table centrifuge.

Procedure using 96-well plate:

Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

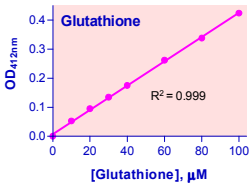
Procedure using cuvette:

Spectrophotometer and cuvets for measuring OD at 412 nm.

GENERAL CONSIDERATIONS

β-mercaptoethanol, dithiothreitol and cysteine are known to interfere in this assay. Avoid using these compounds during sample preparation. Amino acids do not interfere in this assay.

EXAMPLE. 20 µL fresh mouse blood was mixed quickly with 380 µL water. Assays in 96-well plate gave blood glutathione concentration of 1124 ± 8 µM (n = 2)



Calibration curve in 96-well plate

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RayBio®
Human IL-6 ELISA Kit

User Manual
(Revised Dec 16, 2009)

RayBio® Human IL-6 ELISA Kit Protocol

(Cat#: ELH-IL6-001)

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Reagent and Sample Preparation

Bring all reagents and samples to room temperature

Dilute Assay Diluent B 5-fold with distilled water

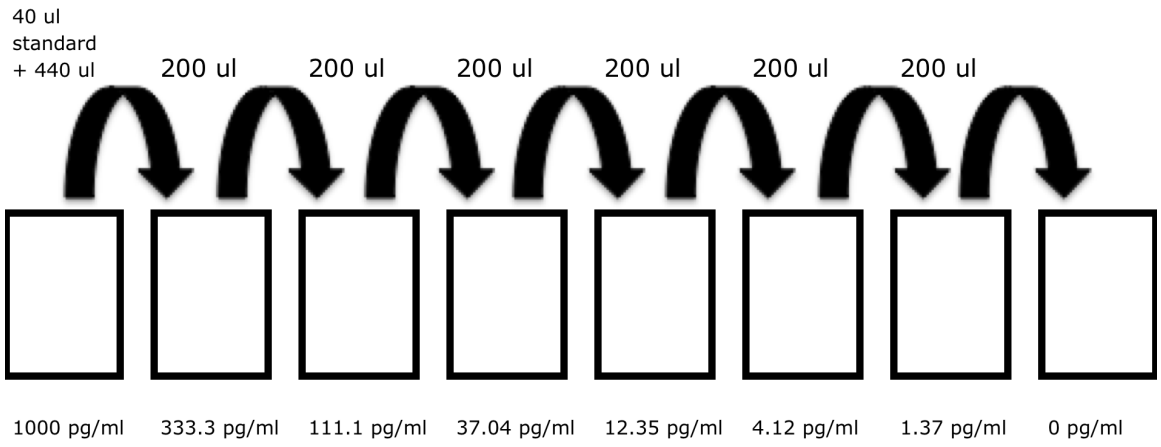
-5-fold = 4 parts distilled water and 1 part Assay Diluent B

Spin vial of Item C (powder)

Add 500 µl Assay Diluent A into item C vial & gently shake. This prepares a 12,000 pg/ml standard.

Add 40 µl IL-6 standard (12,000 pg/ml) from vial C into a tube with 440 µl Assay Diluent A to make a 1,000 pg/ml solution

Use the 1,000 pg/ml solution to make a dilution series (add each 200 ul to 400 ul Assay Diluent already in the tubes



If the wash concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into 380 ml distilled water to yield 400 ml of 1x Wash Buffer

Briefly spin detection antibody vial (Item F).

Add 100 ul of 1x Assay Diluent B in to the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently. The detection antibody concentrate should diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.

Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use.

Add 2 ul of HRP-Streptavidin concentrate into a tube with 198.0 ul 1x Assay Diluent B to prepare a 100-fold diluted HRP-Streptavidin solution. Mix through and then pipette

50 ul of prepared 100-fold diluted solution into a tube with 15 ml 1x Assay Diluent B to prepare a final 30,000 fold diluted HRP-Streptavidin solution.

Assay Procedure

Bring all reagents & samples to room temp.

Add 100 ul of each standard at sample into appropriate wells. Cover and incubate for 2.5 hours at room temperature with gentle shaking.

Discard the solution & wash 4 times w/1x Wash Solution.

Wash by filling each well with Wash Buffer (300ul), using a multi-channel pipette. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Add 100 ul of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

Discard the solution and repeat wash step.

Add 100 ul of prepared Streptavidin solution to each well. Incubate for 45 min. at room temp with gentle shaking

Discard the solution and repeat wash step.

Add 100ul of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

Add 50ul of stop solution to each well. Read at 450nm immediately.

CREATINE KINASE REAGENT KIT AND ASSAY INSTRUCTIONS

POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

Creatine Kinase – MB (CK – MB) Reagent Set

Intended Use
The CK-MB Reagent is for the quantitative determination of the isoenzyme Creatine Kinase-MB (CK-MB) in serum. For *in vitro* diagnostic use only.

Test Summary and Principle
Creatine Kinase exists as dimeric molecules composed of M and B subunits that form the isoenzymes MM, MB, and BB.[1] The subunits M and B are immunologically distinct. CK-MM and CK-MB are distributed primarily in the skeletel muscle and heart muscle, respectively, while CK-BB is present mainly in the brain and in tissues composed of smooth muscle.[2]
Following acute myocardial infarction, CK-MB activity increases significantly and this elevation is highly specific for the laboratory diagnosis of myocardial infarction.[3,4] Although total CK activity usually increases following myocardial infarction, in some patients only the CK-MB activity increases, while the total CK remains in the normal range.[5] In this procedure CK activity is measured in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB, while not affecting the B subunit activity of CK-MB and CK-BB. Due to negligible concentrations of CK-BB in the circulation, the remaining activity, multiplied by a factor of 2, represents the activity of the CK-MB isoenzyme.

Reagents
CK-MB Reagent (when reconstituted)
ADP 2.0 mmol/L, G6PDH 2000 U/L, Creatine Phosphate 20 mmol/L, NAD 2.0 mmol/L, Hexokinase (yeast) 3000 U/L, D-Glucose 20 mmol/L, Anti-human CK-M Antibody (Mouse) - Sufficient amount to inhibit up to 2000 U/L of CK-MM at 37°C. Also contains buffers, activators, surfactants and adenylate kinase inhibitors.

Precautions
1. For *in vitro* diagnostic use only.
2. Normal precautions exercised in handling laboratory reagents should be followed. Bo not pipette by mouth. Reagent contains sodium azide, which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information. Dispose of used or expired reagents according to your laboratory and governmental requirements.

Reagent Preparation
Reconstitute reagent with distilled water as indicated on vial label. Swirl gently to dissolve completely.

Reagent Storage and Stability
1. CK-MB reagent is stable at refrigerated temperature (2-8°C) until the expiration date on the label.
2. After reconstitution, the reagent is stable 5 days when stored at 2-8°C.

Materials Required But Not Provided
1. Spectrophotometer capable of absorbance readings at 340 nm.
2. Accurate pipetting devices.
3. Interval timer.
4. Cuvets and/or Test Tubes.
5. Mixer (Vortex type).
6. Constant temperature bath, or heating block set at 37°C or a temperature controlled cuvet well.

Specimen Collection and Preparation
All specimens used in this test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing. Clear unhemolyzed serum is the recommended specimen sample. No special additives or preservatives are required. Serum CK appears stable for 3 days at 2-8˚C. It is recommended that specimens be assayed immediately after collection. Reconstituted control sera may show a decrease in CK activity in only a few hours.

Interferences
1. Extremely hemolyzed samples are not suitable for testing since they may contain high levels of adenylate kinase, ATP and glucose-6-phosphate which interfere with the assay to yield false results.
2. Drugs and other substances which may interfere with the determination of CK activity have been listed by Young et al.[6]
3. This procedure may overestimate CK-MB values if CK-BB activity in the serum is high. CK-BB activity is usually absent in sera from normal individuals and patients with myocardial infarction.[7]
4. The presence of a macro form of CK-BB in the specimen should be suspected if the CK-MB activity measured by this procedure represents more than 20% of the total CK activity.

Materials Provided
CK-MB Reagent

Procedure (Automated)

Wavelength:	340 nm
Reaction Type:	Kinetic
Reaction Direction:	Increasing
Reaction Temperature:	37°C
Sample/Reagent Ratio:	1:20
Equilibration Time:	20 Seconds
Read Time:	120 Seconds
Lag Time:	300 Seconds
Blank Absorbance Limit:	0.800A
High Absorbance Change/Min:	0.375A/Min.
Factor:	3376
Low Normal:	0 IU/L
High Normal:	24 IU/L
Linearity:	175 IU/L

The above parameters should be employed in programming automated analyzers for CK-MB. Consult your instrument manual for programming instructions. Specific programming applications for many automated analyzers are available from Pointe Scientific, Inc. Technical Service Department.

Procedure (Manual)
1. Allow reagent and specimens to equilibrate to ambient room temperature prior to use.
2. Prepare CK-MB reagent according to instructions (see Reagent Preparation Section).
3. Zero spectrophotometer at 340 nm with distilled water.
4. For each sample and control, add 1.0 mL CK-MB reagent into a cuvet/test tube and warm for approximately 5 minutes at 37°C.
5. Add 50µL of serum to its respective tube, mix well and incubate for 5minutes at 37°C.
6. After the 5 minutes has elapsed, read and record the absorbance.
7. Record the increase in absorbance at 60 second intervals for the next 2 minutes (ΔA/Min). The rate of change should be constant.

APPENDIX G

PEAK ECCENTRIC TORQUE DATA

PERCENT DECLINE IN PEAK ECCENTRIC TORQUE

Placebo		NAC	
Subject	% Decline	Subject	% Decline
1	41.87	11	43.18
2	30.41	12	37.77
3	39.40	13	44.05
4	39.97	14	30.31
5	47.52	15	34.55
6	35.18	16	53.61
7	29.30	17	34.89
8	46.68	18	46.43
9	30.73	19	28.57
10	52.35	20	41.08
		21	29.23
Mean	39.34		
SEM	7.95	Mean	38.05
		SEM	8.21

PLACEBO GROUP PEAK ECCENTRIC TORQUE DATA (Nm)

Eccentric Contraction (Nm)	Subject 1	2	3	4	5	6	7	8	9	10
1	49.41	51.11	44.97	47.56	39.38	57.33	69.00	36.48	35.93	65.18
2	49.22	56.44	44.64	46.87	39.47	61.06	64.89	36.69	36.68	67.75
3	49.49	57.87	42.49	48.77	40.13	62.43	64.21	38.72	37.78	63.96
4	49.96	56.87	41.72	47.39	38.78	66.68	63.36	38.25	38.43	62.26
5	48.36	53.75	40.86	48.92	40.62	61.90	64.82	37.87	35.81	60.39
6	48.00	53.47	41.11	48.73	37.35	25.41	63.54	37.83	34.15	57.47
7	48.15	48.70	40.18	49.52	38.87	66.58	59.05	39.53	36.49	54.81
8	46.96	51.65	37.61	46.98	36.13	65.76	53.68	33.24	34.07	54.88
9	45.81	46.21	42.60	49.78	34.85	66.90	54.58	33.77	34.05	55.81
10	43.81	49.90	37.08	51.36	34.16	64.92	57.00	32.43	36.49	51.71
11	48.26	48.87	36.99	47.69	36.24	62.37	49.83	32.66	32.98	46.68
12	44.09	50.20	34.80	42.71	33.90	60.62	73.07	33.83	28.50	46.43
13	43.14	47.32	33.36	39.38	32.07	65.62	61.23	28.51	32.49	48.29
14	42.41	47.18	34.77	42.19	30.81	51.00	57.59	32.80	33.37	46.63
15	43.91	47.81	33.96	43.67	31.34	56.40	56.17	33.93	32.62	46.55
16	41.93	48.50	30.07	41.49	31.01	50.97	58.38	34.31	32.92	46.37
17	41.14	45.45	33.05	45.59	31.67	54.22	60.89	30.54	31.96	43.24
18	40.47	47.38	31.28	38.86	30.34	54.52	57.50	28.40	30.48	44.46
19	41.70	45.43	34.47	42.63	28.41	52.48	57.30	32.86	26.30	46.00
20	41.16	46.21	32.84	43.58	27.31	53.20	54.16	32.73	27.29	37.56
21	41.82	44.67	32.82	37.33	27.00	56.87	59.89	25.70	25.63	40.67
22	39.95	44.29	31.40	39.22	27.94	56.02	52.34	30.80	25.30	41.45
23	38.77	43.14	29.54	44.99	27.41	51.20	52.34	28.56	27.11	41.06
24	38.14	42.63	25.80	37.84	28.41	48.67	53.52	34.34	29.17	42.07
25	38.35	43.08	29.90	45.67	26.52	47.17	50.59	25.99	30.63	39.02
26	37.52	41.22	34.60	38.53	20.92	49.17	45.86	24.51	30.96	41.96
27	38.95	41.22	33.01	38.36	31.45	48.31	55.60	27.29	34.57	44.59
28	36.95	43.18	32.77	40.40	27.17	48.84	48.11	27.94	31.68	39.13
29	37.41	37.31	32.05	37.94	27.54	45.92	50.11	28.14	28.56	39.68
30	37.30	33.94	29.25	32.98	33.70	46.84	47.47	24.90	28.71	38.19
31	34.40	37.79	32.46	39.55	30.04	41.26	54.90	20.71	32.62	37.01
32	25.03	36.32	33.62	37.03	41.13	38.60	45.52	28.71	24.50	43.87
33	35.48	38.14	33.27	37.54	26.09	47.37	51.04	31.81	26.60	32.36
34	34.13	37.20	30.04	39.95	23.55	41.75	51.87	28.80	28.83	35.23
35	33.66	50.77	28.67	38.21	24.88	41.79	45.51	31.93	28.22	32.60
36	33.82	38.49	27.64	36.59	23.46	42.35	46.51	24.13	29.50	27.36
37	31.42	42.34	34.04	35.20	28.48	45.07	42.97	21.19	24.87	27.94
38	32.49	37.26	26.46	37.21	23.66	41.80	49.36	27.84	24.59	28.88
39	32.35	36.66	29.65	32.33	22.60	35.27	45.02	31.54	24.54	34.67
40	27.81	37.83	28.83	49.02	26.97	42.10	43.63	21.52	27.08	31.02
41	30.37	37.16	25.11	35.17	19.31	40.46	38.50	22.69	26.10	34.43
42	28.92	34.58	26.66	41.20	19.74	37.99	45.78	20.40	26.05	25.92
43	29.74	39.59	23.83	34.63	17.37	39.34	42.36	21.80	28.22	32.83
44	27.51	41.44	22.18	30.33	20.92	38.89	44.07	17.95	26.08	32.82
45	26.86	35.62	24.28	32.78	21.12	27.48	46.10	18.22	24.74	27.03
46	25.37	33.82	30.18	26.89	22.20	38.00	38.38	19.86	24.43	25.84
47	28.25	35.07	24.77	24.74	25.98	33.76	38.51	17.48	21.83	20.40
48	27.92	38.37	28.66	25.03	18.46	33.36	45.65	20.78	23.27	29.13
49	26.24	33.89	21.62	16.56	15.57	32.81	46.68	17.63	25.51	26.41
50	27.34	36.48	23.13	24.32	18.61	30.97	48.18	17.71	23.03	28.34
Mean	37.83	43.48	32.30	39.62	28.82	48.60	52.53	28.52	29.63	41.37
SEM	7.5795	6.6382	5.8739	7.6132	6.8186	11.177	8.1014	6.4068	4.4193	11.556

NAC GROUP PEAK ECCENTRIC TORQUE DATA (Nm)

Eccentric Contraction (Nm)	Subject										
	11	12	13	14	15	16	17	18	19	20	21
1	64.11	44.99	38.31	45.54	36.62	36.50	40.01	38.79	70.09	55.71	46.53
2	62.42	45.20	35.66	44.73	34.54	37.32	38.60	38.83	66.11	57.11	47.77
3	64.83	45.72	36.21	44.61	37.10	40.10	37.95	37.71	66.58	57.99	48.01
4	63.98	41.75	37.15	45.13	35.87	39.59	37.89	44.95	66.95	57.68	42.96
5	63.57	42.09	35.76	44.40	34.18	37.26	36.76	38.73	61.73	56.64	48.43
6	60.07	41.29	35.30	44.09	36.60	36.42	33.19	41.05	63.81	57.99	45.51
7	59.05	42.80	33.16	43.31	36.21	36.12	34.87	31.63	50.52	53.31	43.11
8	59.47	42.76	33.27	42.06	37.98	35.14	36.08	37.81	62.44	56.92	44.44
9	57.63	40.02	32.16	42.83	36.84	34.95	33.21	24.03	62.31	78.17	40.71
10	57.40	38.02	28.79	43.35	31.77	36.70	35.95	24.39	60.44	54.30	41.46
11	57.12	39.82	28.18	43.98	37.86	34.80	35.58	24.39	62.20	50.89	43.77
12	55.67	39.03	27.72	43.68	35.86	32.92	34.25	23.64	60.62	52.68	45.41
13	53.63	36.60	33.73	44.48	36.54	30.72	33.01	37.76	57.31	56.31	42.69
14	54.48	36.65	28.44	41.96	35.77	32.98	32.46	38.73	57.13	53.88	43.34
15	53.30	37.44	26.60	40.82	36.12	31.92	32.47	33.85	57.43	52.29	75.87
16	51.93	36.06	27.41	43.00	34.53	31.01	31.23	43.80	56.23	49.97	42.29
17	51.66	34.92	28.68	45.86	36.38	29.67	30.54	30.23	54.54	51.57	42.18
18	48.53	34.38	27.67	43.12	35.09	29.00	30.73	36.65	58.47	44.74	41.46
19	45.83	35.71	27.52	42.16	36.07	29.53	30.47	29.83	55.45	45.22	42.45
20	46.88	35.08	21.43	40.99	34.80	28.59	29.35	20.29	57.60	48.31	41.41
21	45.96	34.33	28.85	42.31	35.13	26.59	30.03	28.98	51.86	47.99	40.55
22	43.93	35.62	25.40	43.03	34.86	26.87	29.78	25.60	53.92	46.91	40.50
23	42.82	34.87	26.13	43.01	36.58	26.27	29.21	30.44	56.98	47.02	41.68
24	41.27	33.51	23.61	43.63	34.15	25.36	28.60	20.97	50.85	46.37	41.11
25	40.24	30.06	25.83	41.75	34.44	24.31	28.71	25.09	50.12	46.51	38.53
26	46.91	34.56	22.93	38.43	30.33	25.70	29.55	33.40	51.49	44.12	38.53
27	46.19	36.85	13.58	38.79	34.14	23.97	31.70	31.73	55.46	44.52	38.16
28	46.16	35.74	17.86	38.34	36.22	24.34	30.33	36.87	54.98	41.52	38.16
29	46.58	33.28	18.59	37.75	34.15	25.29	30.02	34.31	51.17	44.21	36.66
30	44.37	36.15	12.53	39.07	33.26	22.88	29.60	32.47	51.49	40.66	38.33
31	43.31	31.12	16.96	37.94	35.87	22.48	27.29	22.40	56.28	40.28	36.09
32	43.47	31.86	16.91	38.94	34.86	21.70	27.52	23.87	54.37	35.24	36.75
33	43.70	30.36	17.21	38.79	34.23	20.90	27.42	23.73	46.79	39.64	38.40
34	42.23	31.73	11.76	36.64	33.93	20.49	28.89	23.09	46.38	38.47	37.49
35	40.33	33.28	15.55	39.28	32.69	21.37	27.11	27.02	47.76	37.41	36.89
36	42.31	28.13	8.79	39.99	30.88	21.50	24.79	24.50	43.53	38.17	34.17
37	40.55	32.18	18.72	38.38	30.24	22.70	26.52	23.52	47.36	32.23	32.93
38	39.73	31.50	20.62	37.59	27.68	20.38	23.86	23.87	42.87	36.00	39.29
39	38.40	28.78	19.83	35.34	27.69	18.97	27.41	22.27	49.26	37.44	38.18
40	36.64	25.11	19.51	35.76	26.90	19.15	25.47	18.00	44.64	34.92	38.33
41	38.90	28.71	21.78	34.08	26.52	19.87	22.82	20.39	47.68	36.11	36.82
42	36.39	26.49	23.40	32.24	26.09	20.29	22.95	21.54	49.22	35.05	35.41
43	35.87	30.36	18.50	32.71	23.67	16.75	22.46	15.27	49.21	34.59	33.40
44	36.29	27.45	17.46	33.40	24.27	17.50	25.84	17.28	46.25	36.84	30.62
45	33.96	28.62	18.82	31.56	22.51	17.23	24.67	26.47	45.20	36.63	31.35
46	34.21	24.42	19.40	30.91	22.07	16.37	24.88	20.21	42.44	31.50	31.06
47	33.00	24.27	19.42	30.06	22.52	15.44	24.48	16.02	39.63	31.96	30.57
48	34.21	24.74	18.00	28.27	21.86	17.00	23.65	18.83	47.66	32.73	29.26
49	33.71	25.00	18.36	27.13	22.52	15.73	21.70	18.32	40.20	33.47	28.99
50	31.48	24.18	18.32	26.29	22.11	15.48	23.86	17.42	43.20	36.30	30.23
Mean	46.69	34.07	23.95	39.23	32.18	26.28	29.71	28.02	53.32	45.13	39.76
SEM	9.595	5.889	7.383	5.169	5.101	7.323	4.695	7.963	7.502	9.698	7.254

PERCENT DECLINE IN PEAK ECCENTRIC TORQUE STATISTICAL TABLE

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
perdec	Equal variances assumed	.007	.933	.238	19	.815	.82555	3.47263	-6.44274	8.09384
	Equal variances not assumed			.238	18.808	.815	.82555	3.47272	-6.44796	8.09905

APPENDIX H

MEAN MVC TORQUE DATA
(Nm)

Placebo

Subject	FAM1	FAM2	PRE	POST	1D	3D	7D	10D
1	62.87	62.02	62.07	31.95	32.50	40.79	51.52	59.96
2	55.80	57.53	57.98	26.50	27.48	28.89	41.42	46.58
3	52.75	52.55	52.39	23.08	22.60	33.59	40.60	51.41
4	57.80	58.96	59.42	25.05	26.05	37.01	48.32	52.22
5	37.66	47.41	43.68	24.84	25.75	33.76	39.51	46.64
6	75.75	75.52	73.30	36.26	36.54	43.86	54.67	67.15
7	68.87	79.20	78.08	36.49	50.29	50.10	70.85	71.26
8	46.78	47.24	47.45	21.74	24.12	34.65	46.53	46.08
9	44.35	44.34	45.23	16.46	18.76	25.46	36.87	42.99
10	76.81	74.31	73.98	26.92	28.64	46.35	55.94	65.16
Mean	57.95	59.91	59.36	26.93	29.27	37.45	48.62	54.95
SEM	13.20	12.68	12.49	6.35	8.90	7.78	10.19	10.14

NAC

Subject	FAM1	FAM2	PRE	POST	1D	3D	7D	10D
11	76.94	78.21	79.37	41.97	41.90	52.37	77.10	77.47
12	50.42	50.10	49.77	16.87	22.26	30.10	37.05	44.27
13	42.87	42.65	43.81	20.78	21.29	25.91	31.80	42.22
14	49.30	51.66	51.93	33.16	31.45	39.09	48.87	53.34
15	40.09	43.77	43.93	22.59	30.35	35.60	42.83	43.88
16	41.16	42.52	42.98	13.53	18.24	23.50	34.63	37.25
17	45.15	47.00	47.59	21.80	29.15	37.12	46.18	49.33
18	41.00	38.21	37.84	12.99	17.20	21.79	33.57	33.21
19	76.69	79.69	80.51	41.32	41.52	50.28	59.41	67.09
20	60.34	63.95	64.13	30.58	36.43	47.99	59.57	58.36
21	54.97	54.76	54.33	20.89	31.79	39.69	48.97	51.16
Mean	52.63	53.87	54.20	25.13	29.24	36.68	47.27	50.69
SEM	13.51	14.25	14.49	10.22	8.68	10.63	13.81	13.04

MVC TORQUE AT 90º ELBOW FLEXION
(Nm)

Placebo								
Subject	FAM1	FAM2	PRE	POST	1D	3D	7D	10D
1	63.77	62.61	62.60	31.98	32.85	41.13	51.72	60.27
2	55.15	57.39	57.80	26.32	27.39	28.55	41.64	47.74
3	53.86	52.85	52.93	24.57	21.50	34.06	40.96	51.95
4	56.35	58.61	59.08	25.13	26.29	37.52	48.52	53.27
5	33.47	47.83	43.66	25.17	27.48	33.49	39.87	46.83
6	73.99	75.07	73.21	36.99	34.91	43.89	54.34	67.61
7	63.97	79.21	78.22	36.22	50.52	50.76	71.50	70.66
8	47.72	47.80	48.05	20.98	24.47	35.23	47.11	46.57
9	44.24	44.32	45.24	15.97	18.93	25.74	37.13	42.53
10	78.28	74.33	74.13	27.72	27.89	46.75	56.38	65.59
Mean	57.08	60.00	59.49	27.10	29.22	37.71	48.92	55.30
SEM	13.55	12.52	12.44	6.51	8.84	7.93	10.24	10.01

NAC								
Subject	FAM1	FAM2	PRE	POST	1D	3D	7D	10D
11	82.46	77.95	78.26	41.79	42.68	52.38	77.34	77.20
12	53.76	48.94	48.98	16.71	22.47	26.27	36.34	42.17
13	40.77	43.82	44.02	20.58	21.35	25.89	31.43	42.12
14	52.87	52.25	52.13	32.98	32.33	39.88	48.44	53.70
15	40.48	43.19	43.45	23.21	31.05	35.25	42.05	44.54
16	42.47	43.53	44.03	12.81	18.27	21.62	35.69	37.87
17	43.59	47.54	48.29	22.44	27.51	36.27	44.74	49.70
18	41.67	38.47	37.92	12.64	18.14	21.67	34.46	34.13
19	77.25	80.16	80.49	40.69	40.79	50.35	59.92	68.34
20	57.89	63.94	64.51	31.00	37.11	48.55	60.00	57.62
21	57.93	54.94	54.47	20.57	34.17	40.63	49.84	52.03
Mean	53.74	54.07	54.23	25.04	29.62	36.25	47.30	50.86
SEM	14.60	14.14	14.25	10.23	8.75	11.30	13.92	13.02

MVC TORQUE AT 100º ELBOW FLEXION
(Nm)

Placebo								
Subject	FAM1	FAM2	PRE	POST	1D	3D	7D	10D
1	63.27	61.31	61.33	32.46	32.74	40.62	51.36	59.74
2	57.12	57.85	58.33	26.56	27.79	29.40	41.36	46.05
3	52.58	51.95	52.05	22.83	22.58	33.67	40.68	50.79
4	59.68	59.69	59.87	25.17	26.25	36.71	48.42	52.14
5	39.39	47.97	43.92	24.99	23.82	33.78	39.48	46.37
6	74.00	75.44	73.46	36.57	36.60	43.57	54.89	66.94
7	67.02	79.31	77.73	36.19	50.13	49.77	71.20	72.92
8	46.63	47.05	47.10	21.21	24.06	35.50	46.64	46.05
9	44.26	44.41	45.62	16.87	18.87	25.47	36.51	44.32
10	77.41	74.12	74.24	26.51	29.86	46.13	56.05	65.43
Mean	58.14	59.91	59.36	26.94	29.27	37.46	48.66	55.07
SEM	12.64	12.64	12.44	6.39	8.96	7.56	10.34	10.38

NAC								
Subject	FAM1	FAM2	PRE	POST	1D	3D	7D	10D
11	74.23	78.39	80.06	42.80	40.60	52.44	77.31	77.23
12	50.55	50.77	50.26	17.23	22.68	34.41	36.98	45.50
13	44.29	42.64	42.76	19.49	20.78	26.10	31.22	42.17
14	50.13	51.38	52.26	33.94	32.07	38.20	49.06	52.82
15	39.58	44.37	44.55	22.26	30.40	35.92	43.44	43.67
16	41.03	42.13	42.46	12.27	18.28	25.02	35.21	37.84
17	45.97	46.60	47.09	20.98	29.62	37.79	46.99	49.29
18	41.05	38.29	38.14	12.55	16.07	21.29	34.18	33.08
19	76.30	79.89	80.84	41.66	44.27	51.03	59.34	66.47
20	62.43	63.96	64.03	29.56	36.38	47.47	60.03	58.41
21	53.73	54.67	54.39	21.81	31.30	39.49	47.92	50.41
Mean	52.66	53.92	54.26	24.96	29.31	37.20	47.43	50.63
SEM	12.99	14.31	14.71	10.66	9.10	10.34	13.82	12.82

MVC TORQUE AT 110º ELBOW FLEXION
(Nm)

Placebo

Subject	FAM1	FAM2	PRE	POST	1D	3D	7D	10D
1	61.56	62.15	62.29	31.41	31.92	40.61	51.46	59.87
2	55.14	57.37	57.82	26.61	27.25	28.71	41.25	45.94
3	51.81	52.85	52.18	21.83	23.73	33.03	40.16	51.48
4	57.37	58.58	59.30	24.85	25.60	36.79	48.03	51.26
5	40.12	46.42	43.45	24.35	25.96	34.02	39.18	46.73
6	79.25	76.04	73.25	35.20	38.11	44.11	54.77	66.89
7	75.62	79.08	78.29	37.06	50.21	49.77	69.86	70.22
8	46.00	46.87	47.19	23.03	23.84	33.21	45.85	45.63
9	44.56	44.31	44.84	16.56	18.47	25.15	36.96	42.11
10	74.75	74.48	73.56	26.52	28.17	46.19	55.38	64.45
Mean	58.62	59.81	59.22	26.74	29.33	37.16	48.29	54.46
SEM	13.92	12.90	12.59	6.24	9.01	7.88	10.00	10.08

NAC

Subject	FAM1	FAM2	PRE	POST	1D	3D	7D	10D
11	74.14	78.27	79.80	41.32	42.42	52.27	76.65	77.97
12	46.95	50.58	50.07	16.68	21.61	29.62	37.83	45.14
13	43.55	41.50	44.66	22.25	21.75	25.76	32.76	42.36
14	44.90	51.34	51.40	32.56	29.96	39.18	49.11	53.52
15	40.21	43.76	43.79	22.31	29.61	35.63	43.02	43.43
16	39.97	41.88	42.43	15.51	18.17	23.86	33.00	36.05
17	45.89	46.86	47.38	21.97	30.30	37.30	46.81	48.98
18	40.27	37.88	37.47	13.78	17.41	22.41	32.08	32.42
19	76.54	79.03	80.21	41.61	39.50	49.45	58.97	66.45
20	60.71	63.94	63.85	31.18	35.80	47.95	58.66	59.06
21	53.24	54.68	54.14	20.28	29.91	38.94	49.14	51.03
Mean	51.49	53.61	54.11	25.40	28.77	36.58	47.09	50.58
SEM	13.31	14.32	14.54	9.83	8.36	10.41	13.74	13.32

MVC TORQUE STATISTICAL TABLE

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	17010.822	5	3402.164	229.224	.000
	Greenhouse-Geisser	17010.822	2.772	6135.602	229.224	.000
	Huynh-Feldt	17010.822	3.465	4909.881	229.224	.000
	Lower-bound	17010.822	1.000	17010.822	229.224	.000
time * group	Sphericity Assumed	107.793	5	21.559	1.453	.213
	Greenhouse-Geisser	107.793	2.772	38.880	1.453	.240
	Huynh-Feldt	107.793	3.465	31.113	1.453	.232
	Lower-bound	107.793	1.000	107.793	1.453	.243
Error(time)	Sphericity Assumed	1409.999	95	14.842		
	Greenhouse-Geisser	1409.999	52.677	26.767		
	Huynh-Feldt	1409.999	65.828	21.420		
	Lower-bound	1409.999	19.000	74.210		

PERCENT DECLINE IN MVC TORQUE STATISTICAL TABLE

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	54291.517	5	10858.303	449.064	.000
	Greenhouse-Geisser	54291.517	3.049	17807.993	449.064	.000
	Huynh-Feldt	54291.517	3.888	13962.866	449.064	.000
	Lower-bound	54291.517	1.000	54291.517	449.064	.000
time * group	Sphericity Assumed	141.017	5	28.203	1.166	.331
	Greenhouse-Geisser	141.017	3.049	46.254	1.166	.331
	Huynh-Feldt	141.017	3.888	36.267	1.166	.332
	Lower-bound	141.017	1.000	141.017	1.166	.294
Error(time)	Sphericity Assumed	2297.086	95	24.180		
	Greenhouse-Geisser	2297.086	57.926	39.656		
	Huynh-Feldt	2297.086	73.877	31.093		
	Lower-bound	2297.086	19.000	120.899		

MVC TORQUE DIFFERENCE BETWEEN ANGLES STATISTICAL TABLE

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	50834.329	5	10166.866	649.340	.000
	Greenhouse-Geisser	50834.329	2.752	18472.437	649.340	.000
	Huynh-Feldt	50834.329	2.993	16981.806	649.340	.000
	Lower-bound	50834.329	1.000	50834.329	649.340	.000
time * Angle	Sphericity Assumed	4.635	10	.463	.030	1.000
	Greenhouse-Geisser	4.635	5.504	.842	.030	1.000
	Huynh-Feldt	4.635	5.987	.774	.030	1.000
	Lower-bound	4.635	2.000	2.317	.030	.971
Error(time)	Sphericity Assumed	4697.171	300	15.657		
	Greenhouse-Geisser	4697.171	165.114	28.448		
	Huynh-Feldt	4697.171	179.608	26.152		
	Lower-bound	4697.171	60.000	78.286		

APPENDIX I

RANGE OF MOTION
(degrees of flexion)

NAC

Subject	PRE	POST	1D	3D	7D	10D
1	121.00	96.67	115.00	115.00	120.00	120.00
2	124.00	95.00	100.00	100.00	125.00	120.00
3	120.00	93.33	108.33	100.00	122.00	120.00
4	126.00	86.67	107.00	108.00	122.33	126.00
5	132.33	106.00	105.33	117.00	123.00	129.67
6	125.67	101.00	101.33	113.67	126.33	129.00
7	125.67	95.00	102.67	116.00	120.67	119.67
8	127.33	98.67	114.33	111.00	128.33	128.67
9	136.00	101.33	109.67	118.67	133.67	135.67
10	131.33	100.67	110.67	107.33	125.67	129.67
Mean	126.93	97.43	107.43	110.67	124.70	125.83
SEM	5.02	5.36	5.17	6.72	4.10	5.62

Placebo

Subject	PRE	POST	1D	3D	7D	10D
11	108.00	76.00	92.33	99.00	113.67	115.67
12	123.33	105.33	104.00	100.00	118.67	131.67
13	132.67	100.00	109.00	115.67	123.00	136.00
14	130.00	104.33	112.33	115.33	128.67	129.00
15	127.67	102.67	115.33	119.33	130.00	131.00
16	125.00	92.67	116.00	107.33	121.67	129.67
17	133.00	104.33	110.67	124.00	132.00	132.67
18	124.33	86.67	104.00	107.00	124.00	127.67
19	119.00	102.33	109.67	107.67	120.00	130.67
20	126.67	101.00	113.67	112.67	126.67	127.67
21	126.00	101.33	101.67	105.67	128.33	125.33
Mean	125.06	97.88	108.06	110.33	124.24	128.82
SEM	6.98	9.16	7.05	7.82	5.52	5.21

RANGE OF MOTION STATISTICAL TABLE

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	15387.925	5	3077.585	177.292	.000
	Greenhouse-Geisser	15387.925	3.980	3865.883	177.292	.000
	Huynh-Feldt	15387.925	5.000	3077.585	177.292	.000
	Lower-bound	15387.925	1.000	15387.925	177.292	.000
time * group	Sphericity Assumed	67.939	5	13.588	.783	.565
	Greenhouse-Geisser	67.939	3.980	17.068	.783	.539
	Huynh-Feldt	67.939	5.000	13.588	.783	.565
	Lower-bound	67.939	1.000	67.939	.783	.387
Error(time)	Sphericity Assumed	1649.090	95	17.359		
	Greenhouse-Geisser	1649.090	75.628	21.805		
	Huynh-Feldt	1649.090	95.000	17.359		
	Lower-bound	1649.090	19.000	86.794		

APPENDIX J

VAS MUSCLE SORENESS RATING
(mm)

NAC

Subject	PRE	POST	1D	3D	7D	10D
1	1.33	8.00	38.67	87.33	21.00	8.33
2	2.33	15.67	28.00	74.33	24.33	1.00
3	3.33	11.00	42.00	76.00	12.00	11.67
4	0.00	0.00	19.00	74.00	0.00	0.00
5	0.00	9.67	21.33	75.33	30.33	0.00
6	1.33	32.33	63.67	83.00	12.67	3.00
7	0.00	4.00	19.00	67.33	14.67	0.00
8	0.00	9.00	26.00	74.00	14.33	0.00
9	0.00	10.67	22.00	56.33	12.67	0.00
10	0.00	3.67	12.33	80.33	14.33	0.00
Mean	0.83	10.40	29.20	74.80	15.63	2.40
SEM	1.21	8.89	15.13	8.53	8.17	4.19

Placebo

Subject	PRE	POST	1D	3D	7D	10D
11	0.00	1.33	20.00	62.33	1.33	0.00
12	0.00	6.67	66.67	93.00	15.33	1.67
13	0.00	3.67	26.67	69.67	9.67	0.00
14	0.00	16.67	36.00	74.00	2.00	0.00
15	0.00	2.00	33.33	80.00	11.33	0.00
16	2.67	3.00	31.00	83.67	7.00	2.33
17	0.00	3.33	3.67	68.67	36.33	0.00
18	0.00	0.00	15.00	69.00	12.00	0.00
19	1.67	21.33	36.00	70.00	5.00	0.00
20	0.33	24.33	19.67	63.67	13.00	0.00
21	0.00	3.00	37.67	66.67	14.00	0.00
Mean	0.42	7.76	29.61	72.79	11.55	0.36
SEM	0.90	8.69	16.19	9.27	9.48	0.82

VAS MUSCLE STORENESS STATISTICAL TABLE

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	85190.594	5	17038.119	271.603	.000
	Greenhouse-Geisser	85190.594	1.860	45795.457	271.603	.000
	Huynh-Feldt	85190.594	2.163	39394.138	271.603	.000
	Lower-bound	85190.594	1.000	85190.594	271.603	.000
time * group	Sphericity Assumed	83.620	5	16.724	.267	.930
	Greenhouse-Geisser	83.620	1.860	44.951	.267	.752
	Huynh-Feldt	83.620	2.163	38.668	.267	.784
	Lower-bound	83.620	1.000	83.620	.267	.612
Error(time)	Sphericity Assumed	5959.507	95	62.732		
	Greenhouse-Geisser	5959.507	35.345	168.612		
	Huynh-Feldt	5959.507	41.088	145.043		
	Lower-bound	5959.507	19.000	313.658		

APPENDIX K

ARM CIRCUMFERENCE
(cm)

Placebo						
Subject	PRE	POST	1D	3D	7D	10D
1	25.00	25.20	25.30	25.50	25.70	25.20
2	21.00	21.10	21.30	21.50	22.10	21.30
3	24.00	24.10	24.30	24.50	25.00	24.40
4	27.80	27.85	28.00	28.33	28.50	28.00
5	27.23	27.30	27.48	27.80	28.00	27.35
6	25.80	25.90	25.98	26.17	26.40	25.95
7	40.90	41.00	41.32	41.58	41.88	41.03
8	29.47	29.58	29.67	29.70	29.97	24.62
9	22.83	22.98	23.18	23.35	23.58	22.98
10	25.55	25.65	25.72	25.88	26.27	25.67
Mean	26.96	27.07	27.23	27.43	27.74	26.65
SEM	5.48	5.47	5.50	5.52	5.48	5.42

NAC						
Subject	PRE	POST	1D	3D	7D	10D
11	31.53	31.73	32.00	32.43	32.53	31.73
12	26.52	26.58	26.80	27.10	27.40	26.55
13	23.53	23.62	23.80	24.00	24.60	23.55
14	24.30	24.38	24.50	24.70	25.02	24.37
15	27.00	27.12	27.22	27.48	27.85	27.00
16	33.58	33.68	33.73	33.87	34.08	33.63
17	29.50	29.58	29.68	29.88	30.68	29.58
18	24.87	24.98	25.17	25.50	25.80	24.97
19	30.37	30.52	30.62	30.75	30.98	30.32
20	28.87	28.98	30.02	30.27	30.48	28.88
21	26.07	26.17	26.28	26.48	26.78	26.20
Mean	27.83	27.94	28.17	28.41	28.75	27.89
SEM	3.20	3.22	3.24	3.24	3.18	3.21

ARM CIRCUMFERENCE STATISTICAL TABLE

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	13.730	5	2.746	13.017	.000
	Greenhouse-Geisser	13.730	1.167	11.768	13.017	.001
	Huynh-Feldt	13.730	1.262	10.882	13.017	.001
	Lower-bound	13.730	1.000	13.730	13.017	.002
Time * group	Sphericity Assumed	.484	5	.097	.459	.806
	Greenhouse-Geisser	.484	1.167	.415	.459	.535
	Huynh-Feldt	.484	1.262	.383	.459	.549
	Lower-bound	.484	1.000	.484	.459	.506
Error(Time)	Sphericity Assumed	20.041	95	.211		
	Greenhouse-Geisser	20.041	22.168	.904		
	Huynh-Feldt	20.041	23.974	.836		
	Lower-bound	20.041	19.000	1.055		

APPENDIX L

CREATINE KINASE
(U·L)

Placebo					
Subject	PRE	1D	3D	7D	10D
1	139.09	829.44	1490.29	123.93	27.69
2	141.73	189.68	692.16	222.32	22.74
3	28.84	89.32	924.86	18.13	65.10
4	19.12	38.56	525.05	*	34.61
5	42.52	101.02	718.86	177.65	45.32
6	107.94	113.22	549.11	145.52	54.88
7	145.68	*	*	*	*
8	1586.69	1442.82	1967.05	891.90	48.95
9	17.47	122.94	471.82	31.64	18.95
10	27.52	149.64	334.38	67.73	41.53
Mean	225.66	341.85	852.62	209.85	39.97
SEM	481.29	477.48	537.41	284.51	15.34

NAC					
Subject	PRE	1D	3D	7D	10D
11	*	*	*	*	*
12	86.36	215.72	648.65	285.76	78.61
13	117.83	120.47	337.84	14.01	39.88
14	98.39	137.28	510.22	22.08	43.84
15	33.29	33.45	495.22	*	19.61
16	67.90	66.74	540.21	135.14	72.84
17	184.25	221.66	893.71	39.22	34.61
18	48.45	152.60	639.92	48.45	34.44
19	56.69	89.98	784.61	612.89	140.08
20	26.04	55.21	1620.31	39.22	22.58
21	105.97	136.45	459.96	107.44	57.35
Mean	82.52	122.96	693.07	144.91	54.38
SEM	47.22	63.65	363.58	195.00	35.92

CREATINE KINASE STATISTICAL TABLE

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	5920204.373	4	1480051.093	27.187	.000
	Greenhouse-Geisser	5920204.373	2.307	2566279.854	27.187	.000
	Huynh-Feldt	5920204.373	2.932	2019085.454	27.187	.000
	Lower-bound	5920204.373	1.000	5920204.373	27.187	.000
time * group	Sphericity Assumed	186014.040	4	46503.510	.854	.497
	Greenhouse-Geisser	186014.040	2.307	80633.041	.854	.449
	Huynh-Feldt	186014.040	2.932	63440.081	.854	.470
	Lower-bound	186014.040	1.000	186014.040	.854	.370
Error(time)	Sphericity Assumed	3266438.537	60	54440.642		
	Greenhouse-Geisser	3266438.537	34.604	94395.338		
	Huynh-Feldt	3266438.537	43.982	74267.915		
	Lower-bound	3266438.537	15.000	217762.569		

APPENDIX M

INTERLEUKIN-6
($pg \cdot ml^{-1}$)

Placebo		
Subject	PRE	3D
1	26.50	25.12
2	25.47	23.06
3	24.78	24.78
4	19.63	24.78
5	23.06	22.72
6	23.75	23.41
7	*	*
8	16.19	6.22
9	20.31	21.69
10	20.31	15.85
Mean	22.22	20.85
SEM	3.33	6.16

NAC		
Subject	PRE	3D
11	*	*
12	23.41	28.22
13	26.84	27.19
14	27.87	23.75
15	26.50	23.06
16	21.00	20.66
17	17.56	18.59
18	25.12	25.12
19	28.56	24.44
20	4.50	27.87
21	18.25	15.50
Mean	21.96	23.44
SEM	7.26	4.15

INTERLEUKIN-6 STATISTICAL TABLE

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	.026	1	.026	.001	.973
	Greenhouse-Geisser	.026	1.000	.026	.001	.973
	Huynh-Feldt	.026	1.000	.026	.001	.973
	Lower-bound	.026	1.000	.026	.001	.973
time * group	Sphericity Assumed	19.284	1	19.284	.885	.360
	Greenhouse-Geisser	19.284	1.000	19.284	.885	.360
	Huynh-Feldt	19.284	1.000	19.284	.885	.360
	Lower-bound	19.284	1.000	19.284	.885	.360
Error(time)	Sphericity Assumed	370.362	17	21.786		
	Greenhouse-Geisser	370.362	17.000	21.786		
	Huynh-Feldt	370.362	17.000	21.786		
	Lower-bound	370.362	17.000	21.786		

APPENDIX N

GLUTATHIONE
(μM)

Placebo	
Subject	Serum Glutathione (uM)
1	3.06
2	4.28
3	2.65
4	2.55
5	2.56
6	3.22
7	4.96
8	3.58
9	2.87
10	2.09
Mean	3.18
SEM	0.88

NAC	
Subject	Serum Glutathione (uM)
11	*
12	2.61
13	3.78
14	2.47
15	2.97
16	3.31
17	3.66
18	4.60
19	3.16
20	3.30
21	3.16
Mean	3.30
SEM	0.61

GLUTATHIONE STATISTICAL TABLE

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
gsh	Equal variances assumed	.693	.416	-.042	19	.967	-.01436	.33980	-.72557	.69685
	Equal variances not assumed			-.042	16.945	.967	-.01436	.34411	-.74054	.71181

APPENDIX O

FOOD FREQUENCY QUESTIONNAIRE DATA

Placebo		NAC	
Subject	Score	Subject	Score
1	34.50	11	29.70
2	55.30	12	19.30
3	45.00	13	22.60
4	34.10	14	12.50
5	39.20	15	31.30
6	48.40	16	37.10
7	30.75	17	35.80
8	32.60	18	48.20
9	44.20	19	52.40
10	46.30	20	41.20
		21	8.00
Mean	41.04	Mean	30.74
SEM	8.04	SEM	14.89

APPENDIX P

CALIBRATION DATA

ARM CALIBRATION
(volts)

	Position 2						Position 3						Position 4					
	2.5 (lbs.)	5 (lbs.)	7.5 (lbs.)	10 (lbs.)	12.5 (lbs.)	15 (lbs.)	2.5 (lbs.)	5 (lbs.)	7.5 (lbs.)	10 (lbs.)	12.5 (lbs.)	15 (lbs.)	2.5 (lbs.)	5 (lbs.)	7.5 (lbs.)	10 (lbs.)	12.5 (lbs.)	15 (lbs.)
April 4th	2.44	3.21	3.98	4.75	5.52	6.27	2.16	2.87	3.58	4.29	5.00	5.70	1.80	2.50	3.21	3.91	4.61	5.32
April 5th	2.45	3.22	3.98	4.75	5.52	6.29	2.17	2.87	3.25	4.29	5.00	5.71	1.80	2.50	3.21	3.91	4.62	5.32
April 6th	2.45	3.22	3.99	4.75	5.52	6.29	2.17	2.88	2.92	4.29	5.00	5.71	1.80	2.51	3.21	3.91	4.62	5.32
April 7th	2.27	3.04	3.81	4.57	5.34	6.11	2.00	2.70	3.41	4.12	4.83	5.54	1.63	2.34	3.04	3.75	4.45	5.16
April 8th	2.43	3.20	3.97	4.74	5.50	6.27	2.15	2.86	3.57	4.28	4.99	5.70	1.78	2.48	3.19	3.89	4.60	5.30
April 11th	2.39	3.15	3.92	4.69	5.46	6.23	2.11	2.82	3.53	4.23	4.94	5.65	1.74	2.45	3.15	3.86	4.56	5.27
April 12th	2.35	3.12	3.89	4.66	5.43	6.19	2.07	2.78	3.49	4.20	4.91	5.62	1.72	2.42	3.12	3.83	4.53	5.24
April 13th	2.48	3.25	4.02	4.79	5.56	6.32	2.20	2.91	3.61	4.32	5.03	5.74	1.83	2.54	3.24	3.95	4.65	5.36
April 14th	2.68	3.45	4.22	4.99	5.76	6.53	2.38	3.10	3.81	4.51	5.22	5.93	1.85	2.72	3.42	4.13	4.83	5.54
April 15th	2.24	3.01	3.77	4.54	5.31	6.08	2.07	2.68	3.39	4.10	4.81	5.51	1.72	2.31	3.02	3.72	4.42	5.13
April 18th	2.30	3.07	3.84	4.61	5.37	6.14	2.03	2.74	3.45	4.15	4.86	5.57	1.66	2.37	3.07	3.78	4.48	5.19
April 19th	2.22	2.99	3.76	4.52	5.29	6.06	1.96	2.66	3.37	4.08	4.79	5.50	1.69	2.30	3.00	3.70	4.41	5.11
April 20th	2.28	3.05	3.81	4.58	5.35	6.12	2.01	2.71	3.42	4.13	4.84	5.55	1.64	2.35	3.05	3.76	4.46	5.17
April 21st	2.53	3.31	4.07	4.84	5.61	6.38	2.24	2.96	3.67	4.37	5.08	5.79	1.87	2.59	3.29	4.00	4.70	5.41
April 22nd	2.35	3.12	3.88	4.65	5.42	6.19	2.07	2.78	3.49	4.20	4.90	5.61	1.71	2.41	3.11	3.82	4.52	5.23
April 25th	2.40	3.17	3.94	4.70	5.47	6.24	2.12	2.83	3.54	4.25	4.96	5.66	1.75	2.46	3.16	3.87	4.57	5.28
April 26th	2.45	3.22	3.99	4.76	5.53	6.30	2.16	2.88	3.59	4.30	5.00	5.71	1.80	2.51	3.21	3.92	4.62	5.33
April 27th	2.26	3.03	3.79	4.56	5.33	6.10	2.04	2.70	3.41	4.11	4.82	5.53	1.62	2.33	3.03	3.74	4.44	5.15
April 28th	2.65	3.43	4.20	4.97	5.74	6.50	2.36	3.07	3.78	4.49	5.20	5.91	1.99	2.70	3.41	4.11	4.82	5.52
April 29th	2.45	3.23	4.00	4.77	5.53	6.30	2.17	2.89	3.60	4.30	5.01	5.72	1.80	2.52	3.22	3.92	4.63	5.33
May 2nd	2.32	3.09	3.86	4.62	5.39	6.16	2.05	2.76	3.47	4.18	4.88	5.59	1.68	2.39	3.09	3.80	4.50	5.21
May 3rd	2.51	3.28	4.05	4.82	5.59	6.36	2.22	2.94	3.64	4.35	5.06	5.77	1.77	2.56	3.27	3.97	4.68	5.38
May 4th	2.61	3.39	4.15	4.92	5.69	6.46	2.31	3.03	3.74	4.45	5.16	5.86	1.72	2.66	3.36	4.06	4.77	5.47
May 5th	2.41	2.99	3.76	4.52	5.29	6.06	2.05	2.66	3.37	4.08	4.79	5.50	1.67	2.29	3.00	3.70	4.41	5.11
May 6th	2.37	3.14	3.91	4.67	5.44	6.21	2.09	2.80	3.51	4.22	4.93	5.63	1.73	2.43	3.14	3.84	4.55	5.25
May 9th	2.42	3.18	3.95	4.72	5.49	6.26	2.13	2.84	3.55	4.26	4.97	5.68	1.77	2.47	3.18	3.88	4.59	5.29
May 10th	2.37	3.05	3.82	4.59	5.36	6.13	2.01	2.72	3.43	4.14	4.85	5.56	1.65	2.35	3.06	3.76	4.47	5.17
May 11th	2.55	3.33	4.10	4.87	5.63	6.40	2.26	2.98	3.69	4.39	5.10	5.81	1.89	2.61	3.31	4.02	4.72	5.43
May 12th	2.58	3.36	4.13	4.89	5.66	6.43	2.19	3.00	3.71	4.42	5.13	5.84	1.74	2.63	3.34	4.04	4.75	5.45
May 13th	2.39	3.03	3.80	4.56	5.33	6.10	2.10	2.70	3.41	4.12	4.83	5.53	1.63	2.33	3.04	3.74	4.45	5.15
Mean	2.42	3.18	3.95	4.71	5.48	6.25	2.14	2.84	3.51	4.25	4.96	5.67	1.75	2.47	3.17	3.88	4.58	5.29
SEM	0.12	0.13	0.13	0.13	0.13	0.13	0.11	0.12	0.17	0.12	0.12	0.12	0.09	0.12	0.12	0.12	0.12	0.12
CV	4.94	4.18	3.36	2.82	2.42	2.12	4.94	4.34	4.97	2.89	2.48	2.17	4.96	4.91	3.82	3.13	2.65	2.29

LOAD CELL CALIBRATION

RAW DATA AND DAILY COEFFICIENT OF VARIATION
(volts)

		TRIAL 1			TRIAL 2			TRIAL 3		
	Load (lbs)	**Mean (volts)**	**Standard Deviation**	**CV (%)**	**Mean (volts)**	**Standard Deviation**	**CV (%)**	**Mean (volts)**	**Standard Deviation**	**CV (%)**
January 10th	**2.5**	2.1964	0.0012	0.05	2.1885	0.0001	0.01	2.2103	0.0003	0.01
	5	2.8134	0.0005	0.02	2.8029	0.0004	0.01	2.7930	0.0002	0.01
	7.5	3.4244	0.0003	0.01	3.3687	0.0009	0.03	3.4075	0.0007	0.02
	10	3.9903	0.0002	0.01	4.0361	0.0006	0.01	4.0456	0.0018	0.04
	12.5	4.6294	0.0010	0.02	4.6208	0.0007	0.02	4.6220	0.0002	0.00
	15	5.2886	0.0002	0.00	5.2698	0.0014	0.03	5.2710	0.0011	0.02
January 11th	**2.5**	2.1244	0.0005	0.02	2.1231	0.0006	0.03	2.1211	0.0005	0.02
	5	2.7244	0.0010	0.04	2.7256	0.0005	0.02	2.7366	0.0000	0.00
	7.5	3.2991	0.0007	0.02	3.3077	0.0004	0.01	3.3019	0.0002	0.01
	10	3.9278	0.0006	0.01	3.8994	0.0003	0.01	3.9266	0.0003	0.01
	12.5	4.4897	0.0009	0.02	4.5165	0.0004	0.01	4.5061	0.0002	0.00
	15	5.1852	0.0014	0.03	5.1617	0.0003	0.00	5.1774	0.0005	0.01
January 12th	**2.5**	2.0213	0.0003	0.01	2.0353	0.0003	0.01	2.0460	0.0016	0.08
	5	2.6000	0.0008	0.03	2.6270	0.0002	0.01	2.6406	0.0003	0.01
	7.5	3.1775	0.0004	0.01	3.1944	0.0003	0.01	3.2221	0.0002	0.01
	10	3.8019	0.0004	0.01	3.8001	0.0002	0.00	3.8213	0.0007	0.02
	12.5	4.3356	0.0016	0.04	4.3926	0.0052	0.12	4.3892	0.0008	0.02
	15	4.9803	0.0010	0.02	5.0367	0.0007	0.01	5.0380	0.0012	0.02
January 13th	**2.5**	1.9859	0.0051	0.26	1.9672	0.0108	0.55	1.9705	0.0007	0.03
	5	2.6121	0.0029	0.11	2.5803	0.0011	0.04	2.5814	0.0015	0.06
	7.5	3.1848	0.0061	0.19	3.1490	0.0008	0.02	3.1523	0.0011	0.04
	10	3.7927	0.0043	0.11	3.7355	0.0029	0.08	3.7579	0.0008	0.02
	12.5	4.3310	0.0015	0.03	4.3242	0.0009	0.02	4.3044	0.0003	0.01
	15	4.9371	0.0027	0.05	4.9841	0.0017	0.03	4.9561	0.0011	0.02
January 14th	**2.5**	2.0509	0.0010	0.05	2.0303	0.0001	0.01	2.0425	0.0002	0.01
	5	2.6332	0.0013	0.05	2.6333	0.0002	0.01	2.6186	0.0006	0.02
	7.5	3.2132	0.0028	0.09	3.2142	0.0009	0.03	3.1763	0.0001	0.00
	10	3.7647	0.0037	0.10	3.7793	0.0005	0.01	3.7989	0.0001	0.00
	12.5	4.3735	0.0027	0.06	4.3891	0.0006	0.01	4.3789	0.0005	0.01
	15	4.9899	0.0016	0.03	5.0055	0.0008	0.02	5.0376	0.0014	0.03
January 15th	**2.5**	2.0448	0.0007	0.04	2.0302	0.0005	0.03	2.0294	0.0003	0.02
	5	2.6583	0.0016	0.06	2.6370	0.0001	0.00	2.6505	0.0003	0.01
	7.5	3.2367	0.0020	0.06	3.2254	0.0004	0.01	3.1877	0.0002	0.01
	10	3.8174	0.0027	0.07	3.8538	0.0003	0.01	3.8579	0.0008	0.02
	12.5	4.4273	0.0023	0.05	4.3977	0.0014	0.03	4.3959	0.0014	0.03
	15	5.0973	0.0018	0.04	5.0985	0.0005	0.01	5.0479	0.0012	0.02
January 16th	**2.5**	1.9782	0.0004	0.02	1.9510	0.0002	0.01	1.9753	0.0003	0.01
	5	2.5557	0.0010	0.04	2.5763	0.0003	0.01	2.5811	0.0009	0.03
	7.5	3.1177	0.0016	0.05	3.1556	0.0008	0.02	3.1552	0.0004	0.01
	10	3.7365	0.0025	0.07	3.7491	0.0003	0.01	3.7629	0.0003	0.01
	12.5	4.3208	0.0019	0.04	4.3419	0.0009	0.02	4.2753	0.0003	0.01
	15	4.9363	0.0021	0.04	4.9826	0.0011	0.02	4.9481	0.0009	0.02

LOAD CELL CALIBRATION

DAILY COEFFICIENT OF VARIATION BETWEEN LOADS

	Load (lbs)	Mean (volts)	Standard Deviation	CV (%)
January 10th	2.5	2.1984	0.0111	0.50
	5	2.8031	0.0102	0.36
	7.5	3.4002	0.0286	0.84
	10	4.0240	0.0296	0.73
	12.5	4.6241	0.0046	0.10
	15	5.2765	0.0105	0.20
January 11th	2.5	2.1229	0.0017	0.08
	5	2.7289	0.0067	0.25
	7.5	3.3029	0.0044	0.13
	10	3.9179	0.0161	0.41
	12.5	4.5041	0.0135	0.30
	15	5.1748	0.0120	0.23
January 12th	2.5	2.0342	0.0124	0.61
	5	2.6225	0.0207	0.79
	7.5	3.1980	0.0225	0.70
	10	3.8078	0.0118	0.31
	12.5	4.3725	0.0320	0.73
	15	5.0183	0.0329	0.66
January 13th	2.5	1.9745	0.0100	0.51
	5	2.5913	0.0180	0.70
	7.5	3.1620	0.0198	0.63
	10	3.7620	0.0288	0.77
	12.5	4.3199	0.0138	0.32
	15	4.9591	0.0236	0.48
January 14th	2.5	2.0413	0.0104	0.51
	5	2.6284	0.0084	0.32
	7.5	3.2012	0.0216	0.67
	10	3.7810	0.0172	0.45
	12.5	4.3805	0.0079	0.18
	15	5.0110	0.0243	0.49
January 15th	2.5	2.0348	0.0086	0.42
	5	2.6486	0.0108	0.41
	7.5	3.2166	0.0257	0.80
	10	3.8430	0.0223	0.58
	12.5	4.4070	0.0176	0.40
	15	5.0812	0.0289	0.57
January 16th	2.5	1.9681	0.0149	0.76
	5	2.5710	0.0135	0.53
	7.5	3.1428	0.0218	0.69
	10	3.7495	0.0132	0.35
	12.5	4.3127	0.0340	0.79
	15	4.9556	0.0241	0.49

LOAD CELL CALIBRATION

COEFFICIENT OF VARIATION BETWEEN DAYS

Load (lbs)	Mean (volts)	Standard Deviation	CV (%)
2.5	2.0535	0.0819	3.99
5	2.6562	0.0819	3.09
7.5	3.2320	0.0899	2.78
10	3.8407	0.0989	2.58
12.5	4.4172	0.1112	2.52
15	5.0681	0.1192	2.35