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Relationship Between Metabolic By-Products and Nervous System Failure/Fatigue

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RELATIONSHIP BETWEEN METABOLIC BY-PRODUCTS AND NERVOUS SYSTEM FAILURE/FATIGUE

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ABSTRACT

Fatigue has generally been defined as an acute impairment of exercise/sport performance that includes both an increase in the perceived effort necessary to exert a desired force or power output, and the inability to produce the desired force or power output. The majority of research to date looking at fatigue has focused upon substrate utilization, however, what is relatively unknown is the contribution the nervous system has upon fatigue. Therefore, the purpose of this dissertation was to investigate potential mechanisms that relate to neural fatigue. An additional purpose was to determine if there were any relationships between metabolic by-products and EMG characteristics following exercise. The first investigation sought to determine changes in EMG M-wave amplitude of the gastrocnemius following the calf raise exercise. There were no significant changes in M-wave EMG amplitude following exercise. The second investigation compared changes in muscle contractile properties and EMG characteristics of the VL, RF, and VM following a high-intensity exercise. There was a significant decrease in MDF of the VL only. Additionally, there was a decrease in peak force and rate of force development. The last investigation utilized the same exercise protocol as the second investigation, but added the supplementation of aspartate and sodium bicarbonate. Both supplements were effective in reducing ammonia concentrations following exercise. Additionally, supplementation with sodium bicarbonate resulted in an increase in rate of force development following exercise. As for EMG characteristics, there was a significant decrease in MDF for the RF, but not the VL. There were no significant changes in PF or EMG amplitude. Currently, no relationship between the metabolic and nervous systems during times of fatigue can be determined at this point.

CHAPTER 1. INTRODUCTION

Performance during athletic competition is initially dependent upon both the rate of force development and the maximum force produced (57). Ultimately, however, fatigue will set in during competition or exercise, thus, decreasing performance or leading to a loss during competition. Fatigue has generally been defined as an acute impairment of exercise/sport performance that includes both an increase in the perceived effort necessary to exert a desired force or power output, and the inability to produce the desired force or power output (14). The majority of research to date investigating fatigue has focused upon substrate utilization, however, what is relatively unknown is the contribution the nervous system has upon fatigue, specifically, within the peripheral nervous system (PNS).

Within the PNS, many factors can have the potential to affect the motor unit (MU). It can be speculated that factors may affect separately the motor neuron (MN) or the muscle fibers innervated by the MN. On the other hand, both may be affected simultaneously (46). For example, presynaptic fatigue may be an indication of failure of action potential propagation along the axon or an inadequate release of acetylcholine (ACh). At the post-synaptic level, fatigue may be due to insufficient depolarization of the muscle membrane or failure of action potential propagation along the sarcolemma. However, all proposed theories lack sufficient description of the magnitude of their effect.

Muscle fatigue is a multi-faceted process that arises in a task dependent manner (31, 44). The unfortunate fact is that fatigue will begin to develop at the onset of exercise and/or sport competition, thus, ensuring a subsequent decrement in performance.

Previous research regarding muscle fatigue and the underlying mechanisms are a matter of debate, however, the common element to all fatigue based investigations is the transient decline in muscle force-generating capacity (44). Specifically, a decreases in force or power production have been observed by an accompanying increase in the level of effort required to perform the exercise until task failure occurs (21). In addition to impacting a fiber's capacity for maximum force generation, fatigue also decreases the maximum velocity of shortening and slows relaxation time (4). Consequently, if force and/or velocity are negatively impacted, then power output will be ultimately impacted negatively as well. The ability to generate force quickly is considered functionally important during situations where time to develop force is limited (53), such as re-stabilizing the body following a loss of balance or during explosive sport activities (1, 16, 49, 52). It is thought that force production is influenced by several neural, mechanical and architectural parameters, however, the exact mechanisms regarding explosive force production remains unknown. This may include agonist neural drive, maximal strength, fascial length, fascial angle, and muscle-tendon unit (MTU) stiffness (5, 8, 9, 16).

Electromyography (EMG) analyses have been used extensively to correlate EMG characteristics to manifestations of muscle fatigue during exercises of maximal voluntary contractions (20). Electromyography has been related to fatigue since the early 1980s, with the main characteristics studied being the changes in the mean/median frequency and/or changes in muscle activation via amplitude (38). Using a spectral analysis, results have shown a decrease in mean and median frequency (7, 18), which has been attributed to decreases in action potential conduction velocity (10), changes in action potential shape (26), and reduced muscle relaxation rates (34). On the other hand, an increase in

EMG amplitude following a fatiguing activity has been thought to indicate greater motor unit recruitment, firing frequency, and/or synchronization (43, 50).

It is difficult to document motor unit recruitment strategies during voluntary contractions in humans (37). A few authors, however, have attempted to do so in recent years. Cook and colleagues reported EMG amplitude to increase from the first five to the last five repetitions during three sets to failure of leg extension at 70% and 20% peak torque (13). Not surprisingly, it was reported that EMG amplitude was higher at 70% peak torque compared to 20%. Lastly, it has been reported that a greater EMG amplitudes was observed following an intensity of 75% 1RM compared to 30% (45). These studies have investigated EMG amplitude either following a single set of resistance exercise (2, 45), or during the initial five and final five repetitions of multiple sets (13). A spectral analysis of the EMG signal is useful in providing additional information regarding muscle fatigue, for a change in mean/median may indicate muscle fatigue (26). Jenkins et al. showed the median frequency to decrease significantly following three sets to failure of the leg extension exercise (30). Additionally, the authors reported EMG amplitude to increase following the three sets to failure.

The relationship between metabolic by-products and fatigue via a decline in maximal force generating capacity or rate of force development has been investigated for decades. Despite the volume of literature regarding this relationship, the exact mechanism still remain poorly understood (3, 23). In terms of surface electromyography (sEMG), results are consistent in that characteristics and manifestations of muscle fatigue during exercise are related to increases in amplitude (38) or shifts in sEMG median frequency to lower frequencies (7, 18). However, the literature is inconclusive in that

these changes are related to increases in metabolic by-products. Intramuscular bioenergetic changes have been related to limiting a muscle's ability to maintain a given level of peak force and/or power (28, 35). Declines in muscle tension have been noted due to changes in muscle phosphocreatine stores and a concomitant cytoplasmic inorganic phosphate accumulation (15, 27), changes in muscle lactate or pH (32), and adenylate depletion and subsequent increase in the concentration of muscle IMP (36) and blood ammonia (24). Although many have attempted to investigate muscle fatigue via electrophysiological and biochemical changes individually, only a limited number of studies have investigated the effects metabolic by-products have on EMG characteristics.

It is believed that a decline in pH, or increased acidosis, contributes to fatigue through an inhibition of various metabolic processes, such as impairing muscle glycolytic pathways and/or muscle shortening characteristics, including rate of force development (RFD) and conduction velocity (10, 19, 29, 32, 48). Decreases in maximum force production and rate of force development suggest that intramuscular pH affects mechanisms involved in rapid activation and shortening of the muscle during maximal voluntary contractions (47). Investigations have shown in isolated single non-human fibers that a decline in pH significantly reduces velocity of shortening and the rate of ADP release from myosin (17, 33). It is believed that these membrane-bound processes and actomyosin interactions are negatively impacted by the accumulation of potassium (K^+) (12) and inorganic phosphate (Pi) (41). Additionally, central drive also plays an important role in rapid force production (55), however, whether or not central drive is affected by changes in pH is unknown.

In addition to an increased acidosis, another theory that is being revisited is the formation of ammonia (56). Tashiro in 1922 was the first to investigate whether there is a link between the production of ammonia and fatigue among isolated nerve fibers after electrical stimulation (39, 51). Ammonia accumulation within the systemic circulation comes from sources such as the gut, muscle, kidney, or brain (22), with at rest the majority of systemic ammonia being released from the gut or gastrointestinal tract. Once inside the gastrointestinal tract, ammonia is delivered to the liver to be incorporated into either urea via ureagenesis or glutamine.

During exercise, the body will continue to use adenosine triphosphate (ATP) as needed, and as ATP is hydrolyzed for energy, an accumulation of adenosine diphosphate (ADP) phosphorylation will begin to occur (25, 40). Once the rate of ATP hydrolysis exceeds the rate of ADP, then the ATP content of the muscle fiber will become depleted. However, this decrease in ATP does not necessarily appear as a stoichiometric increase in ADP concentration (54). The reason for this is due in part to myokinase system operating to produce an ATP and adenosine monophosphate (AMP). As AMP builds up, AMP deaminase will hydrolysis AMP into inositol monophosphate (IMP) and an ammonia ion, NH_4^+. The myokinase system is beneficial for it helps to produce more ATP, however, the downside is the removal of an adenine nucleotide (54). As stated, it is commonly believed that fatigue only corresponds to changes in substrate utilization or inadequate energy formation. However, while the cell is producing adequate ATP, it may also be producing ammonia as a way to alleviate the production of AMP. Nonetheless, the goal of any athlete enduring their training program would be to maximize ATP formation while decreasing the production of ammonia.

Parnas and colleagues in the 1920s were the first to report the relationship between ammonia production and anaerobic work (42). Since the early work of Parnas, research has shown an intensity dependent relationship between plasma ammonia concentration and exercise with ammonia production increasing rapidly once intensity exceeds 60% of VO_{2max} (6, 11). The mechanisms behind this increase in ammonia have been identified as part of the purine nucleotide cycle (PNC). Within this cycle, adenosine monophosphate (AMP) is converted to inositol monophosphate (IMP) and an ammonium ion (NH_4^+) via the enzyme AMP deaminase (25). This enzyme is highly present in fast-twitch muscle fiber types, which is beneficial for the help of removing the AMP buildup and ridding the system of ammonia. However, as AMP deaminase forms ammonia, an adenine nucleotide is removed through the formation of IMP. This step is irreversible, thus, increasing fatigue by reducing the available substrates for ATP formation. Since the majority of sports incorporates fast muscle fiber types with high repeated stimulation of these fiber types, it can be assumed that high concentrations of AMP deaminase within these fiber types may be playing a role with the onset of fatigue.

Unfortunately, with respect to human performance and fatigue, the nervous system has been largely neglected. The importance of the nervous system upon performance has been understood by strength and conditioning coaches for decades, however, research that helps to develop an understanding of the nervous system during times of fatigue is lacking. Therefore, it is the purpose of this dissertation to investigate potential mechanisms that relate to neural fatigue. An additional purpose was to determine if there was a relationship between metabolic by-products during exercise and EMG characteristics. To answer these questions, three projects were conducted:

1.) Examine changes in muscle activation via M-wave recordings following multiple resistance training sessions. This project was titled "The impact of weight lifting upon neurotransmission failure within trained males and females."

2.) Examine changes in force production, EMG amplitude, and power spectrum shifts following a high intensity exercise session. This project was titled, "The effects of high-intensity exercise on isometric strength parameters."

3.) Examine the effects of an ammonia scavenger (aspartate) and pH buffer (sodium bicarbonate) on force production, EMG amplitude and power spectrum shifts following multiple high intensity exercise sessions. This project is titled, "Impact of aspartate and sodium bicarbonate supplementation upon central and peripheral fatigue."

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CHAPTER 2. THE IMPACT OF WEIGHT LIFTING UPON NEUROTRANSMISSION FAILURE WITHIN TRAINED MALES AND FEMALES

2.1 Introduction

Human performance is largely dependent upon both force and power generation, however, sustained muscle contraction is limited by fatigue (19). Fatigue is defined as the inability to produce a desired force or power output (6), and can involve both the central (14) and peripheral nervous systems (11). Central fatigue includes processes starting from the activation of the motor cortex to the recruitment of motoneurons at the spinal level. As for peripheral fatigue, this includes the action potential transmission along the motor nerve axon, sarcolemma, and T-tubules. In addition, peripheral fatigue includes the function of the neuromuscular junction and the efficiency of cross-bridge force production (11). One way to investigate both central and peripheral components to fatigue is through noninvasive transcutaneous nerve stimulation and electromyography (EMG) combined with voluntary muscle activation (19). Specifically, peripheral fatigue is manifested in alterations of the twitch properties of the fatigued muscle, while central fatigue is displayed with the twitch interpolation technique as a measure of central activation deficit (13)

The human nervous system is highly adaptive and can be modified in response to a variety of different motor experiences (3, 34). Neural adaptations have been reported with both endurance (30, 35), and strength training (1, 8, 10). Additionally, these neural adaptations have been thought to contribute to enhanced motor performance (10, 30). Many attempts have been made to determine the exact mechanisms regarding these adaptations; however, a conclusion still remains to be elucidated. Two responses that

have been measured to investigate muscle activation and the coinciding neural adaptations are the Hoffman (H) -reflex and M-wave. (16, 21). Both of these responses are affected by a common neural mechanism, however, the H-reflex is more sensitive to altered presynaptic inhibition and motoneuron excitability (22, 26, 34). On the other hand, the M-wave is more sensitive to changes distal to the neuromuscular junction, particularly within the sarcoplasmic reticulum or t-tubules of the muscle (1, 9).

The M-wave signal is present following an electrical stimulation that is applied to a mixed peripheral nerve. From here, an orthodromic signal along a motor neuron axon results in an activation of the target muscle. Increases in M-wave amplitude have been observed in responses to resistance training (1, 16). However, not all investigations have observed a corresponding change in maximal voluntary contraction (MVC) (32). Therefore, the M-wave amplitude may indicate changes that occur within the muscle (1).

Therefore, the purpose of this investigation was to determine pre-to-post exercise amplitude changes of the M-wave following the calf-raise exercise. An additional purpose involved comparing the changes in M-wave amplitude following either a single repetition or five repetitions of the said exercise. Similar to the results presented by Aagaard et al. (1), it was hypothesized that M-wave amplitude would increase from pre- to post-exercise and a further increase would be present following multiple repetitions.

2.2 Methods

2.2.1 Experimental Overview

The study consisted of participants performing a calf-raise (CR) exercise on three separate days to determine changes in muscle activation characteristics following

electrical nerve stimulation. Participants first completed a 5-repetition max (5RM) calf-raise exercise to determine the amount of weight which would be used on the remaining testing days. Following the initial 5RM, participants completed either one repetition (rep) or five repetitions (reps) in a randomized order and separated by one week. The weight recorded for each participant's 5RM was used for both testing days. For each testing session, M-wave amplitude was obtained prior to exercise and immediately post-exercise.

2.2.2 Participants

Twelve participants were recruited (age: 21.1±1 y; height: 1.69±0.093 m; weight: 76.5.4±12.9 kg), however, two had to be removed from data analysis. The remaining participants completed all aspects of this study. Participants had at least six months of weightlifting experience, and were recruited from activity courses at Louisiana State University, as well as from gyms located in the Baton Rouge area. The Louisiana State University Institutional Review Board approved the study, and each participant signed a written consent form after receiving an explanation of the purpose, potential risks, and benefits of participation in the study. Participants were excluded from the study if they reported a history of any knee and/or back injuries. In addition, all potential participants completed a Physical Activity Readiness Questionnaire (PAR-Q) before inclusion into the study. Only those individuals who answered "no" to all PAR-Q questions qualified as research participants.

2.2.3 5RM Testing Procedures

The first day of testing consisted of completing each participant's 5RM for the CR, what was used for the subsequent two testing sessions. The procedures for acquiring

a 5RM for the CR were performed according to the guidelines outlined by the National Strength and Conditioning Association®(25). Upon arrival to the lab, participants performed a standardized warm-up of five min on a stationary cycle ergometer. Following the warm-up, participants were then set up in a power-rack for proper height fitting for the CR. The movement was executed by standing flat footed with the barbell placed on the upper trapezius and plantar-flexing as high as possible, followed then by returning to starting position of feet flat on the ground. The first set consisted of performing the CR for 10 reps with an empty bar. An estimated weight that the participants believed they could perform five reps easily was then placed on the bar. Following this lift and a two min rest, 10-20% more weight was added to bar. If the participant completed five reps successfully, a two min rest was given and additional weight was added in similar increments. This process continued until the participant was unable to complete five reps or five reps were completed with improper technique.

2.2.4 Testing Protocol

Testing began one week following the initial visit where participants determined their 5RM. Each participant performed the CR at their 5RM consisting of a set number of repetitions, one or five, in a randomized order. All testing sessions, including the 5RM testing, used a standard Olympic style barbell (45 lbs.) and bumper plates. The Olympic bar and bumper plates were used to allow participants to drop the bar when performing the CR. Prior to all sessions, participants began with a five-minute warm-up via a stationary cycle ergometer. Following the five-minute standard bike warm-up, participants performed an un-weighted barbell warm-up of the CR exercise as described above. Following the un-weighted warm-up, each participant began by performing the

CR with either 1-rep or 5-reps at 50% and 75% of their 5RM. There was a three min rest between each warm-up set. Finally, participants performed the CR with for either 1-rep or 5-reps at 100% of their 5RM. Stimulation of the tibial nerve and EMG recordings were performed immediately pre-exercise and immediately following the final set.

2.2.5 M-wave amplitude

Muscle EMG data were collected at 2000 Hz. The EMG recordings were filtered at a band width of 30-1000 Hz. Participants were shaved and wiped with an antiseptic alcoholic wipe to cleanse the desired locations, and self-adhering Ag-AgCl bipolar surface electrodes were placed with an inter-electrode distance of two centimeters. The electrodes were secured over the lateral gastrocnemius muscle belly in line with the muscle fibers using adhesive tape, and then were wrapped using a self-adhesive elastic sports bandages. The electrode placement of the gastrocnemius was determined from the Surface EMG Non-Invasive Assessment of Muscles (SENIAM) guidelines.

Stimulation of gastrocnemius was performed by electrically stimulating the tibial nerve in the popliteal fossa with the negative electrode proximal to the positive electrode. as. Stimulation was administered using a BSLSTM Voltage Stimulator connected to a MP36 data acquisition unit (Biopac Systems, Inc., Goleta, CA). Participants laid prone on a bench with the voltage of the stimulation starting at 40 V and increased until a plantar flexor response was observed at the foot, or stimulation maxed out at 100 V. Three stimulations were delivered at pre-exercise and immediately post-exercise with each stimulation separated by 10 sec. M-wave amplitude was calculated as the difference between peak maximum and peak minimum of the M-wave amplitude response. Data

were averaged with a minimum of two out of three measures, and any data with a presence of low frequency noise or non-responsive EMG signals were removed.

2.2.6 Data Analysis

Microsoft Excel was used to perform a paired t-test for the M-wave amplitude recordings from pre- to post-exercise for both the one-rep and five-rep exercise of the CR. Statistical significance was determined at $P < 0.05$.

2.3 Results

Pre- to post-exercise M-wave amplitude was not statistically significant for either one-rep ($P = 0.2$) (Fig. 2.1) or five-reps ($P = 0.24$) (Fig. 2.2).

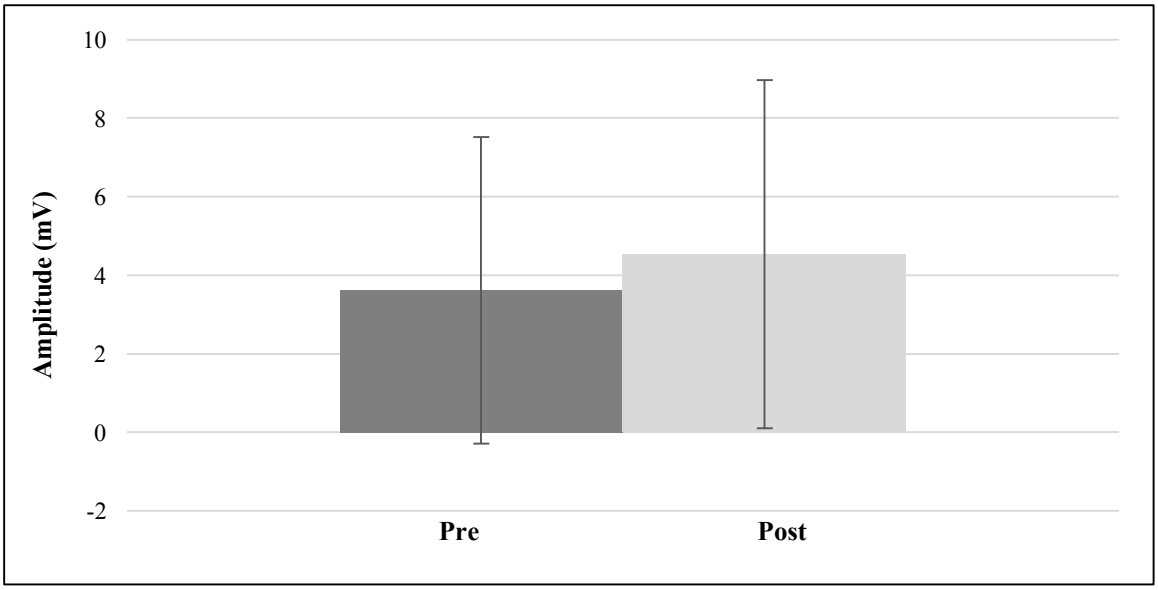

FIGURE 2.1. M-wave Amplitude Following One-Rep of the CR. Values are mean ± standard deviations.

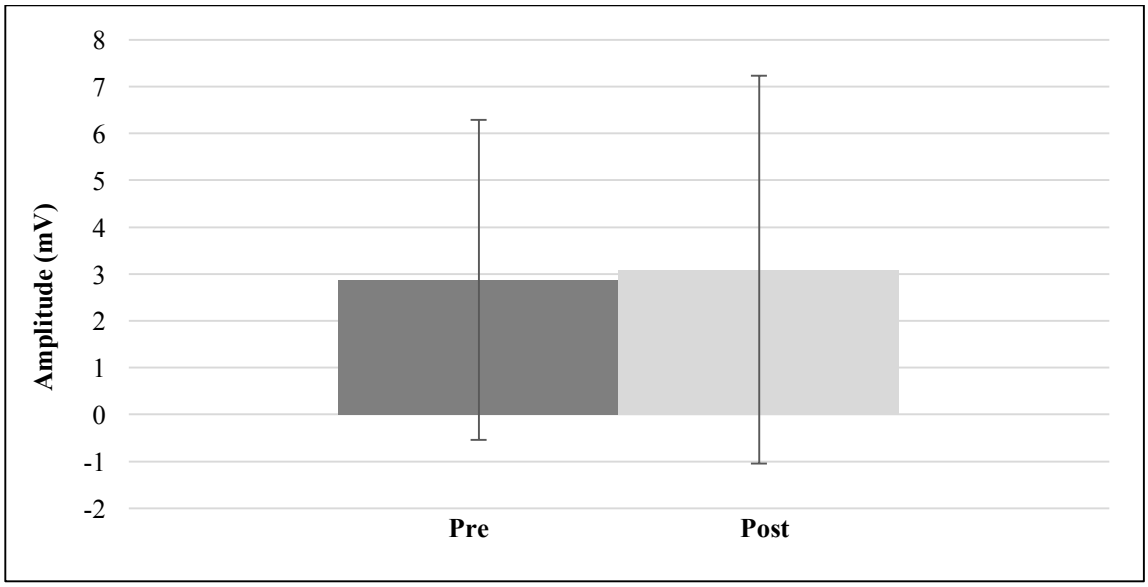

FIGURE 2.2. M-wave Amplitude Following Five-Reps of the CR. Values are mean ± standard deviations.

2.4 Discussion

The purpose of this investigation was to electrically stimulate the gastrocnemius and to determine changes in M-wave amplitude following a resistance training exercise. An additional purpose was to compare the M-wave amplitude changes following one rep versus five reps of the CR. There were no statistically significant changes in M-wave amplitude following the CR when performed for either one rep or five reps. The hypothesis of this investigation was that M-wave amplitude would increase following both testing sessions, with a greater increase seen after more repetitions.

Resistance training sessions are known to induce acute decreases in muscle strength and changes in the neuromuscular system (17). However, with respect to changes in M-wave amplitude, results remain inconsistent (19). For example, some have reported a decrease in M-wave amplitude following maximal (4) or submaximal contractions (24), while others reported no changes following maximal (23) or

submaximal contractions (29). Neural adaptions are task dependent and may be one reason in the discrepancy amongst M-wave amplitude results (2, 28, 36). For example, the majority of investigations have utilized maximal voluntary contractions (MVC), however, holding a contraction maximally may not be sufficient enough of an exercise stimulus to elicit neural adaptations. The current investigation tried to deliver a strong exercise stimulus by having participants perform a plantar-flexing movement with high loads. However, following researchers who observed no changes in M-wave amplitude (23, 29), the current protocol may not have been sufficient enough of an exercise stimulus to the gastrocnemius to warrant any neural adaptions.

Changes in M-wave amplitude could demonstrate a failure to maintain neuromuscular propagation at a normal level (15), with major sites of failure stemming from a decrease in sarcoplasmic reticulum or t-tubule excitability (1). Therefore, changes in membrane excitability would subsequently influence the motor unit by either recruiting more motor units or altering the firing frequency of the motor neuron (27). Additionally, a change in M-wave amplitude may result from decreased activity and/or efficacy of the Na^{+}-K pumps (7). In agreement with our findings, others have found an unchanged M-wave amplitude after a fatiguing exercise (5, 12).

Lastly, fiber type may have played a role in the outcome of the current study (18). Fast-twitch motor units are more fatigable compared to slow twitch motor units, and are recruited more during high-intensity contractions (20). Although not statically significant, the small increase in M-wave amplitude of gastrocnemius following our protocol could be the result of an increase in motor unit recruitment, however, this is only speculative (31, 33). It has been suggested that increases in amplitude could indicate a greater

synchronization of the motor units, however, it appears that motor unit recruitment is the primary method of neural adaptions (11). The exact mechanisms behind how the body makes neural adjustments are still unclear.

In conclusion, there were no significant changes in M-wave amplitude following two resistance training exercise sessions. M-wave amplitude did show an increasing trend from pre-to-post exercise following both training sessions, however, no statistical significance was found. Researchers should continue investigating the M-wave amplitude in order to better understand how the body alters the nervous and muscular system under fatiguing conditions to ensure proper force/power production.

2.5 References

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CHAPTER 3. THE EFFECTS OF HIGH-INTENSITY EXERCISE ON ISOMETRIC STRENGTH PARAMETERS

3.1 Introduction

Explosive muscular force production is an integral aspect to performance in sport (34, 41), and force adaptions can be attributed to increases in muscular strength (24, 25). Research has shown that a variety of athletes rely upon both isometric maximal strength and rate of force development (RFD), and these two factors have been documented within track cyclists (53), track and field athletes (52), college wrestlers (35) and weightlifters (54). Although there is no consensus within strength and conditioning professionals regarding how much strength is needed for optimal performance (55), research does support that the importance of maximum isometric strength is underestimated in a variety of athletic populations (51, 52, 54). For example, it was shown that maximum strength was able to discriminate between athletes of different performance levels within the sport of American football (17), however, this relationship could be dependent on position and type of test used (7).

A validated method of testing peak force (PF) and RFD within the strength and conditioning profession is the isometric mid-thigh pull test (33). The isometric mid-thigh pull test was first described by Haff et al. (20), and since its introduction to research, it has been shown to correlate well with 1-repetition maximum (1RM) testing of the back squat within college wrestlers (35), college track and field athletes (40), soccer players (36), American Football players (34, 40), and weightlifters (19, 54). Moreover, the isometric mid-thigh pull test is a highly reliable test that correlates well with the maximal values obtained using jumping and 1RM tests (52, 53).

Electromyography characteristics have been related to fatigue within exercise and sport, with the majority of investigations reporting decreases in the median frequency (MDF) and increases in amplitude (38, 45, 57). These two characteristics have been related to fatigue for at least three decades, thus, making them a useful technique to investigate potential mechanisms related to neural fatigue (6, 12). Decreases in the MDF have been attributed to decreases in action potential conduction velocity (10), changes in action potential shape (21), and reduced relaxation rates of muscles (31). An increase in EMG amplitude following a fatiguing activity is thought to indicate greater motor unit recruitment, firing frequency, and/or synchronization (45, 57).

Numerous experiments have investigated PF, RFD, EMG amplitude and MDF in various conditions, however, little research has emphasized these measurements when investigating factors considered to be important in terms of training, i.e. exercise volume, total work, and/or time under tension. Electromyography amplitude has been shown to increase following exercise (2, 22, 49), while the MDF to decrease (22). Moreover, the majority of research conducted has utilized exercise protocols of either moderate intensities or isometric contractions. However, lacking are investigations that incorporates changes in EMG characteristics following a dynamic exercise protocol.

Therefore, the purpose of this investigation was to determine changes in PF, RFD, EMG amplitude (measured as the root mean square amplitude or RMS), and median power frequency (MDF) following a high-intensity exercise protocol. These measurements were utilized to gain new insights into how both neural and muscle contractile properties change following a high-intensity exercise training session. The isometric mid-thigh pull test was chosen to describe any changes in muscle contractile

properties due to the high reliability and strong correlation to an individual's absolute strength. Based upon current literature, it was hypothesized that a decrease would be observed in PF, RFD, and EMG MDF following the high-intensity exercise. Additionally, it was hypothesized that there would be an increase in EMG amplitude following the high-intensity exercise.

3.2 Methods

3.2.1 Experimental Overview

One day of testing was conducted to investigate the changes in the aforementioned muscle contractile characteristics of healthy, exercise trained males. An isometric mid-thigh pull test was conducted with EMG analysis pre and post-exercise to determine changes in muscle activation. Peak force production (PF) and rate of force development (RFD) were also measured during the isometric mid-thigh pull test. Each participant performed one high-intensity exercise protocol consisting of barbell thrusters, squat jumps, lunges jumps, and forward jumps.

3.2.2 Participants

Eleven participants completed all aspects of this study (age: 22.1±1.6 y; height: 1.75±0.05 m; weight:79.8±8.4 kg). Participants had at least six months of current resistance training experience, and were recruited from activity courses at Louisiana State University, as well as from gyms located in the Baton Rouge area. The study was approved by the Institutional Review Board at Louisiana State University. The purpose, potential risks, and benefits of participation in this study were fully explained to each participant, and signed consent was obtained before enrollment. Participants were

excluded from the study if they reported any history of knee and/or back injuries. In addition, all potential participants completed a Physical Activity Readiness Questionnaire (PAR-Q) before inclusion into the study. Only those individuals who answered "no" to all PAR-Q questions were used as research participants.

3.2.3 Isometric Mid-Thigh Pull Test

As mentioned above, the isometric mid-thigh pull test was performed to measure PF and RFD (20, 34, 52), and was derived from the vertical ground reaction force data collected at 3000 Hz using an AMTI force platform. Force plate data were low pass filtered offline at 50 Hz using a second order, zero-lag Butterworth filter. Participants were instructed to pull on an immovable bar (located in a power rack with pins) as quickly as possible, and to maintain this maximal effort for five seconds. Specific instructions were to pull on the bar as "hard and fast as possible" in order to produce optimal results for recording of PF and RFD (5, 20, 47). Participants performed three sets of five seconds with a three-minute rest, both before and immediately after the exercise session. Bar height was adjusted for each person so that both the hip and knee angles were between 130 and 150 degrees, respectively. Participants were instructed to maintain these angles throughout the duration of the trial. Force-time curves were analyzed for RFD during the mid-thigh pull from the ΔForce/ΔTime. Analysis for the RFD started at the onset of muscle contraction and stopped at the following time points: 50 ms (0-50 ms), 150 ms (0-150 ms), and 250 ms (0-250 ms). Peak force, also obtained during the mid-thigh pull test, was defined as the highest value produced during the five second isometric contraction. The highest values produced out of the three trials for both PF and RFD were used for further analysis.

3.2.4 Electromyography (EMG)

Muscle EMG data were collected from the vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM) during the isometric mid-thigh pull test at 3000 Hz using a 16 channel, MA300 system (Motion Lab Systems, Baton Rouge, LA). The electrode placement for each recorded muscle was determined using the Surface EMG Non-Invasive Assessment of Muscles (SENIAM) guidelines. To prepare and clean the desired EMG electrode placement locations, participants were shaved and wiped with an antiseptic alcoholic wipe. The electrodes used were self-contained Ag-AgCl electrodes (Model: MA-411, Motion Lab Systems, Baton Rouge, LA). All electrodes were secured over the muscle belly in line with the muscle fibers using adhesive tape, and all of the electrodes on the thigh were then wrapped with a self-adhesive elastic sports bandage to restrict their movement during the high-intensity exercise.

Electromyography recordings were band-pass filtered at 10-750Hz. A Fast Fourier Transform (FFT) was applied to the EMG data during a one second time period surrounding PF (one-half second before PF and one-half second following PF). The MDF was defined as the frequency that divided the area under the power spectrum into two equal parts. The EMG amplitude was expressed as a root mean square amplitude (RMS), and was also analyzed during the same one second time period surrounding PF. An average of a minimum of two out of three trials for both pre- and post-exercise were used in analysis for both MDF and amplitude. Any EMG data which included the presence of low frequency noise or non-responsive signal was removed.

3.2.5 High-Intensity Exercise Protocol

Each participant performed one high-intensity exercise protocol consisting of four exercises: barbell thrusters, squat jumps, lunge jumps, and forward jumps. These exercises were selected due to the high contribution of the quadriceps muscles throughout each movement. Exercises began immediately following the initial isometric mid-thigh pull test. Participants were instructed to perform the exercises in the order listed above. The exercise protocol consisted of performing each exercise three times for a period of 20 s with a 30 s rest between each exercise. The completion of all four exercises and their corresponding rest times was designated as one round. Each participant completed three rounds of exercises with a one min. rest between each round. Upon completion of the three rounds, the post-exercise isometric mid-thigh pull test was performed.

The barbell thruster was performed by first completing a front squat with an unloaded bar (45 lb.), and then while the participant stood from the squat, the bar was pushed overhead, ending with the bar balanced above the head and over the participant's heels. The jump squats, lunge jumps, and forward jumps were all performed unloaded. The jump squats were performed by lowering the body downward into a regular squat, followed by jumping up explosively. When the participant landed, they lowered their body back into the squat position which completed one rep. The lunge jumps were performed by stepping forward with one leg while simultaneously lifting up onto the ball of the back foot. Participants then jumped and switched legs, ending with the opposite leg in the forward position. The forward jumps were performed by jumping forward over a hurdle that was measured at a height equivalent to each participants' patella.

3.2.6 Data Analysis

Microsoft Excel was used to perform a paired t-test (pre- vs. post-exercise) for all EMG characteristics and force plate variables. Statistical significance was determined at $P < 0.05$.

3.3 Results

Peak force (Fig. 3.1) was significantly lowered from pre-exercise to post-exercise ($P < 0.001$). The RFD time points of 0-150 ms (Fig. 3.3) and 0-250 ms (Fig. 3.4) both decreased significantly from pre-to post-exercise ($P < 0.02$ & $P < 0.04$, respectively). However, no statistical significance was found from pre- to post-exercise for the 0-50 ms RFD time point (Fig. 3.2) ($P = 0.3$).

No statistically significant changes in EMG amplitude were identified for the VL ($P = 0.07$) (Fig. 3.5), RF ($P = 0.12$) (Fig. 3.6), or VM ($P = 0.34$) (Fig. 3.7). Median frequency of the VL decreased significantly from pre-to post-exercise ($P < 0.03$) (Fig. 3.8). On the other hand, the RF ($P = 0.1$) (Fig. 3.9) and the VM ($P = 0.4$) (Fig. 3.10) were not statistically significant from pre-to post-exercise.

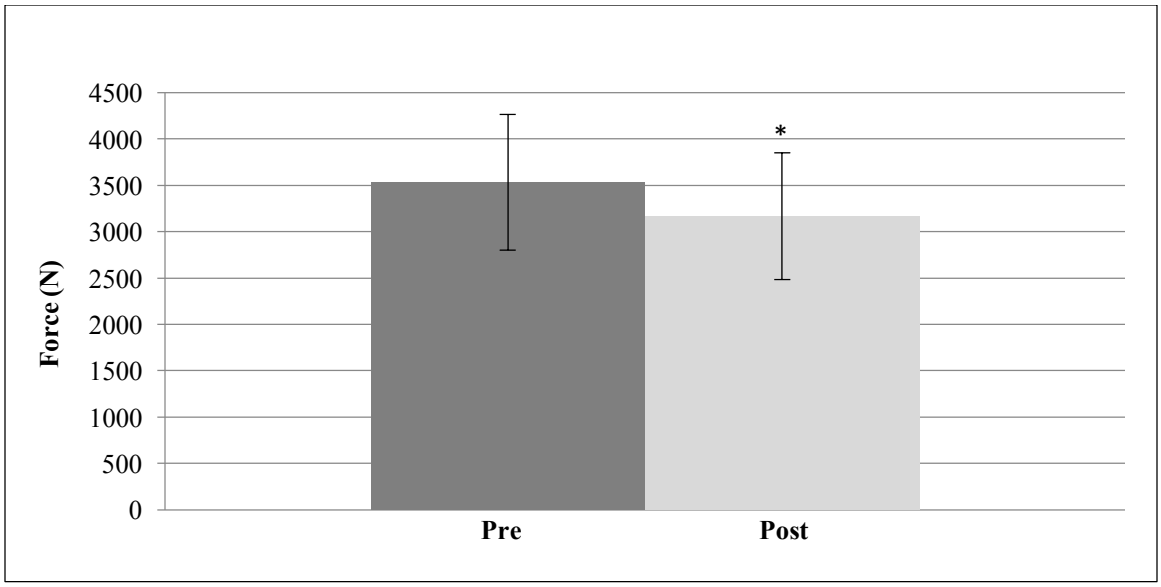

FIGURE 3.1. Peak Force. Values are mean ± standard deviations. * Post-exercise was different from baseline testing, $P < 0.001$.

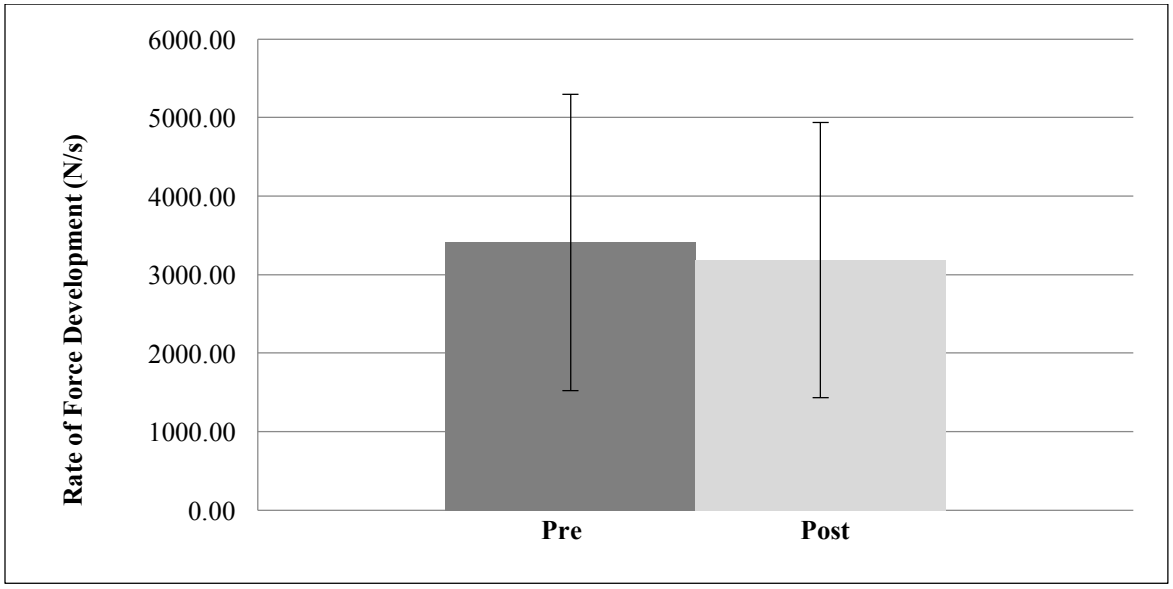

FIGURE 3.2. RFD from the Onset of Contraction to 50 ms. Values are mean ± standard deviations.

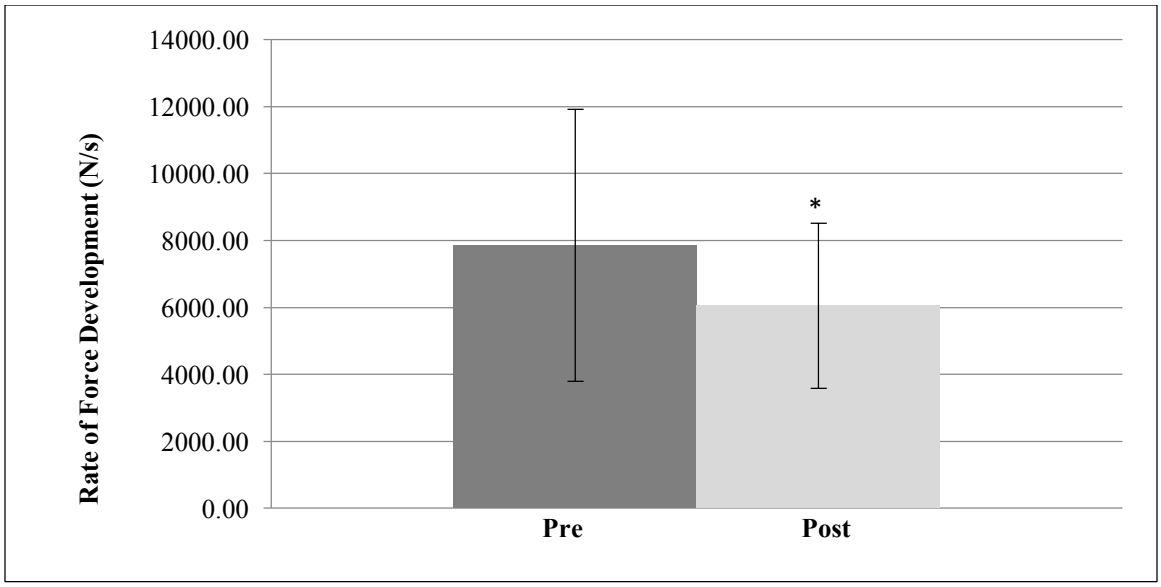

FIGURE 3.3. RFD from the Onset of Contraction to 150 ms. Values are mean ± standard deviations. * Post-exercise was different from baseline testing, $P < 0.02$.

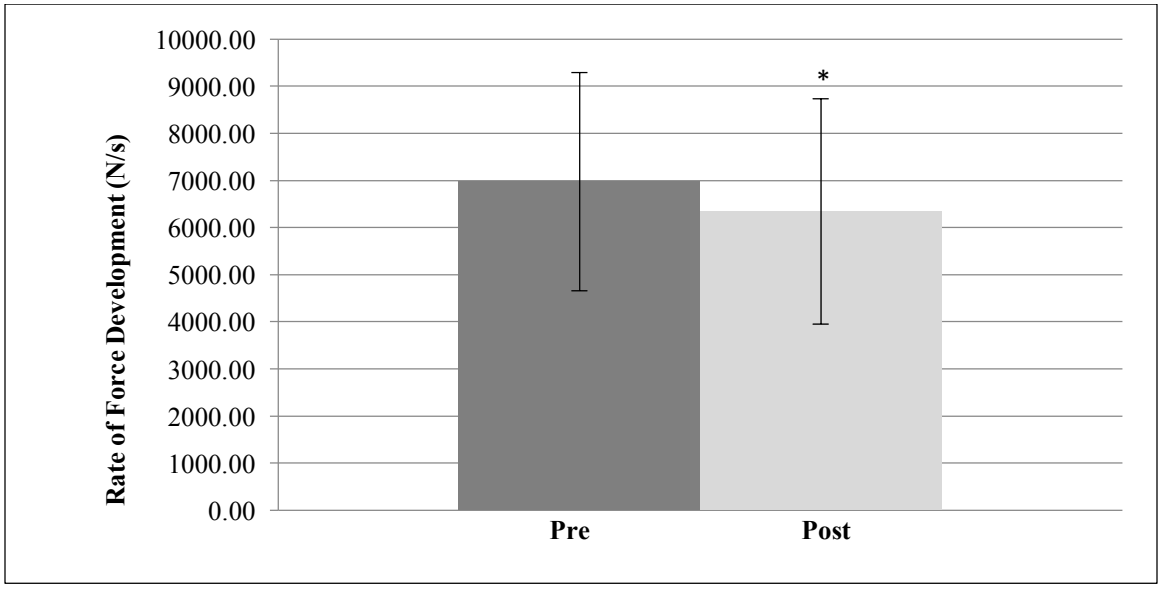

FIGURE 3.4. RFD from the Onset of Contraction to 250 ms. Values are mean ± standard deviations. * Post-exercise was different from baseline testing, $P < 0.04$.

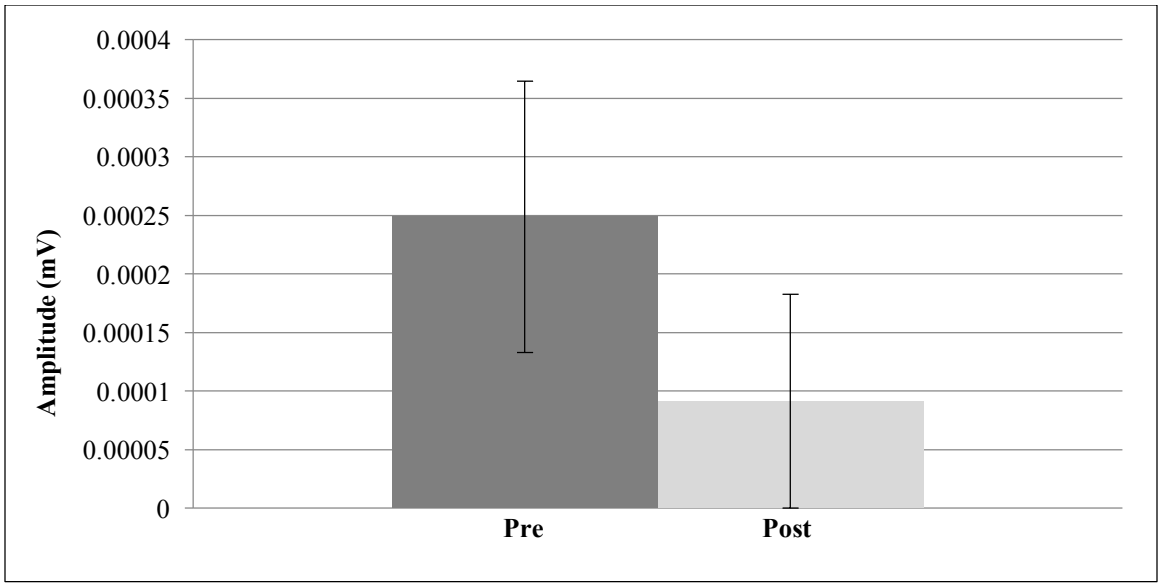

FIGURE 3.5. EMG Amplitude of the Vastus Lateralis. Values are mean ± standard deviations.

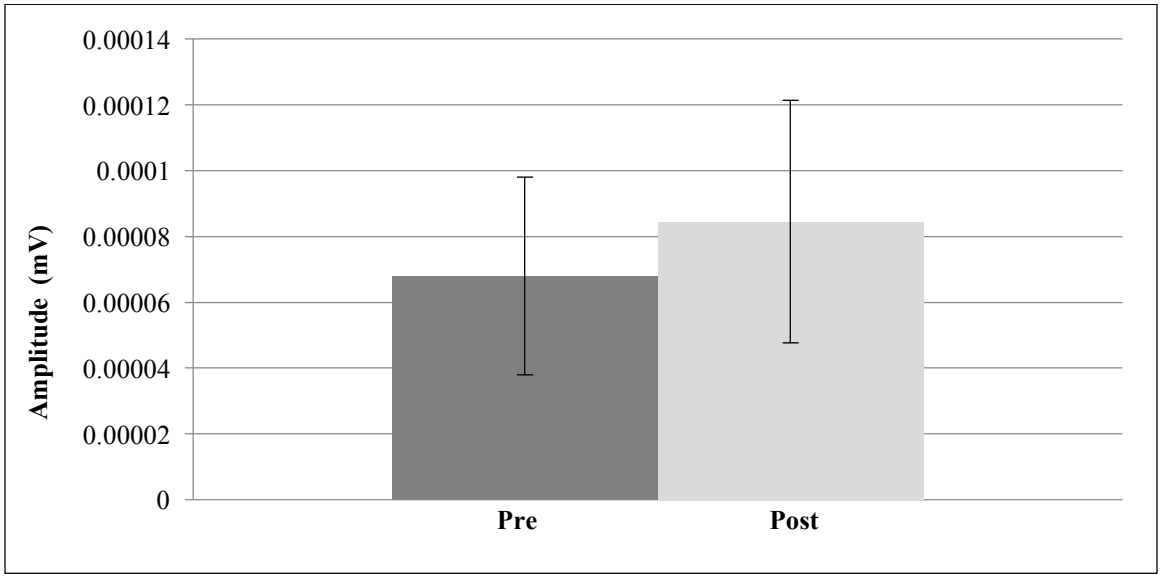

FIGURE 3.6. EMG Amplitude of the Rectus Femoris. Values are mean ± standard deviations.

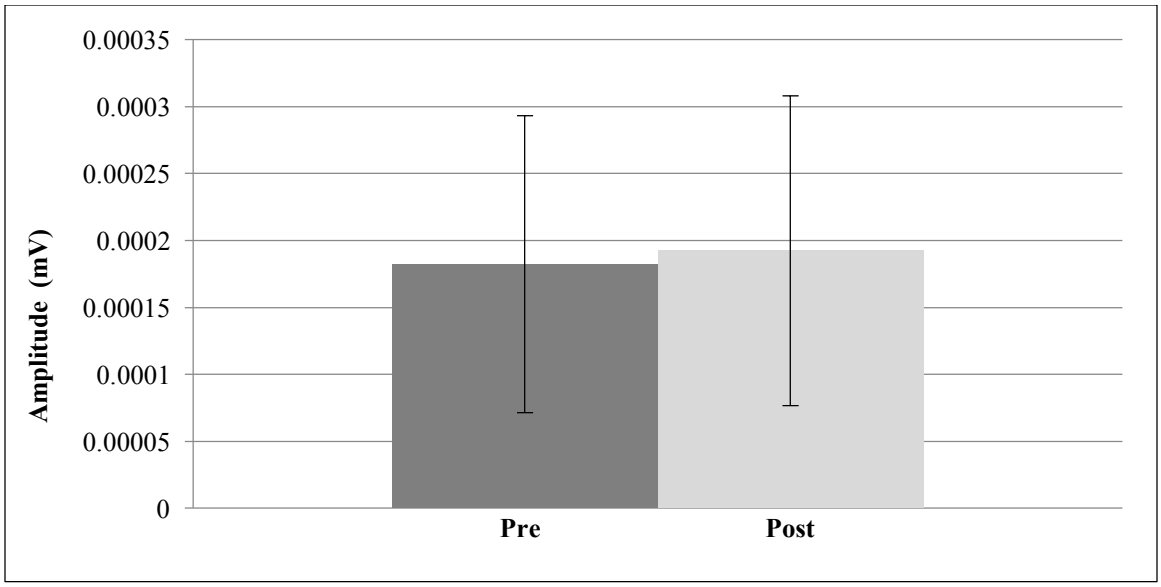

FIGURE 3.7. EMG Amplitude of the Vastus Medialis. Values are mean ± standard deviations.

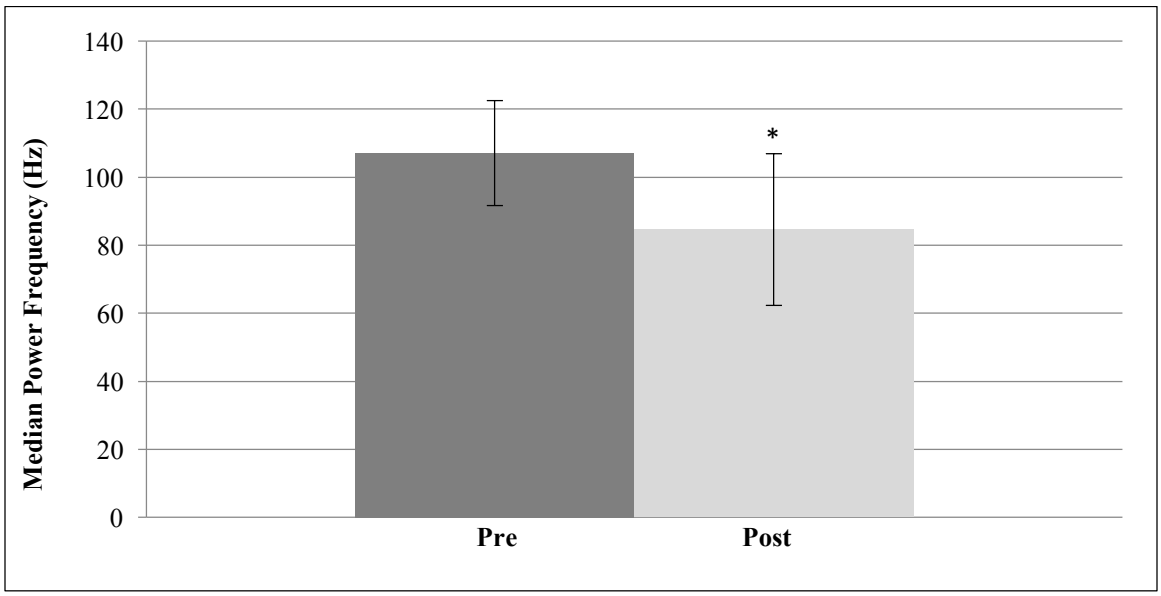

FIGURE 3.8. EMG Median Frequency of the Vastus Lateralis. Values are mean ± standard deviations * Post-exercise was different from baseline testing, $P < 0.03$.

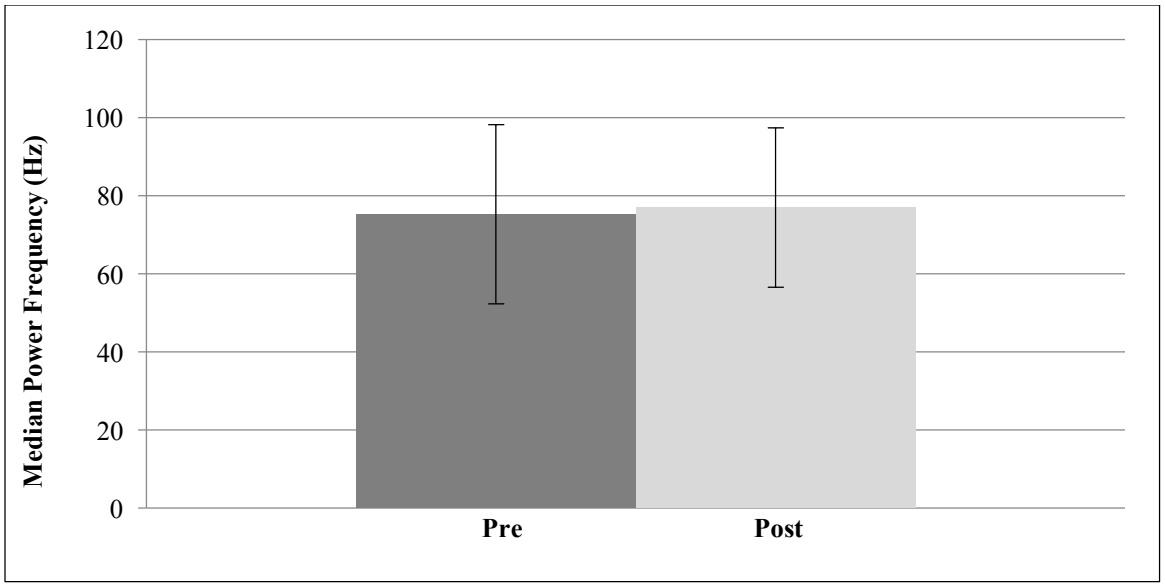

FIGURE 3.9. EMG Median Frequency of the Rectus Femoris. Values are mean ± standard deviations.

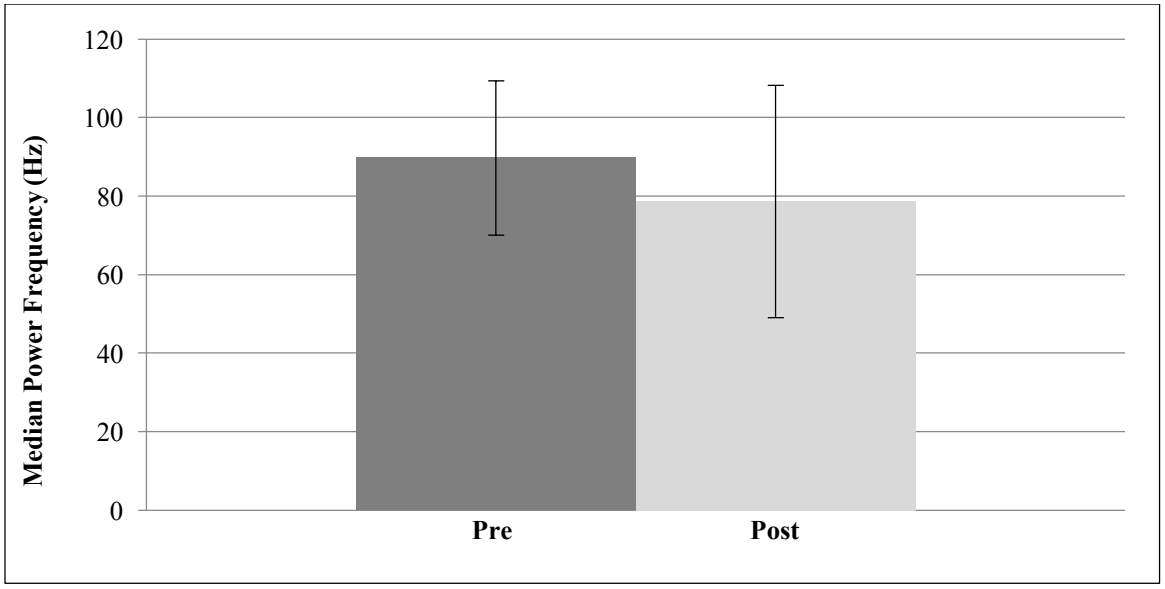

FIGURE 3.10. EMG Median Frequency of the Vastus Medialis. Values are mean ± standard deviations.

3.4 Discussion

The main findings of this investigation were that PF and RFD significantly decreased following one high-intensity exercise protocol. Additionally, the VL had a significant pre-post decrease in MDF following one high-intensity exercise protocol. The

pre-post MDF of the VM and RF were not statistically different following exercise. Lastly, pre-post EMG amplitude did not change significantly across all three muscles.

Isometric strength, absolute strength, and power production are all important components to athletic performance (17, 35, 50, 52-54). Therefore, any loss in strength and/or power would be unfavorable, especially during athletic competitions (26, 32). Along with high force production, it is also functionally important to be able to quickly develop force when time is limited (60), such as when re-stabilizing the body following a loss of balance or when explosively generating power during sport activities (1, 11, 56, 59). The exact mechanisms of explosive force production are unknown, however, it is thought that explosive force production is influenced by several neural, mechanical and architectural parameters, such as agonist neural drive, maximal strength, fascial length, fascial angle, and muscle-tendon unit (MTU) stiffness (4, 8, 9, 11). Nonetheless, this investigation was able to report that fatigue took place as made evident by the decreases in both PF and RFD following exercise. Despite finding in this study of a reduction in force production, the mechanisms behind a loss in force remain unclear and cannot be fully elucidated within this investigation.

The majority of research investigating changes in EMG activation patterns have reported EMG amplitudes to increase following a fatiguing exercise protocol (30, 43, 58). An increase in EMG amplitude following a fatiguing activity has been thought to indicate greater motor unit recruitment, firing frequency, and/or synchronization (45, 57). This form of neural adaption is beneficial because adjustments are being made to compensate for the deficiency in contractility resulting from impairment of the fatigued motor units (16, 37). Although not significant, there was a trend of increasing EMG amplitude for

both the RF and VM. Interest in the neuromuscular activation of the three superficial quadriceps (QF) muscles during fatiguing tasks have been studied for over two decades (13, 14, 27, 28, 43, 58), however, authors have argued that the RF is more fatigable compared to the other QF muscles during knee extension movements, however, this may depend on the type of contraction (isometric, isotonic, or isokinetic) (3, 27, 46). There is no agreement as to why the RF is more fatigable, but one explanation could be that out the four QF muscles, the RF is the only muscle that is biarticulate.

The current investigation was able to report a decrease in the MDF of the VL. A decrease in the MDF is of importance because it represents a shift in the power spectrum to lower frequencies (21, 22), and has been attributed to decreases in action potential conduction velocity (10), changes in action potential shape (21), and reduced relaxation rates of muscles (31). Additionally, a decrease in the relaxation rate of the muscles could be attributed to build-up of metabolic by-products, such as an increased inorganic phosphate (Pi) accumulation, decreased intramuscular pH, or altered sarcolemmal ion gradient (10). This study's findings are in accordance with others that reported a decrease in the MDF (2, 22). Although not measured, the buildup of metabolic by-products may have resulted in a decrease MDF of the VL. Therefore, incorporating the buildup of metabolic by-products alongside changes in MDF warrants further investigation.

Lastly, the metabolic capacity of the fiber types found within the QF muscles could have impacted the EMG activation patterns following exercise (2, 48). Johnson et al. and Edgerton et al. have shown that across the four QF muscles, type II fibers range from 53 to 71% (15, 23). Shifts in frequency are influenced by muscle membrane properties and fiber types (18) (29), with lower values being reported for the VM

compared to the VL and RF (39, 42, 44). Although slow-twitch fibers are deemed as more fatigue resistant, it is not known whether or not slow-twitch fibers are able to withstand metabolic by-product buildup. On the other hand, the same can be said for fast-twitch fibers. Once again, the decrease in muscle activation could be explained by an increase in metabolic by-product buildup. However, this cannot be concluded with certainty on account that no metabolic by-products were investigated within this project.

In conclusion, the present investigation reported performance variables such as PF and RFD to decrease following a high-intensity exercise protocol. The decrease in force production could partly be explained by the changes in EMG characteristics as seen by the decrease in the MDF of the VL. What cannot be determined at this point is whether or not any metabolic by-product buildup had an impact upon the nervous system and the subsequent EMG activation patterns. Future studies should aim to investigate if any relationship exists between metabolic by-product buildup and changes in neural activity.

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CHAPTER 4. IMPACT OF ASPARTATE AND SODIUM BICARBONATE SUPPLEMENTATION UPON CENTRAL AND PERIPHERAL FATIGUE

4.1 Introduction

Researchers have been trying to diminish exercise-induced hyperammonaemia (EIH) for over 50 years, with the first studies dating to Professor Henri Laborit's laboratory (28, 60). Ammonia has been known to hasten fatigue, with high accumulations arising during exercise from the deamination of adenosine monophosphate (AMP) to inositol monophosphate (IMP) via AMP deaminase (42). Briefly, when adenosine triphosphate (ATP) consumption exceeds supply, the ATP/ADP (adenosine diphosphate) ratio decreases. At this point, myokinase will transfer one energy-rich phosphate group from one ADP to another, forming one ATP and one AMP. Once AMP begins to accumulate, AMP deaminase will convert AMP to IMP and ammonia (22). The deamination of AMP is the main source of ammonia during high-intensity exercise (26). On the other hand, during prolonged submaximal exercise, the catabolism of branched chain amino acids (e.g., leucine, isoleucine, and valine) serves as the main producer of ammonia (55, 65). Differences in ammonia production and AMP deaminase activity have been shown to exist in different muscle fiber types (39). Skeletal muscle fibers that are mitochondria-rich seem to produce less ammonia during exercise compared to skeletal muscle fibers that are mitochondria-poor (39).

Laborit was the first to utilize aspartate supplementation to attenuate EIH and reported an increase in endurance within swimming rats (28, 29, 60). The rationale for using aspartate during exercise was its potential transformation to oxaloacetate by aspartate transaminase, followed then by its subsequent ability to be used in the Krebs

cycle (60). Additionally, aspartate has also been shown to be involved in the malate-aspartate shuttle by arriving at the mitochondria as oxaloacetate via a series of steps involving nicotinamide-adenine dinucleotide (NAD) and the reduced form of NAD (NADH). Lastly, aspartate is involved in the urea cycle by combining with citrulline to form argininosuccinate, which is then converted into arginine and fumarate. From here, fumarate is then transported into the mitochondria to be oxidized in the Krebs cycle to form oxaloacetate (60).

Unfortunately, there have been limited investigations examining the impact of aspartate on either endurance or resistance exercise. Investigations have shown that when exercising at intensities between 60-75% VO_{2max}, endurance times improved by 50.5% (1), 22.2% (52), and 15.7% (64) when supplementing with aspartate. However, Maughan et al. reported no improvement in endurance performance with aspartate supplementation (36). Contrary to endurance protocols and aspartate supplementation, studies utilizing resistance training and aspartate supplementation have been less conclusive (14, 19, 59, 61). Along with ammonia accumulation, research has shown that metabolic acidosis contributes to fatigue during high-intensity exercise (11, 13, 38, 47). Due to the increase in metabolic acidosis, alkalizing substances have been investigated to blunt the rise in acidosis since the 1930s (16). Sodium bicarbonate ($NaHCO_3$) has been one ergogenic aid that has been effective in blunting the rise of metabolic acidosis by increasing the level of bicarbonate (HCO_3^-). Sodium bicarbonate is a natural buffer that works by accepting a proton to form carbonic acid in the blood (43), and supplementation with $NaHCO_3$ has been shown to promote a greater extracellular efflux of H+ and lactate (53, 62, 67). Oral administration of $NaHCO_3$ will result in roughly a 5-6 mmol/l increase in extracellular

blood HCO_3^- with a corresponding shift in extracellular pH from 7.4 to ~7.5 (54). Additionally, $NaHCO_3$ supplementation appears to be most effective when ingested at doses between 0.2 and 0.3 g/kg body weight, with ingestion being administered between 60 and 120 minutes prior to exercise (38). Sodium bicarbonate supplementation has shown to be effective in sprint exercise, with results showing an increased time to failure (4, 8, 13, 30, 38, 43, 63).

The effects of metabolic acidosis and $NaHCO_3$ supplementation upon electromyography (EMG) amplitude have been investigated, however, there is limited work investigating the changes amongst the EMG median frequency (MDF) following $NaHCO_3$ supplementation. Additionally, the impact ammonia has upon the nervous system during exercise is relatively unknown, with mixed results being reported following aspartate supplementation. Thus, any relationships between blood ammonia production and/or metabolic acidosis upon these EMG characteristics are unknown. Additionally, the combined effects of the two supplements have not been investigated. Therefore, the purpose of this investigation was to determine if a relationship existed between exercise-induced metabolic by-product accumulation and EMG characteristics in trained men following aspartate and $NaHCO_3$ supplementation when administered independently and combined. It was hypothesized that ammonia concentrations would be lowered following supplementation of aspartate and $NaHCO_3$. Additionally, it was hypothesized that force production, RFD, and EMG characteristics would not decrease as much with supplementation of aspartate and $NaHCO_3$.

4.2 Methods

4.2.1 Experimental Overview

Five days of testing, each separated by one week, were conducted to investigate the changes in muscle contractile characteristics within healthy, exercise trained males. Additionally, two supplements (aspartate and $NaHCO_3$) were administered to help blunt the rise of ammonia and metabolic acidosis, respectively. The first testing day was a familiarization session, while the remaining four days were considered the testing sessions. For each day of testing, a pre and post-exercise isometric mid-thigh pull test with EMG analysis were performed to measure peak force production (PF), rate of force development (RFD), and EMG characteristics.

4.2.2 Participants

Twelve participants were recruited for the study (age: 21.9±1.5 y; height: 1.77±.068 m; & weight: 82.4±8.4 kg); however, one had to be removed from data analysis due to gastrointestinal tract complications resulting from the supplements. The remaining 11 participants completed all aspects of the study. All participants had a minimum of six months of current resistance training experience, and participants were recruited from activity courses at Louisiana State University, as well as from gyms located in the Baton Rouge area. The study was approved by the Institutional Review Board at Louisiana State University. The purpose, potential risks, and benefits of participation in this study were fully explained to each participant, and signed consent was obtained before any assessments were completed. Participants were excluded from the study if they reported a history of any knee and/or back injuries. In addition, all

potential participants completed a Physical Activity Readiness Questionnaire (PAR-Q) before inclusion in the study. Only those individuals who answered “no” to all PAR-Q questions were used as research participants.

4.2.3 Isometric Mid-Thigh Pull Test

The isometric mid-thigh pull test was performed to measure PF and RFD (21, 37, 56). The isometric mid-thigh pull test is considered one of the best methods to assess strength due to its high correlations to 1RM strength (21). Vertical ground reaction force data were collected at 1800 Hz using an AMTI force platform. Force plate data were low pass filtered offline at 50 Hz using a second order, zero-lag Butterworth filter. Participants were instructed to pull on an immovable bar (performed in a power rack with pins) as quickly as possible and to maintain such effort for five seconds to ensure maximal force production. Specific instructions were to pull on the bar as “hard and fast as possible” in order to produce optimal results for recording PF and RFD (6, 21, 50). Participants performed three sets of five seconds with a three-minute rest before and after a high-intensity exercise protocol. The highest values at the respected time points for both PF and RFD were used for later analysis. Bar height was adjusted accordingly so that both the hip and knee angles were between 130 and 150 degrees, respectively. Participants were instructed to maintain these angles throughout the duration of the trial. The RFD was determined using the slope of the force-time profile during the time period starting at 55 ms from the onset of muscle contraction and extended until 250 ms. Peak force was also obtained during the mid-thigh pull, and was defined as the highest value produced during the five second isometric contraction.

4.2.4 Electromyography (EMG)

Muscle EMG data were collected from the vastus lateralis (VL) and rectus femoris (RF) during the isometric mid-thigh pull test at 1800 Hz using a 16 channel, MA300 system (Motion Lab Systems, Baton Rouge, LA). The electrode placement for each recorded muscle was determined using Surface EMG Non-Invasive Assessment of Muscles (SENIAM) guidelines. To prepare and clean the desired EMG electrode placement locations, participants were shaved and cleansed with an antiseptic alcoholic wipe. The electrodes used were self-contained Ag-AgCl electrodes (Model: MA-411, Motion Lab Systems, Baton Rouge, LA). All electrodes were secured over the muscle belly in line with the muscle fibers using adhesive tape, and then were wrapped using a self-adhesive elastic sports bandages.

Electromyography recordings were bandwidth filtered at 10-750Hz. A Fast Fourier Transform (FFT) was applied to the EMG data during the 250 ms RFD time period, and a one second time period surrounding PF (one-half second before PF and one-half second following PF). The MDF was defined as the frequency that divided the area under the power spectrum into two equal parts. The EMG amplitude was expressed as the root mean square values (RMS), and was also analyzed during the 250 ms RFD time period, and the same one second time period surrounding PF. An minimum of two out of three trials for both pre- and post-exercise were averaged in analysis for both MDF and amplitude. Any EMG signal with a presence of low frequency noise or a non-responsive signal was removed.

4.2.5 High-Intensity Exercise Protocol

Each participant performed five high-intensity exercise sessions, with each session consisting of four exercises: barbell thrusters, squat jumps, lunge jumps, and forward jumps. These exercises were selected due to the high contribution of the quadriceps muscles throughout each movement. The first exercise session was a familiarization session, and the subsequent four sessions were considered the testing sessions. Exercises began immediately following the pre isometric mid-thigh pull test. Participants were instructed to perform the exercises in the order listed above with the completion of all four exercise and rest times between each exercise being designated as a round. Each participant completed three rounds of exercises with a one min rest between each round. Upon completion of the three rounds, the post isometric mid-thigh pull test was performed.

The first testing session consisted of performing each exercise for a period of 20 sec with a 30 sec rest between each exercise. The subsequent three testing sessions were equated for total amount of work that was performed in the first testing sessions by having each participant perform the same number of repetitions for each exercise as they did in the first session. Time to complete repetitions for each exercise was used in data analysis, and is described as total time of work. Rest times of 30 sec between each exercise and one min between each round were the same for all four testing sessions. Rate of perceived exertion (RPE) was recorded using the Borg scale following each exercise, with the final value provided by the participant at the end of each session used for subsequent data analysis.

The barbell thruster was performed by first completing a front squat with an unloaded bar (45 lb.), and then then while the participant stood from the squat, the bar was pushed overhead, ending with the bar balanced above the head and over the participant's heels. The jump squats, lunge jumps, and forward jumps were all performed unloaded. The jump squats were performed by lowering the body downward into a regular squat, followed by jumping up explosively. When the participant landed, they lowered their body back into the squat position which completed one rep. The lunge jumps were performed by stepping forward with one leg while simultaneously lifting up onto the ball of the back foot. Participants then jumped and switched legs, ending with the opposite leg in the forward position. The forward jumps were performed by jumping forward over a hurdle that was measured at a height equivalent to each participant's patella.

4.2.6 Supplementation

Two supplements were investigated within this project: aspartate and sodium bicarbonate ($NaHCO_3$). For each experimental condition, the participants were given two bags of a powder mixture consisting of one of the following: placebo (PLA), 30 g of a sports drink powder (Gatorade) (control); aspartate (ASP), 30 g of the sports drink powder and 12.5 g of L-Aspartate powder (Source Naturals-ASP); $NaHCO_3$ (SBC), 30 g of the sports drink powder and 0.3g/kg body mass $NaHCO_3$ powder (Arm & Hammer); and combination (CBO), 30 g of the sports drink powder and both doses of L-Aspartate powder and $NaHCO_3$ powder. All subjects received all conditions during this project. Participants were instructed to mix each condition's powder mixture into ~20 oz. of water for each visit. For each testing session, participants were given instructions to consume

one packet 23 hrs. prior to visit. The second packet was to be consumed one hr. before the test session. A one-week "wash-out" period was performed for each treatment. Each participant received the placebo as the first condition, with the remaining three conditions administered in a randomized order. The placebo was administered first to establish a baseline session volume to be followed in the subsequent testing sessions.

4.2.7 Blood Collection and Biochemistry

Blood samples were collected into lithium-heparinized vacuum tubes via venipuncture. Approximately 9 ml of blood was taken from participants for all testing sessions before and following the high-intensity exercise. Whole blood was immediately centrifuged, then processed for plasma and stored at -30° C until analyzed. Determination of ammonia concentration was measured using a commercially available assay (Sigma Ammonia Assay Kit, Sigma Aldrich), and measured at 340 nm with an Evolution 60S UV-Visible Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA).

4.2.8 Data Analysis

Peak force, RFD, and ammonia concentrations data were analyzed using a four (condition)-by-two (time) repeated measures (RM) analysis of variance (ANOVA). Significant main and interaction effects were further analyzed using Student's *t* test where appropriate. All EMG data, RPE, and time to exercise completion were analyzed using a one-way RM-ANOVA with significant main effects being further analyzed using Student's *t* test where appropriate. Correlations between variables were analyzed using the Pearson product moment correlation coefficient adjusted for inter-individual variability. Statistics were performed in JMP statistical software 12 (SAS Institute Inc.,

Cary, NC). Data are displayed as mean ± standard deviation and significant differences were declared at $P < 0.05$.

4.3 Results

4.3.1 Ammonia

There was a significant time effect (P =0 .004) (Fig. 4.1) for ammonia concentrations from pre- to post-exercise, and interaction effect (P = 0.04) amongst the post values with the aspartate and $NaHCO_3$ having a lower post ammonia concentration compared to the placebo. However, there was no condition effect (P = 0.08) for ammonia concentrations.

4.3.2 Isometric Mid-Thigh Pull

Rate of force development (Fig. 4.2) did show an interaction effect (P = 0.01) with the placebo pre values reporting a higher RFD compared to the aspartate, $NaHCO_3$, and combination. However, there were no condition effects (P = 0.22) or time effects (P = 0.36). There was a condition effect for PF (P = 0.05) (Fig. 4.3), with the aspartate and combination reporting a lower peak force compared to the placebo. However, there were no time effects (P = 0.1) or interaction effects (P = 0.42) for peak force.

4.3.3 Electromyography (EMG)

No statistically significant changes in RFD EMG amplitude were identified for the VL (P = 0.16) (Fig. 4.4), or the RF (P = 0.49) (Fig. 4.5) from pre- to post-exercise. As for the RFD EMG MDF, there was no statistical significance observed for the VL (P =0.25) (Fig. 4.6). A condition effect was observed for the RFD EMG MDF of the RF (P

= 0.01) (Fig. 4.7) with the placebo decreasing significantly from the $NaHCO_3$, and the combination decreasing significantly from the aspartate and $NaHCO_3$ treatments. There were no statistically significant correlations found between changes in ammonia concentrations and changes in RFD ($P = 0.08$), or changes in any EMG characteristics during the RFD time period: EMG RMS of the VL ($P = 0.7$), EMG RMS of the RF ($P = 0.7$), EMG MDF of the VL ($P = 0.8$), or EMG MDF of the RF ($P = 0.2$).

No statistically significant changes in PF EMG amplitude were identified for the VL ($P = 0.7$) (Fig. 4.8) or the RF ($P = 0.75$) (Fig. 4.9). As for PF EMG MDF, the VL (Fig. 4.10) was not statistically significant different across the four supplements ($P = 0.54$). A condition effect was found for the PF EMG MDF of the RF ($P = 0.04$), with the placebo decreasing significantly from the $NaHCO_3$, and the combination decreasing significantly from both aspartate and $NaHCO_3$ (Fig. 4.11). There were no statistically significant correlations found between changes in ammonia concentrations and changes in PF ($P = .7$), or changes in any EMG characteristics during the PF time period: EMG RMS of the VL ($P = .7$), EMG RMS of the RF ($P = 0.6$), EMG MDF of the VL ($P = .7$), or EMG MDF of the RL ($P = .3$).

4.3.4 Work Intensity

There was no statistical significance reported for RPE between the four supplements ($P = 0.41$) (Fig. 4.12). Total time of work (TTW) was statistically reduced ($P < 0\ .0001$) (Fig. 4.13) with the aspartate, $NaHCO_3$, and combination trails compared to the placebo. However, when only the aspartate, $NaHCO_3$, and combination treatments were analyzed together, no statistical significance was observed ($P = 0.56$).

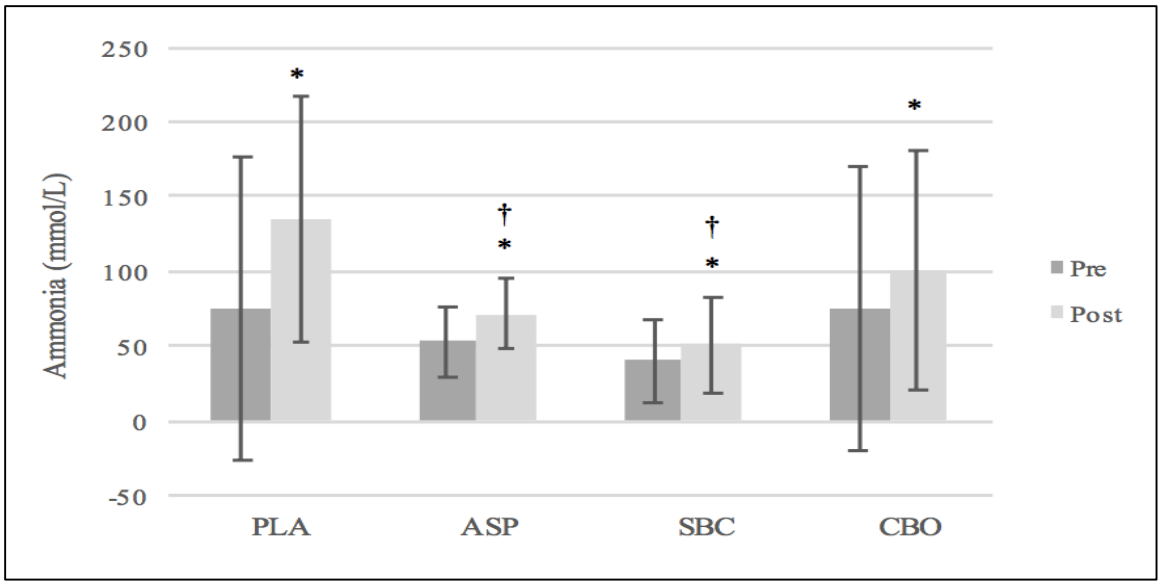

FIGURE 4.1. Ammonia Concentrations. Values are mean ± standard deviations. * Time effect from pre- to post-exercise (P = .004); † Interaction (time x supplementation treatment) effect (P = .04) between post values following supplementation with either placebo (PLA), aspartate (ASP), $NaHCO_3$ (SBC) or ASP+ $NaHCO_3$ combined (CBO).

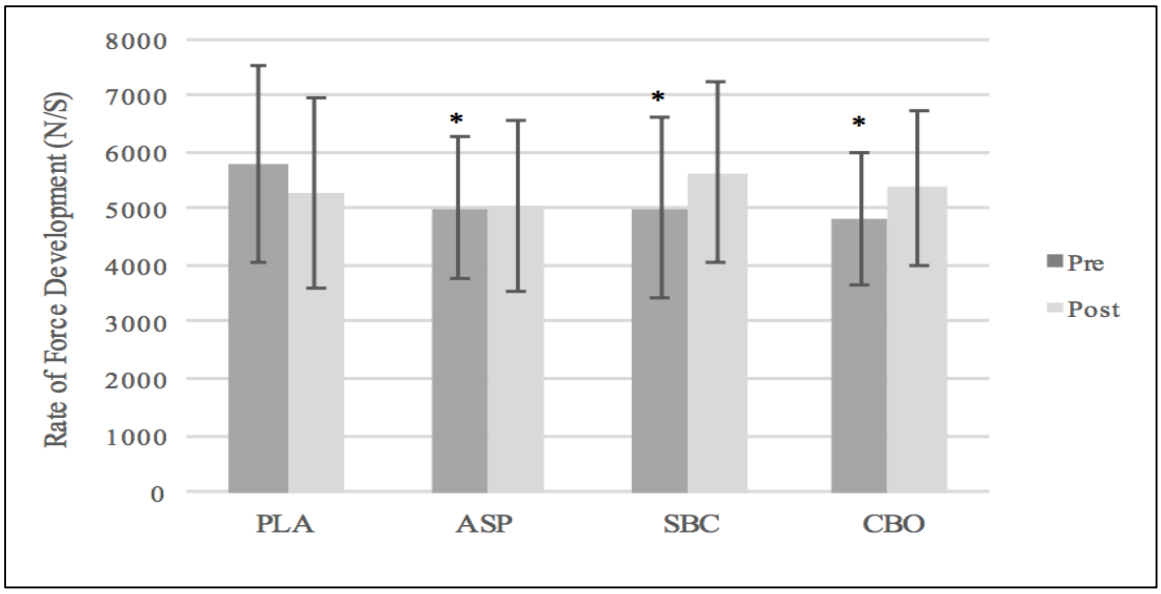

FIGURE 4.2. Rate of Force Development. Values are mean ± standard deviations. *Values significantly (P = .01) higher at pre-exercise for the placebo (PLA) compared to the other 3 pre-exercise time points.

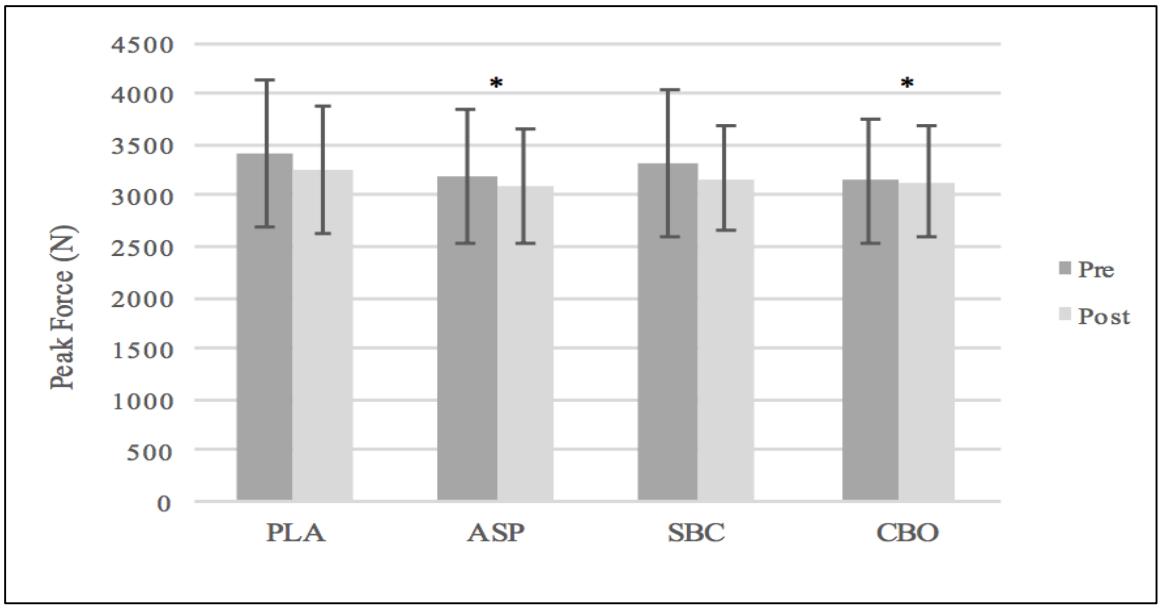

FIGURE 4.3. Peak Force. Values are mean ± standard deviations. *Values are higher for the placebo (PLA) compared to the aspartate (ASP) and combination (CBO) treatments.

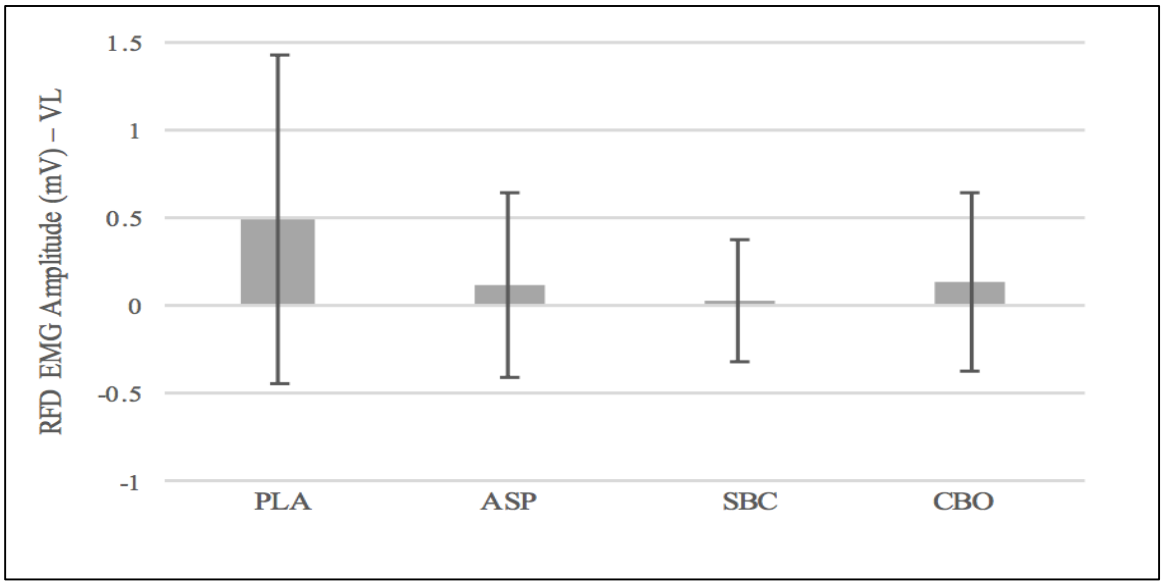

FIGURE 4.4. RFD EMG Amplitude of the Vastus Lateralis. Values are mean ± standard deviations.

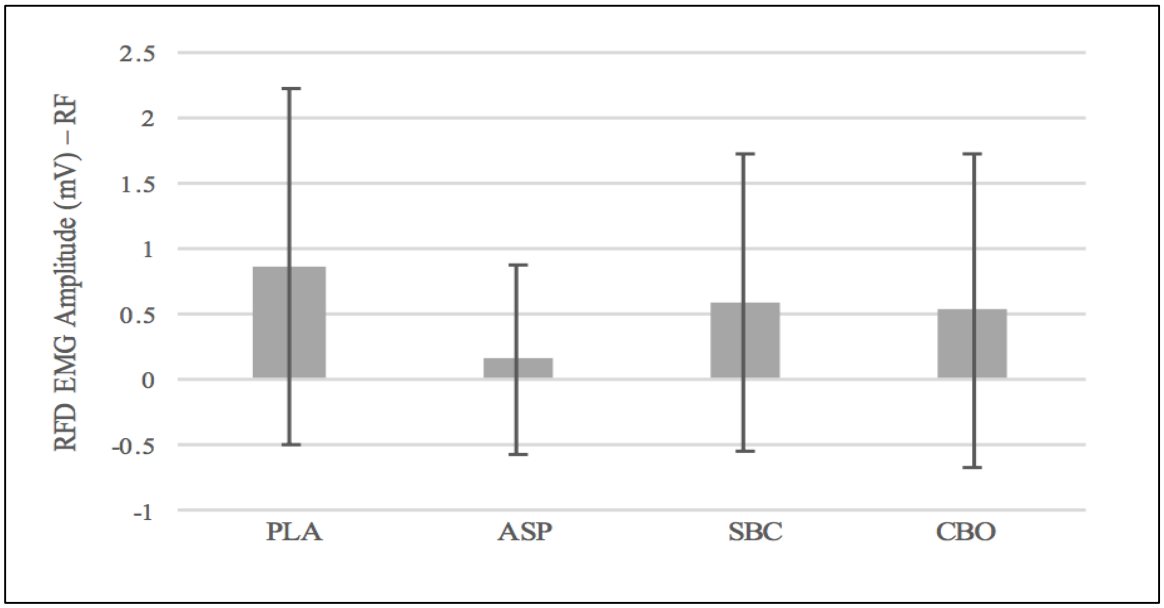

FIGURE 4.5. RFD EMG Amplitude of the of the Rectus Femoris. Values are mean ± standard deviations.

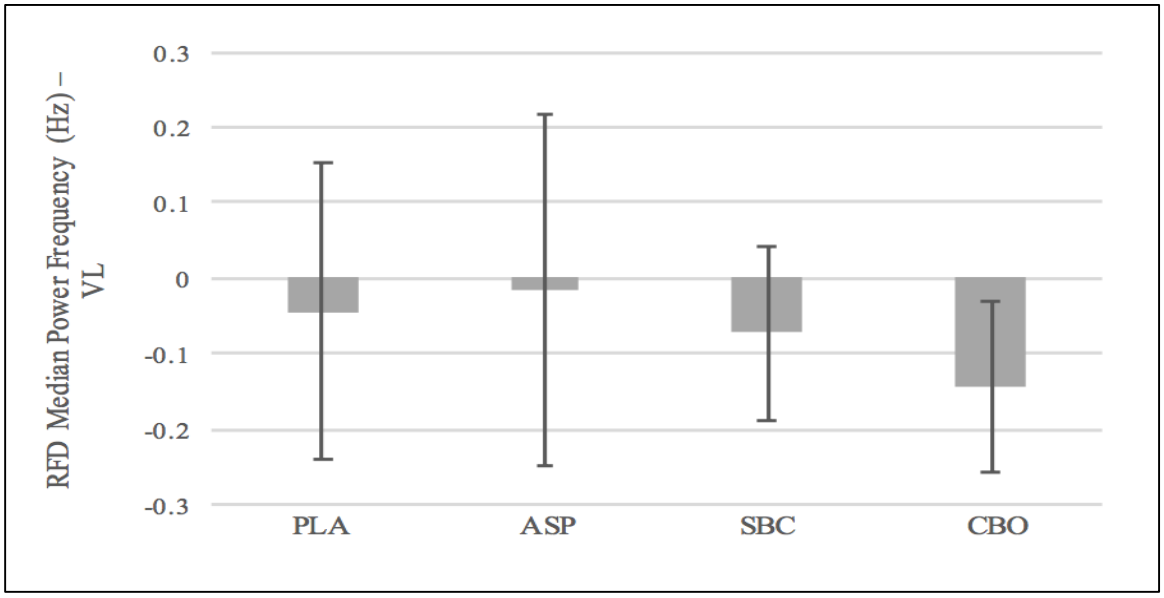

FIGURE 4.6. RFD EMG Median Frequency of the Vastus Lateralis. Values are mean ± standard deviations.

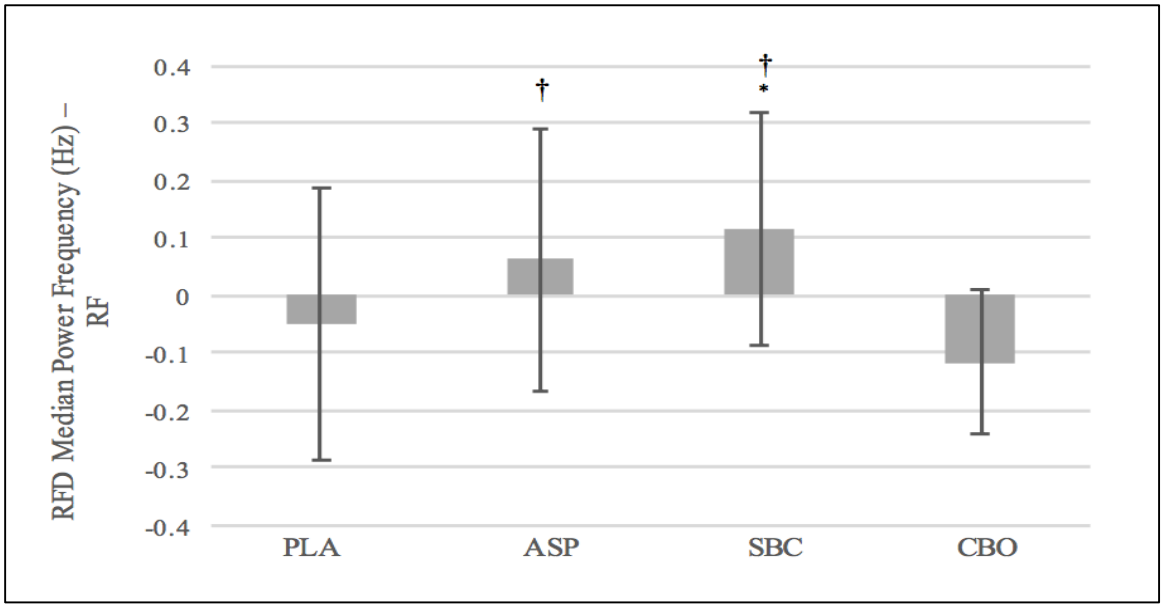

FIGURE 4.7. RFD EMG Median Frequency of the Rectus Femoris. Values are mean ± standard deviations. * Values differ significantly ($P = .01$) for the placebo (PLA) treatment compared to $NaHCO_3$ (SBC). † Values differ significantly for combination (CBO) treatment compared to aspartate (ASP) & sodium bicarbonate (SBC).

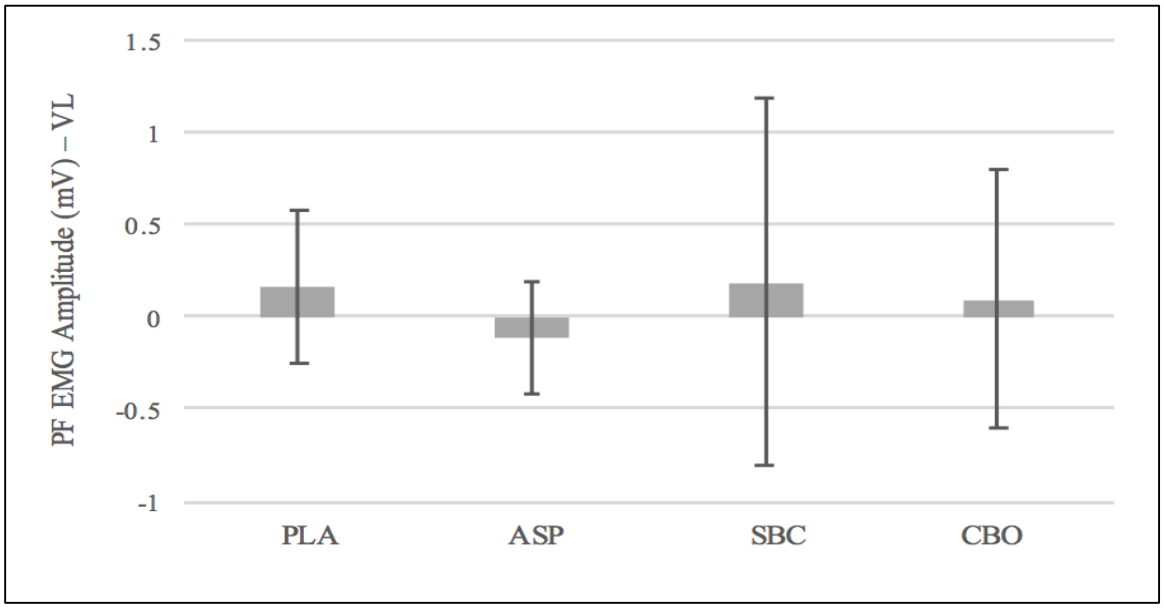

FIGURE 4.8. PF EMG Amplitude of the Vastus Lateralis. Values are mean ± standard deviations.

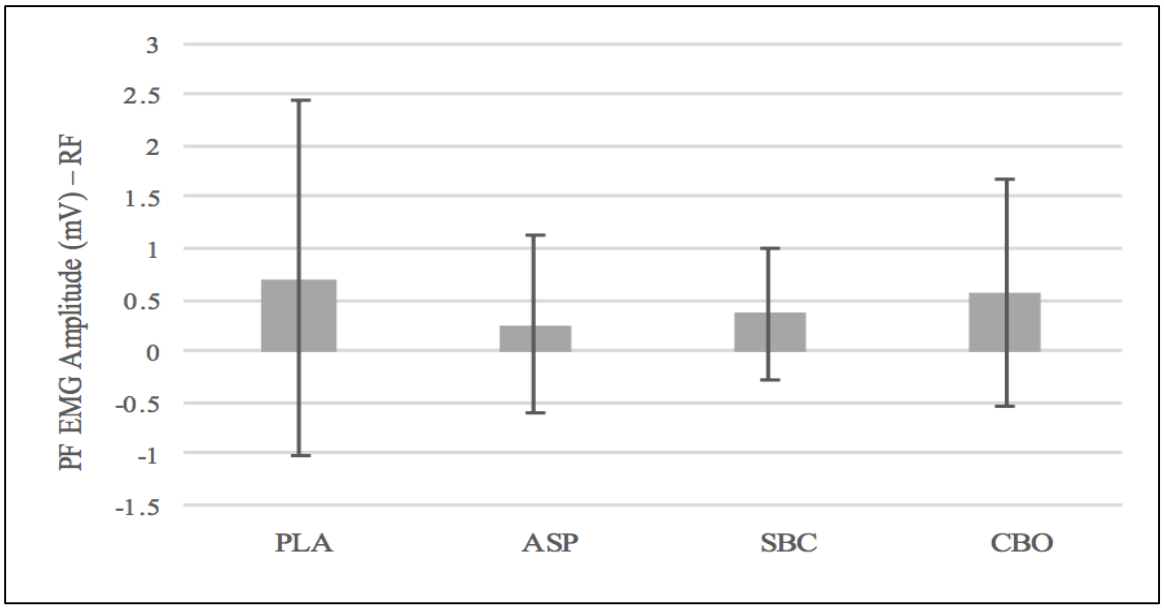

FIGURE 4.9. PF EMG Amplitude of the Rectus Femoris. Values are mean ± standard deviations.

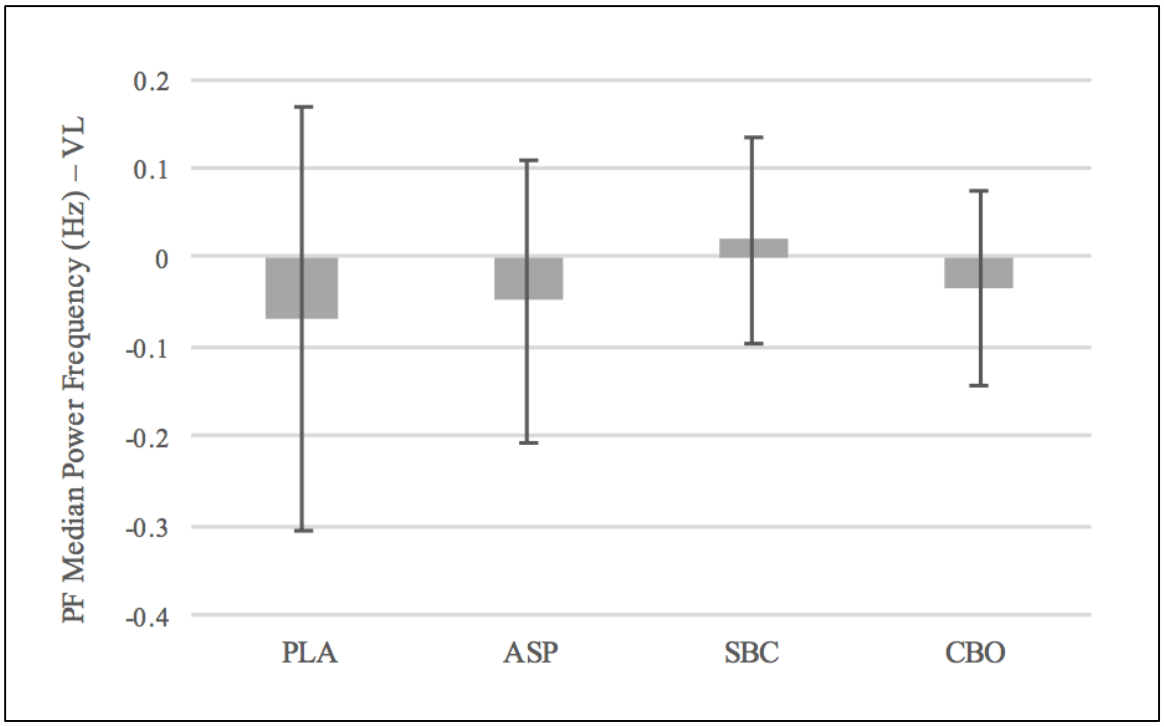

FIGURE 4.10. PF EMG Median Frequency of the Vastus Lateralis. Values are mean ± standard deviations.

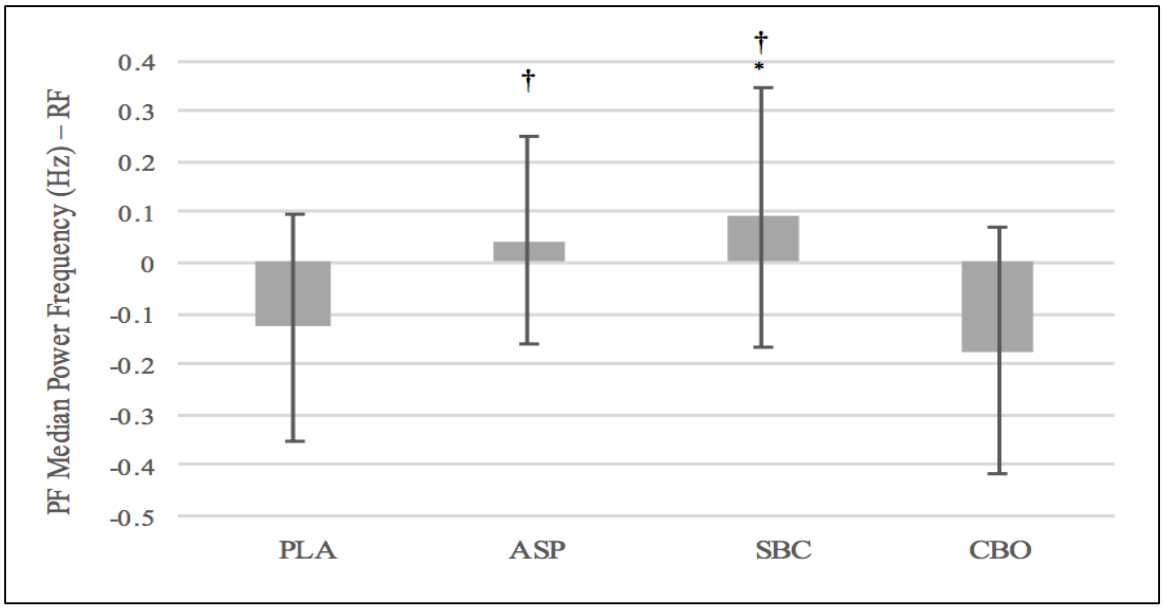

FIGURE 4.11. PF EMG Median Fequency of the Rectus Femoris. Values are mean ± standard deviations. * Values differ significantly ($P = .04$) for the placebo (PLA) treatment compared to sodium bicarbonate (SBC). † Values differ significantly ($P = .04$) for combination (CBO) treatment compared to aspartate (ASP) & $NaHCO_3$ (SBC).

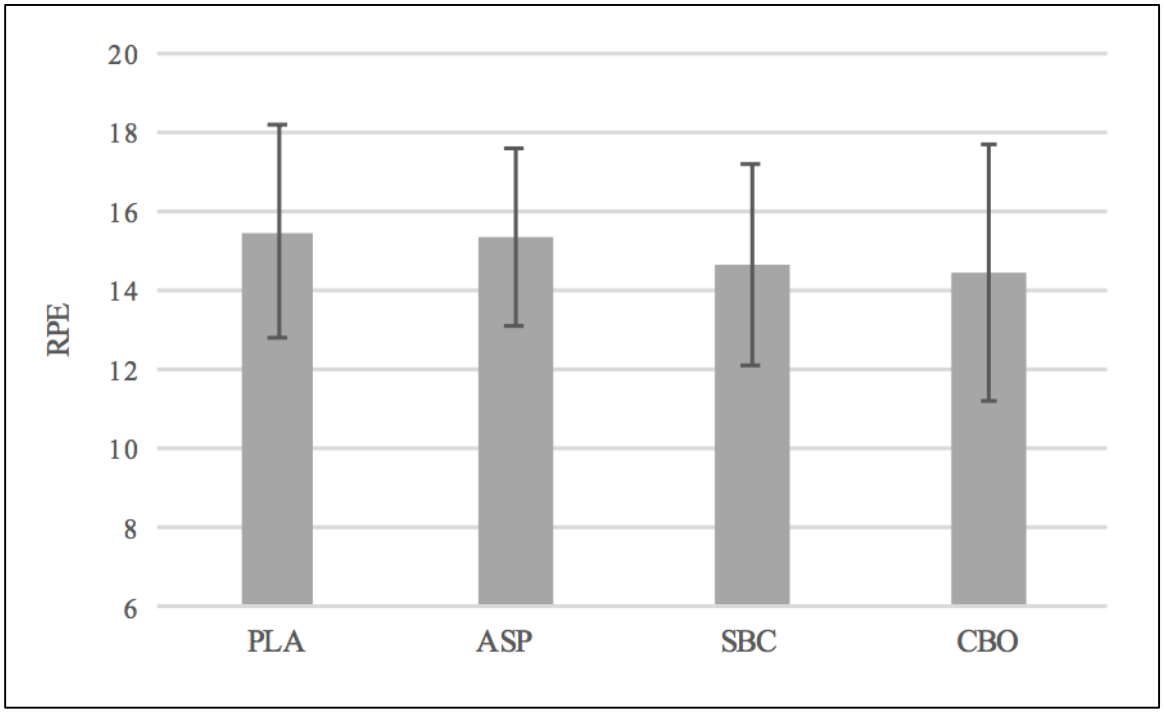

FIGURE 4.12. Rate of Percieved Exertion. Values are mean ± standard deviations.

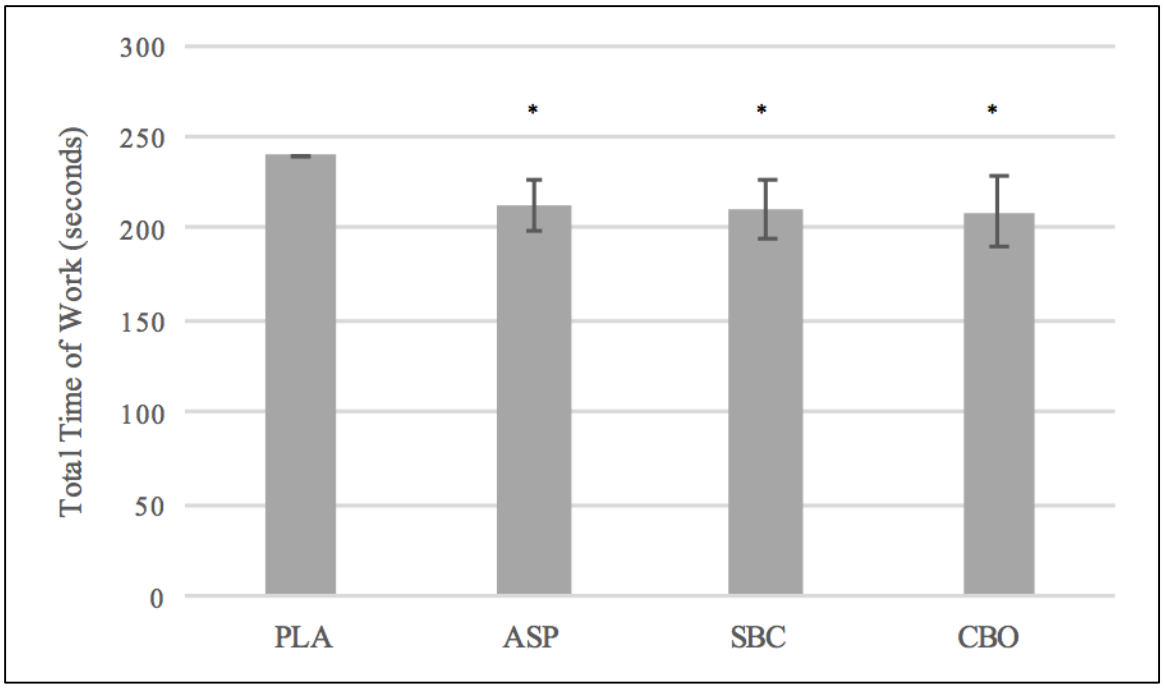

FIGURE 4.13. Total Time of Work in seconds. Values are mean ± standard deviations. *Values differ significantly ($P < .0001$) for the placebo (PLA) treatment compared to the aspartate (ASP), $NaHCO_3$ (SBC), and combination (CBO) treatments.

4.4 Discussion

The purpose of this investigation was to determine if a relationship existed between exercise-induced metabolic by-product accumulation and EMG characteristics in trained men following aspartate and $NaHCO_3$ supplementation when administered independently and combined. Participants were supplemented with aspartate and $NaHCO_3$ to determine if reducing either ammonia buildup (aspartate) or acidosis ($NaHCO_3$) would help to indicate this relationship between metabolic by-products and EMG characteristics. Results showed that ammonia values were raised significantly from pre- to post-exercise across conditions, with the placebo having the greatest increase in ammonia concentration following exercise. Lower pre-to-post exercise peak force values were observed with acute supplementation of aspartate and the combination of aspartate

and $NaHCO_3$ compared to the placebo. Conversely, only the pre values were statistically significant with RFD across all four supplements, with the placebo reporting the highest pre value compared to the other three conditions. The MDF of the RF did decrease significantly during both the RDF and PF time periods No statistical significance was found for the EMG amplitude for either the VL or the RF. This was the first study that sought to determine if a relationship was apparent among changes in MDF and blood ammonia concentrations. Based on the findings of this study, no relationship can be determined between metabolic by-products and changes in EMG characteristics.

The current investigation's exercise protocol was successful in elevating ammonia concentrations, with the placebo treatment having the greatest increase in ammonia concentrations compared to the aspartate and $NaHCO_3$ supplements. The results within the current investigation are in accordance with others who have reported a significant rise in ammonia post-exercise (5, 10, 48). Additionally, the findings are in accordance with researchers who found aspartate to be effective in lowering ammonia concentrations post-exercise (1, 52, 64). However, our findings contradict others that have reported no increase in endurance performance or strength with aspartate supplementation (14, 19, 36, 59, 61). For instance, Maughan et al. had participants cycle at 75% VO_{2max} till exhaustion, and reported no difference in work capacity when compared to a placebo (36). Triplett et al. reported that aspartate supplementation was not effective in reducing ammonia concentrations when the back squat was performed at five sets of ten repetitions with heavy loads, and concluded that the intensity of the back squat may have been too great for aspartate to reduce ammonia concentrations. The current protocol was based on the findings of Rogatzki et al. who reported ammonia to rise greater when the back squat

exercise was performed with more repetitions, low loads, and shorter rest times (48). Increases in ammonia result when ATP and PCr decrease. At this point, the cell will begin to produce ATP by converting two ADPs into an ATP and AMP via the enzyme myokinase. Once AMP builds up, it will then be converted into IMP and ammonia. It is believed that aspartate is able to scavenge for ammonia by helping to convert ammonia into Krebs cycle intermediates. However, the exact mechanism behind aspartate's ability to lower ammonia concentrations remains unclear.

Ammonia accumulation during exercise should be of particular interest to exercise physiologists because a high concentration of ammonia has been known to hasten muscular fatigue (12, 42). Yuan et al. and Kantanista el al. have even suggested that ammonia is a better biomarker than lactate to monitor for changes sport performance (26, 66). A dose dependent relationship exists between plasma ammonia concentrations and exercise intensity whereby as intensity rapidly increases above 60% of VO_{2max}, ammonia production increases as well (5, 10). Ammonia released from the muscle during exercise has been said to impact local fatigue via a change in membrane excitability, and the additional ammonia released into the blood can have direct access to brain tissue where there is a relatively free exchange across the blood-brain barrier (33). During times where blood ammonia is elevated, the brain can extract ~45% of the ammonia in circulation (31), and once inside the brain, ammonia has been described to have negative effects upon the central nervous system by altering neurotransmitters release (42). Only one study has investigated the role ammonia plays upon mechanical functions, and was performed by treating frog sartorius muscles with both ammonium ions and sodium ions (23). It was reported that when muscles were treated with sodium ions, twitch tension

was potentiated. However, as the sodium ions were switched with ammonium ions, the twitch tension slowly decreased. Therefore, Heald postulated that one site of impact ammonia may have had upon was the surface membrane of the muscle rather than the sarcoplasmic reticulum (23). The proposed theory of ammonia accumulation on membrane excitability could explain some of our findings, however, more research needs to be conducted in order to validate such proposals.

One interesting finding from the current investigation was that when supplemented with $NaHCO_3$ alone or in combinations with aspartate, RFD values increased from pre- to post-exercise. A full explanation to these findings cannot be elucidated at this time. However, since aspartate was successful in lowering ammonia, the conclusion of Heald leads to our speculation that when ammonia is reduced, membrane excitability is increased (23). Both motor unit recruitment and firing frequency help to modulate force production (7), therefore, force production would be unaffected if either motor unit recruitment or firing frequency were unaffected. However, due to the limitations of the current investigation, motor unit recruitment and firing frequency were not measured. Therefore, no conclusion is able to be made regarding if force was altered due to a change in the motor unit.

Another finding from the current investigation was a decrease in MDF of the RF during both the RFD and PF time periods. The placebo or combination treatments had the greatest decrease in MDF compared to the aspartate and $NaHCO_3$ supplements. Decreases in MDF have been attributed to decreases in action potential conduction velocity (9), changes in action potential shape (24), and reduced relaxation rates of muscles (34). Although beyond the scope of this investigation, it can be speculated that

changes in MDF could be a result of decreased Na^+/K^+ pump activity. Both the nerve and muscle require ATP to run the Na^+/K^+ pumps, and as ATP decreases, these pumps will in turn begin to operate at a slower rate. Hence, an increase in ammonia will lower the amount of ATP, which could lead to the decrease in Na^+/K^+ pump activity (15). We cannot say for certain that this is the exact mechanism, however, conclusions by Heald would indicate our hypothesis is possible due in part to aspartate supplementation improving force production.

The metabolic capacity found within the fiber types of the quadriceps muscles may have also led to the decreases found within the MDF following exercise (2, 51). Johnson et al. (25) and Edgerton et al. (17) have reported that across the four quadriceps muscles, type II fibers range from 53 to 71%. Muscle membrane properties are a major factor leading to shifts in frequency (20, 27), with lower values being reported for the vastus medialis (VM) compared to the VL and RF (41, 44, 45). The decreases in MDF of the RF could be explained by the RF having a greater amount of fast-twitch fibers than the VL, and it has been argued that the RF is more fatigable than the other quadriceps muscles during knee extension movements. There is no agreement as to why the RF if more fatigable, but one explanation could be because it is the only bi-articulate muscle among the four quadriceps muscles (58).

Lastly, there is strong evidence that metabolic-sensitive group III/IV muscle afferent fibers are discharged more frequently in an acidic state (49), and this could influence the descending central drive to the muscle (3, 35). Although not significant, we did notice a trend of increasing EMG amplitude across the four treatments for both PF and RFD, with the placebo treatment seeing the greatest increase. The majority of

research investigating changes in EMG activation patterns have reported EMG amplitudes to increase following a fatiguing exercise protocol (32). An increase in EMG amplitude following a fatiguing activity has been thought to indicate greater motor unit recruitment, firing frequency, and/or synchronization (46, 57). This form of neural adaption is beneficial because adjustments within the nervous system are being made to compensate for the deficiency in contractility resulting from impairment of the fatigued motor units (18, 40). There is a possibility $NaHCO_3$ could have reduced any metabolic acidosis, thus, resulting in less group III/IV muscle afferent fibers discharging. If the above is correct, then further support is supplied for the reason as to why PF and RFD were not affected when supplementing with $NaHCO_3$, either alone or in combination with aspartate.

In conclusion, PF, RFD, and EMG MDF were all negatively impacted with higher ammonia concentrations. Additionally, aspartate supplementation was effective in lowering ammonia concentrations and resulted in a lesser change in force production as well as a decrease in MDF of the RF. No strong relationships can be determined at this point or any mechanisms, therefore, more research is needed. Nonetheless, the current investigation does provide evidence of a connection between what is produced via the metabolic system and the subsequent impact upon the EMG characteristics. Also, this investigation provides support that supplementing with aspartate and sodium bicarbonate may be beneficial during sport or competition where reducing fatigue is of the utmost importance.

4.5 References

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CHAPTER 5. CONCLUSIONS

The purpose of this dissertation was to investigate potential mechanisms that relate to neural fatigue. An additional purpose was to determine if a relationship exists between metabolic by-products produced during exercise and EMG characteristics. Currently, we are unable to make any conclusions of said relationship. Fatigue, whether metabolic or neural, is a complex area, one that gets even more compounded when two systems are merged together. Nonetheless, despite being complex, it is still an important research topic due to its impact upon not only sport and exercise success, but also performing many daily living tasks. The unfortunate fact is that despite the relevance, the interaction between the metabolic and nervous systems is a very understudied area. Thus, it was the goal of this dissertation to begin exploring the interaction between the two systems during times of fatiguing exercise.

The first investigation was conducted to better understand muscle fatigue via EMG amplitude of the M-wave recordings. Although a trend of increasing amplitude following exercise was noticed, we, however, were unable to report any significant changes within EMG amplitude. Despite the non-significant findings, the M-wave still presents itself to be one of the better avenues to investigation fatigue within the muscle and nerve. The reason being that it can demonstrate a failure to maintain neuromuscular propagation at a normal level. If a better understanding of the M-wave can be established, then this can help improve the understanding of the nerve, muscle, neuromuscular junction, and the important role each plays in human movement. There have been good investigations done with the M-wave, however, the mechanisms still remain to be elucidated. At the present, not much has been investigated concerning different metabolic

by-products and changes in M-wave amplitude. Therefore, this is one avenue for future research, and could help better understand how the two systems interact with one another.

The second investigation incorporated performance variables and EMG characteristics, and sought to determine how these variables were altered following one high-intensity exercise session. We were able to report a significant decrease in MDF of the VL. Additionally, we reported a loss in peak force and rate of force development. On the other hand, we weren't able to report a significant change in EMG amplitude of any muscle. The rationale of this study was to gain a better understanding of neural changes following a common exercise regime that is used by both strength and conditioning coaches as well as in the general fitness arena. Additionally, the isometric mid-thigh pull test still remains a good way to measure performance variables for the strong reliability and correlations to strength found throughout the literature.

The third investigation built off the second investigation by incorporating metabolic by-products, and their potential impact upon nervous system failure/fatigue. To investigate the relationship between these by-products and fatigue, this study selected ammonia accumulation and whether or not this accumulation had any impact upon neural failure/fatigue. To prevent ammonia accumulation, aspartate was administered either alone or in conjunction with sodium bicarbonate. We were able to report that ammonia was raised significantly following exercise. Additionally, when supplementing with aspartate, force production and EMG characteristics appeared to be better than when only the placebo was administered. Although we cannot conclude definitively that ammonia had an impact upon the nervous system, our results do show that the supplements may have been effective in limiting metabolic by-products due in part to better performance.

Therefore, research should continue to investigative the impact ammonia has upon fatigue within the nervous system.

Future research should continue to determine if a relationship exists between metabolic by-products and changes in muscle contractile properties. Also, it would be helpful to investigate how motor unit recruitment and firing frequency are altered following an accumulation of metabolic by-products. We were only able to use surface EMG, and thus, not able to determine exactly how individual motor units or firing frequency were altered during times of fatigue or in the presence of metabolic by-product buildup. Surface EMG is a good technique to use within exercise physiology, however, information is limited in that it only provides a global snapshot of what is actually happening within the muscle. Therefore, future research should investigate how metabolic by-product accumulation influences changes in motor unit recruitment and/or firing frequency.

In conclusion, any relationship between metabolic by-products and nervous system failure/fatigue could not be fully determined within the confines of this dissertation. Despite the lack of determining a relationship, this dissertation was able to provide more information into the area of neuromuscular fatigue through the administration of aspartate and sodium bicarbonate. These supplements may prove beneficial for those engaging in sport. At the present time, any mechanisms leading to neuromuscular fatigue still remains unclear, however, this dissertation was able to provide ideas as to where future research should be directed.

APPENDIX 1. GENERAL EXAMINATION

RELATIONSHIP BETWEEN METABOLIC BY-PRODUCTS AND NERVOUS SYSTEM FAILURE/FATIGUE

A review of the literature for the General Examination
Submitted to the graduate faculty of the
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in
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by
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GENERAL EXAMINATION OUTLINE

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CHAPTER 1. GENERAL PHYSIOLOGY

1.1 Introduction

Performance during athletic competition is initially dependent upon both the rate of force development (RFD) and the maximum force produced (182). Ultimately, however, fatigue will set in during competition or exercise, thus, decreasing performance or leading to a loss during competition. Fatigue has generally been defined as an acute impairment of exercise/sport performance that includes both an increase in the perceived effort necessary to exert a desired force or power output, and the inability to produce the desired force or power output (48). The majority of research to date looking at fatigue has focused upon substrate utilization, however, what is relatively unknown is the contribution the nervous system has upon fatigue, specifically, within the peripheral nervous system (PNS). Within the PNS, many factors can have the potential to affect the motor unit (MU). It can be speculated that factors may affect separately the motor neuron (MN) or the muscle fibers innervated by the MN. On the other hand, both may be affected simultaneously. At the peripheral level, there are several proposed pre- and postsynaptic mechanisms that can be impaired (157). For example, presynaptic fatigue may be an indication of failure of action potential propagation along the axon (branch point) or an inadequate release of acetylcholine (ACh). At the postsynaptic level, fatigue may be due to insufficient depolarization of the muscle membrane or failure of action potential propagation along the sarcolemma. However, all proposed theories lack sufficient description of the magnitude of their effect.

Burke et al. distinguished three types of motor units: fast-twitch, fatigable (FF); fast-twitch, fatigue-resistant (FR); and slow-twitch (S), which is the most resistant to fatigue (30). As stated earlier, the maximum amount of force produced greatly impacts performance. Force is modulated during voluntary actions by a combination of MU recruitment and changes in MU activation frequency (rate coding) (126). Hence, force will be greater when the greater the number of MUs are recruited along with a greater discharge frequency. Moreover, fast-twitch, fatigable fiber types are also classified into fast-twitch, glycolytic or Type IIx fibers. These fibers are generally the larger muscle fibers, which allows for greater force output. However, the downside is that they are extremely fatigable. The majority of sports utilize such fiber types, since explosive movements are responsible for success in these sports. All of which increase the potential for fatigue. As stated, this may come from a decrease in rate coding or decrement in MU recruitment.

Many authors have hypothesized potential factors that may contribute to PNS fatigue (48, 175, Wilkinson, 2010 #896). Some examples include: buildup of peripheral toxins or metabolic by-products; reduced centrally mediated self-regulation; increased inflammatory cytokine production; altered neurotransmitter metabolism; or reduced periphery regulated central drive control (181). Therefore, the purpose of this review paper is to highlight the current understanding of fatigue as it pertains to both muscular and metabolic fatigue.

1.2 Muscle Fatigue

1.2.1 Defining Muscular Fatigue

As stated earlier, fatigue has generally been defined as an acute impairment of exercise/sport performance that includes both an increase in the perceived effort necessary to exert a desired force or power output, however, there is an inability to produce the desired force or power output (48). Definitions will tend to vary based upon the questions posed by the investigators, thus, the causes of fatigue will be depend on the model studied, experimental conditions, and task imposed on the muscle. For example, *in vitro* experiments typically attempt to manipulate conditions to determine directly the cause(s) of contractile failure, since fatigue is related to a loss in force or power of a single cell or whole muscle. As for *in situ* studies, a similar approach can be applied which would more closely mimic physiological conditions compared with the more basic *in vitro* studies. Lastly, *in vivo* studies tend to involve variations in the mechanisms and magnitude of fatigue. This often depends on the task imposed, which has been termed "task specificity" (107). No matter the activity, fatigue is a fundamental functional characteristic of skeletal muscle. An advantage to studying muscle fatigue is that it is an acute, quantifiable event (107). Generally speaking, fatigue research will report the magnitude of the fall in force or power in regards to a specific contraction protocol designed to produce fatigue. Also within muscle fatigue research, another approach often used is that of quantifying fatigue as the duration a contraction or series of contractions can be maintained at a specific, submaximal target tension (107).

Muscle fatigue can refer to a motor deficit, a perception or a decline in mental function, or a gradual decrease in the force capacity of muscle or the endpoint of a sustained activity (60). Measuring muscle fatigue can be done so via a reduction in muscle force, a change in electromyography activity, or an exhaustion of contractile function. Due to the complexity of defining muscle fatigue, a more focused definition would be an exercise-induced reduction in the ability of muscle to produce force or power whether or not the task can be sustained (22). Unraveling the mechanisms of fatigue is a difficult area to explore, especially when trying to decide whether fatigue arises from metabolic by-products produced from exercise or from neuromuscular failure.

Many factors come into play when looking at both neuromuscular and metabolic fatigue; thus, it can be difficult to ascertain which has a greater impact. Therefore, it can be assumed that many factors will influence the onset of fatigue; a greater question to be explored may be how one system influence another system. Specifically, how do metabolic by-products produced during exercise impact the nervous system, thus, leading to nerve failure? For instance, an investigation can look into what is known as branch point failure, which is a blockage occurring at sites of axonal bifurcations (164). Motor neurons are highly branched with varying lengths, therefore, at high stimulation frequencies, these longer branches are unable to repolarize as fast as the rest of the axon. When this happens, neurons will be unable to depolarize, and manifests as a failure of a single motor unit's individual muscles cells to contract. This failure is referred to as branch point failure. As with most areas of muscle fatigue, branch point failure is rarely discussed and appears to be relatively unknown to the scientific community. This review

will discuss branch point failure and other fatigue-inducing factors by investigating the relationship of metabolic by-products on nervous system failure/fatigue.

Some researchers believe that fatigue originates within the periphery, while others tend to argue that fatigue is more located within the central nervous system (CNS) (175). Although the CNS more than likely has a major impact upon fatigue, this review will explore fatigue within the PNS. The idea of muscle fatigue being the initial site of fatigue over brain fatigue being the initial site dates back to the Italian physiologist Angelo Mosso. Mosso defined within his seminal book on muscle fatigue by stating, "Being unable to separate the study of the muscles from that of the nervous system, I have thought well to restrict myself in this book to study of brain fatigue…I shall speak of muscular fatigue and of the changes which take place in the muscle only so far as may be necessary for a better understanding of fatigue in the brain," (59). This statement was made over 100 years ago, and despite vast amounts of research, muscular fatigue and its mechanisms are still wildly undefined. Anecdotally, a case for working muscles having a fatiguing effect upon the brain could be made. But, it is imperative to develop a working knowledge of muscular fatigue and all that encompasses it while under fatiguing conditions. Additionally, the same amount of research has been conducted over the last 100 years pertaining to metabolic fatigue research as muscular fatigue, and unfortunately, the results are just as undefined.

It must be mentioned that there is a distinction between muscle fatigue and the ability to continue the task. Muscle fatigue is the point in which there is a decrease in maximal force or power that a given muscle(s) can produce, as of which develops gradually soon after the onset of the sustained physical activity (60). An important point

to keep in mind is that muscle fatigue is not the point or moment of task failure. Many tend to categorize muscle fatigue with task failure, however, this is incorrect for fatigue takes place over a given duration from the beginning of activity.

1.2.2 Quantification of Fatigue

The mechanisms leading to muscle fatigue are immensely diverse, thus multiple measuring techniques and timing of measures can be beneficial for determining the causes of fatigue (36). When looking at mechanical fatigue, measures are obtained using test contractions that could differ from those contractions that induce fatigue. An example of this would be a maximum voluntary isometric contraction (MVC) following a bout of submaximal dynamic exercise (26). Determining which measuring technique will be based upon if one is seeking to investigate mechanisms (i.e., physiological processes) or to assess performance (i.e., to have functional relevance). Generally, measurements will be obtained before and after fatiguing activity, however, values can be obtained during activity to provide time-course information. Post-exercise data should be obtained within a timely fashion for the speed of recovery can be an issue if measurements are not made immediately on exercise cessation, which means that the extent of fatigue may be underestimated (26). Peak MVC force has been utilized quite extensively with human because it determines the volitional force-generating capacity of a muscle under relatively standard conditions (26, 69, 99, 130). The problem with MVC measurements is that it bears little resemblance to a bout of dynamic exercise, despite helping to determine mechanisms of whole muscle fatigue. Specifically, one main issue with MVC investigations utilizing submaximal contractions is that only about <50% muscle mass is activated at one time. Trying to interpret the results from MVC investigations in relation

to fatigue within an athletic event or general exercise is contradictory for there are hardly any instances where an individual only performs submaximal isometric contractions at <50% of muscle mass. Also, RFD has shown to be unaffected following repetitive arm movement, despite a 30% decline in MVC (78). This demonstrates why MVC may be a poor performance measure.

Measuring fatigue via force or velocity tests tends to give more applicable results when utilizing dynamic movements. Maximum shortening velocity can be assessed for it slows during fatigue, and is helpful in addressing mechanisms associated with maximal rates of cross-bridge cycling (99, 177). One main issue is that maximum shortening velocity is normally measured under an unloaded condition, which is in contrast to real exercise where there is always some form of a loaded condition. Power is one measurement that is widely utilized for fatigue, and can be determined using force-velocity measures in an electrically stimulated muscle fiber, motor unit, or whole muscle (99, 177). Additionally, other tests utilized are single muscle group protocols, which generally include repeated knee extensions (8). Other studies have used isokinetic dynamometry, however, caution should be taken due to isokinetic contractions do not exist in nature and are considerably slower than the peak limb velocities (99). From this, power measurements tend to be questionable. For instance, there have been reported declines in peak power during maximal isokinetic sprint cycling, but only at higher pedal rates (110-129 rpm), which resembles the cadence of competitive cycling (1). Peak muscle power falls considerably more than isometric force after bouts of stimulated contractions in humans. This is not surprising because force and velocity determine power, therefore, power will be less affected when only one variable is changed.

Peak power can decline markedly (to <50% initially), however, peak MVC force seldom falls by more than 30% during dynamic exercise (26, 78, 99). In contrast, peak tetanic force can be reduced by 10-50% of initial levels following stimulation induced fatigue (1, 35, 177). At this point, excitation and Ca2+ release from the sarcoplasmic reticulum are impaired. Additionally, very prolonged MVCs (over several minutes) or repeated contractions in the body with occluded blood flow can have peak forces falling to 40-60% of initial levels (69). Good muscle perfusion in dynamic exercise may protect against severe force fatigue, but, it cannot be excluded that some fibers undergo a large force depression while other fibers are fatigue resistant. Therefore, future studies are warranted to investigate how some fibers are protected against fatigue while others succumb to fatigue.

1.2.3 Contraction Types and Patterns

When looking specifically at contraction types, the majority of research has utilized isometric contractions, which are easily mimicked (36). In contrast, dynamic exercise involves shortening and lengthening contractions, and often a pre-stretch is followed immediately by shortening, that is, a stretch-shortening cycle (SSC) is employed (1, 130). A greater force loss results following repeated shortening contractions when compared to repeated isometric contractions, for fatigue may be induced when stimulation includes lengthening or contains a pre-stretch followed by shortening (1, 99). The latter can reflect SSC actions, but without reflex contributions, therefore, many features of the contraction types occurring during real exercise can be represented in stimulation models. Contraction patterns involve the duration of each contraction, how often each contraction occurs, the number of contractions (or exercise duration), and

whether fatigue runs should be repeated to mimic intermittent exercise (36). Biomechanical or electromyography patterns obtained during exercise can help to determine contraction patterns.

1.2.4 Stimulation Frequency and Patterns

Dynamic protocols need to evoke a frequency and pattern that mimics the motor unit firing patterns such as those found during voluntary contractions (i.e., involve trains of action potentials and in consequence tetanic contractions) (69, 78)**(FIG1-Cairns)**. Initially, these patterns include a doublet of action potentials, usually at 50-200 Hz, followed by sustained firing rate during MVCs ranging from 10-60 Hz (69, 78). During prolonged MVCs, firing rates may decline by up to one half in some motor units while others are unaffected. Firing rates <20 Hz may even increase, and there may even be dropout of excitation for periods. Stimulation patterns depend on the muscle or motor unit activated, and on the duration and type of contraction involved. Future investigations should incorporate variable frequency trains in order to mimic physiological activation rather than using a constant frequency train.

Maximal force is evoked under a constant stimulation frequency of 50-200 Hz (24, 35, 69), which exceeds the sustained rates during MVCs. What isn't taken into account is the initial doublet, which can increase force rapidly and can make use of the catch-like properties of some muscles (25). Variable frequency trains along with an initial doublet may convey some fatigue resistance compared with constant frequency trains. Additionally, peak force declines more rapidly following constant high-frequency stimulation compared to prolonged MVC. This rate of decline of MVCs can be mimicked with a progressive fall in stimulation frequency (1).

1.2.5 Fatigue Approaches with Real Exercise

Real exercise can be loosely defined as either sport or physical activity of daily living, and is dependent on subject selection, fatigue quantification system, and fatigue protocol (36). Fatigue investigations utilizing data from within competition may involve video analysis (kinematics), and power or force which can be assessed in a manner that does not compromise performance (e.g., for cycling or rowing). Common testing procedures include exercising to exhaustion at a standardized submaximal power, completion of the maximum volume of work in a set time, undertaking a set volume of work as fast as possible, and short-duration all-out effort. What is starting to gain a lot more interest with fatigue is the use of weight lifting. The benefit of utilizing weight lifting as a modality is its practicality, for all athletes and most gym goers all use some form of weights to train. Another benefit is the physiological systems are intact, for there is no surgery or anesthesia to be undertaken. The majority of resistance training protocols have utilized isometric contractions, often using MVCs (36). One main issue with using isometric contractions and trying to apply to real life is that these contractions seldom happen in normal life. Isometric contractions tend to be sustained for a longer time period (5-7 s), as compared to dynamic movements (<500 ms) (140). Based upon this, future research should attempt to utilized dynamic movements that are more applicable to real life, that is, that can be applied to sport and exercise.

1.2.6 High Frequency Fatigue vs. Low Frequency Fatigue

Fatigue can take place from multiple causes, however, one such area that deserves some acknowledging is a failure of excitation-contraction coupling. It is understood that

fatigue can be caused by a failure of action potential propagation along the surface membrane and t-tubular system, a failure of the coupling mechanisms between action potential and release of calcium, or a failure of calcium regulation at the level of the contractile elements (100). Additionally, adjustments in skeletal muscle function are generally characterized by changes in voluntary maximal or sustained force-generating capacity, however, they should also be characterized by modifications in electrically induced contraction torque (139). This can be from evoked mechanical twitch (peak twitch, maximal rate of muscle contraction and relaxation), and low- and high-frequency titanic torque response. Two types of failure associated with ECC failure is a preferential loss of force at high frequencies of stimulation (high-frequency fatigue) and the other affecting force development at low frequencies (low-frequency fatigue). Specifically, high-frequency fatigue occurs as a result of alteration in action potential propagation over the sarcolemma or t-tubules, while low-frequency fatigue is associated with Excitation-Contraction Coupling (ECC) failure (139).

High-frequency fatigue is shown when force production declines rapidly while the muscle is stimulated continuously at a frequency close that that which gives maximal force (6). High-frequency fatigue is characterized by a recovery that is very rapid, with a component of recovery that has a time course of only 1-2 s (21, 101). This type of contraction would be seen within humans when lifting a heavy object, and is generally recognized at the onset and recovery of fatigue are rapid. The investigations of Bigland-Ritchie et al. 1979 (21) and Jones et al. 1979 (101) lead to the recognition of the following features of high-frequency fatigue: 1) loss of force after stimulating at high frequencies is reversed rapidly by reducing the frequency, 2) loss of force is accompanied

by a loss of amplitude and slowing of the waveform of the muscle action potential, and 3) loss of force is exacerbated if extracellular [Na^+] is decreased or [K^+] increased. Low-frequency fatigue occurs when force from low-frequency stimulation decreases following physical activity (40). It has been discussed that training involving high volume causes low-frequency fatigue, particularly, training that impairs calcium ion transient when MU activation level is low (56). As with high-frequency fatigue, Bigland-Ritchie et al. 1979 (21) and Jones et al. 1979 (101) discussed the main features of low-frequency fatigue as follows: 1) the forces at low frequencies of stimulation are the most severely affected, 2) recovery is slow, taking hours or, in severe cases, days for a full recovery, and 3) the effect persists in the absence of gross metabolic or electrical disturbance to the muscle. As Jones points out, low-frequency fatigue is caused by a variety of forms of activity and is characterized by loss of force when tested at low frequencies of stimulation (101). This type of fatigue is not caused by low-frequency stimulation.

1.3 Bioenergetics & Ammonia Production

1.3.1 Introduction

Metabolic fatigue has classically been defined as the decreased ability of an organism or one of its parts to respond or function because of prolonged exertion or repeated stimulation (128). As with muscular fatigue, multiple factors will more than likely contribute to metabolic fatigue. These may include:

1) Substrate depletion of adenosine triphosphate (ATP), carbohydrate (CHO), phosphocreatine (PC)
2) Accumulation of waste products including CO_2, Hydrogen ions (H^+), NH_3, free radicals
3) Inhibition of metabolic pathways, and/or
4) Central factors such as NH_3 uptake within the brain

The majority of sports nutrition physiology has focused on enhancing performance and minimizing fatigue by enhancing ATP production, glycogen storage, and sports-specific training. The ability to use energy is the hallmark of all living organisms, with ATP being the high-energy compound found within cells that provide this energy for physiological functions. Within the cell, ATP is only stored in small amounts, about 3 mmol per kilogram in most tissues to about 6 mmol per kilogram in skeletal muscle. Due to this low storage, specific metabolic pathways must be ramped up to digest food components in order to produce ATP for energy. These components include carbohydrates for glycolysis, triacylglycerol derivatives for beta-oxidation, and amino acids for intermediaries during all pathways. Metabolism whether at rest or during activity is all based upon rates, and as ATP utilization increases, so does the demand for replenishment increase. Additionally, waste products such as NH_3, H^+, and CO_2 increase along with ATP utilization and replenishment. The amount of waste products generated depends greatly on the source, speed, and amount of substrate consumed. Astrand and Rodahl (10) explained how substrates for ATP production are mediated by: exercise intensity, duration, modality, type (continuous or intermittent); state of training; diet; state of health; and/or availability and source of substrate. The table below displays the various biochemical pathways, energy sources, production characteristics, and waste products of ATP metabolism:

Metabolic Pathway(s)	Energy Source	Estimated Rate (moles/min)	Estimated Capacity (moles)	Waste Products
ATP-PC	Intramuscular ATP Phospho-creatine	Very Fast (> 3.6 ATP/min)	Very Low (0.5 ATP)	P_i NH_3
Glycolysis (exclusively)	Glucose Glycogen	Fast (1.5 ATP/min)	Low (500g CHO – 9 ATP)	H^+
Glycolysis + TCA + ETC	Glucose Glycogen	Somewhat Fast (1 ATP/min)	Med (500g CHO - 114 ATP)	CO_2
Gluconeogenesis; Transamination/ Oxidation – TCA Cycle, ETC	BCAA's Glycerol Lactate Alanine	Variable	High (2500g PRO-175 ATP)	NH_3 CO_2
β-Oxidation - TCA Cycle, ETC	Fatty acids Glycerol Ketones	Very Slow	Very High (>15,000g Fat – 8,000 ATP)	CO_2

High-intensity activity helps to accelerate ATPase, adenylate kinase, and AMP deaminase, all of which help to produce different waste products (10). Additionally, during high-intensity activity, there is about a 50% reduction in muscular [ATP], which will yield up to a 20-fold change in [ADP] (adenosine diphosphate) and 100-fold increase in [AMP] (adenosine monophosphate) (10). In summary, fatigue can be generalized by

the demand for ATP, availability of substrate, and status of training. Any disruption in the above factors will result in an unfavorable ratio of waste products. Thus, an accumulation of these waste products will result in a decrease in performance.

1.3.2 ATP – PC System

Active muscle derives its energy from ATP, which consists of a base (adenine), a sugar (ribose), and three phosphates. During high-intensity activity, fatigue will begin to develop due to an inefficiency to replenish ATP stores. Many believe that fatigue is a result of a lack of ATP, however, a closer look into exercise biochemistry will reveal that it is the cell's inability to regenerate ATP that will lead to fatigue development (6). A feature of fast muscle is that it can consume ATP much faster than it can regenerate, with the unfortunate result is an increase in ADP and inorganic phosphate (P_i). Two reactions in particular are in close equilibrium: the creatine kinase (PCr + ADP $\leftrightarrow$ Cr + ATP) and the adenylate kinase (ADP + ADP $\leftrightarrow$ ATP + AMP). Due to this close equilibrium, the net consumption of ATP leads relatively stereotyped changes in the concentration of ATP, ADP, P_i, phosphocreatine (PCr), creatine (Cr), and AMP (7). During activity and net consumption of ATP, [ATP] is initially unchanged while the net effect is a fall in [PCr] and rises in [Cr] and [P_i]. This can be measured via muscle biopsies and nuclear magnetic resonance (NMR) studies (34). When [PCr] reaches low levels (<10 mM), [ATP] starts to fall and [ADP] rises substantially. Adenosine diphosphate concentration should normally be around 10 μM under controlled conditions, however, during high-intensity activity it can rise to 100-300 μM, which could result in unfavorable waste product formation. When [ADP] reaches such levels, the [AMP] also becomes

significant, and can be broken down by AMP deaminase to NH_3 and inositol monophosphate (IMP). Ammonia is a deamination by-product of the ATP-PC pathway for ATP production, utilization, and regeneration (151). Schmidt (1928) was first to report that AMP deamination in response to ATP hydrolysis was a major source of ammonia production in active muscle (156). The production of NH_3 by AMP deaminase ($AMP + H_2O \Rightarrow IMP + NH_3$) is the primary source of ammonia production during short-term intense activity (151, 156). The production of ammonia can be summarized by:

1) $ATP + H_2O \Leftrightarrow ADP + P_i$ (*ATPase*)
2) $ADP + ADP \Leftrightarrow AMP + ATP$ (*myokinase*)
3) $AMP + H_2O \Rightarrow IMP + NH_3$ (*AMP deaminase*)

1.3.3 Purine Nucleotide Cycle

The proper management of adenine nucleotide metabolism in skeletal muscle is central to the role of muscle as a transducer of chemical energy into mechanical work (172). This is because adenine nucleotides are important in nearly all energy transfer reactions within the cell, and this precise control is extremely important during exercise when there is occurring a large, abrupt increase in the rate of energy transfer. During steady-state exercise, energy rates are controlled to match the energy expenditure. However, as intensity begins to increase, so do the unwarranted by-products (reactions 1, 2, & 3 above). As stated, fatigue is more a result of a decreased ability to replenish ATP than an actual decrease in the availably of ATP. Thus, a net result is the formation of AMP when ATP hydrolysis exceeds rephosphorylation. What needs to be kept in mind is that the AMP concentrations do not rise, but are quickly converted into IMP and NH_3 (reaction 3). Additionally, it should be noted that AMP deaminase is mostly present in

fast muscle cells, and mostly non-existent in slow muscle cells. Besides the increase in by-products, another direct result of this reaction is the depletion of adenine nucleotide pool. Banister and colleagues pointed out that the maintenance of the total amount (TAN) adenine nucleotides (ATP + ADP + AMP) is essential to the prevention of fatigue (15). The step involving AMP deamination is irreversible, however, AMP may be regenerated via the purine nucleotide cycle (PNC). The reactions go as follows:

1) AMP + H_2O => IMP + NH_3 (*AMP deaminase*)

2) IMP + Aspartate + GTP => Adenylosuccinate + GDP + P_i (*adenylosuccinate synthetase*)

3) Adenylosuccinate <=> AMP + Fumarate (*adenylosuccinase*)

Thus, one complete turn of the PNC in which there is no net loss of AMP results in the deamination of aspartate to fumarate and ammonia and the consumption of energy (GTP – Guanine triphosphate => GDP – Guanine diphosphate). The AMP produced during reamination becomes part of the muscle ATP pool through the myokinase reaction (reaction 2). Lowenstein (114) first proposed that the PNC has several possible functions, including the:

1) maintenance of high ATP:ADP ratio by shifting the myokinase equilibrium (reaction 2) toward ATP by removing AMP through deamination to IMP;
2) regulation of phosphofructokinase activity by the ammonium ion ($NH4^+$) concentration;
3) regulation of phosphorylase *b* activity by the IMP concentration;
4) replenishment of citric acid cycle intermediates by production of fumarate;
5) deamination of amino acids for oxidative metabolism

Others have also proposed the same functions of the PNC (115, 154, 167). The first three proposed functions are dependent on the initial reaction of the PNC. This is because this reaction effects the ATP:ADP ratio, which increases the cellular concentration of NH_4^+ and IMP. Proposed functions (4) and (5) would require the complete functioning of the PNC because the reaminating limb of the PNC is essential for fumarate production and deamination of aspartate.

Studies dating back to the 1960's have demonstrated that the recruitment of fast-twitch muscle fibers (vs. slow-twitch) increases AMP deaminase activity and ammonia production (73, 124, 142). Essentially, AMP deaminiase is activated by high intramuscular [ADP], [AMP], and a fall in pH. Conversely, it is inhibited by high [ATP], [GTP], and [P_i]. Wheeler and Lowenstein demonstrated this inverse relationship of AMP deaminse (179). In contrast to fast-twitch muscle fibers, slow-twitch muscle fibers contain low amounts of AMP deaminase, thus, produces less ammonia (73, 124, 142). Therefore, mitochondria-rich muscle (slow oxidative) will either produce less ammonia via AMP deaminase or there is less production in general during high-intensity exercise compared to mitochondria-poor (fast-glycolytic) muscle. In the 1970's, Heald demonstrated via *in situ* an inhibition of skeletal muscle contractile properties when saturated in a concentrated ammonium ion solution (88). Collectively of this section, the recruitment of fast-twitch muscle fibers accelerates ATP hydrolysis, AMP deamination, PNC activity, and intramuscular [NH_3]. Additionally, the impact of waste product formation should be a priority within fatigue research, especially the formation of ammonia and its toxicity.

1.3.4 Glycolysis and Pyruvate Oxidation

Rates are the primary factor when dealing with metabolism, and once the ATP-PC system is unable to keep up with ATP demand, glycolysis will begin to be the primary pathway for energy production. Glycolysis is a sequence of enzyme-catalyzed reactions for the breakdown of CHO (glucose), as of which is the principal route for CHO breakdown within all cells, especially during exercise (170). Looking specifically at skeletal muscle, there are two starting substances for the glycolytic pathway, glucose and glycogen. Both starting products of glycolysis will result in pyruvate formation, however, the difference is that a glucose unit from glycogen will produce one more net ATP than a free glucose unit. If beginning with glucose, glycolysis will generate a net of two ATP (i.e. 4 ATP created, minus 2 ATP utilized). Conversely, if beginning with glycogen, glycolysis will generate a net of three ATP. The following reactions display those reactions taking place during glycolysis, beginning with glucose and then continuing with glycogen:

$$Glucose + 2ADP + 2P_i + 2NAD^+ \rightarrow 2\ pyruvate + 2ATP + 2NADH + 2H^+$$

$$Glycogen + 3ADP + 3P_i + 2NAD^+ \rightarrow Glycogen_{n-1} + 2\ pyruvate + 3ATP + 2NADH + 1H^+$$

The end product of glycolysis is the formation of pyruvate, which is the point when glycolysis ceases. From this point, there are two primary fates of pyruvate: the conversation into lactate or the conversion into acetyl-CoA for TCA functions. A more detailed description of the fate of pyruvate being converted into acetyl-CoA will follow in the next section. On the other hand, when the cell is unable to use the pyruvate that is generated due the rate of production, pyruvate must be converted into lactate via the

enzyme lactate dehydrogenase (LDH). A more detailed explanation of lactate production will be addressed in subsequent sections. But, what must be remembered is that the formation of either lactate or pyruvate will help keep the cell operating in an efficient manner assuming production doesn't become overwhelming. Assuming the cell is operating efficiently, both the production of lactate and pyruvate will help keep ATP levels maintained, and regenerate ATP that is hydrolyzed during activity.

1.3.5 Tricarboxylic Acid (TCA) Cycle

The prime function of the TCA cycle is to completely oxidize (i.e., remove electrons) form acetyl groups in a way that will result ATP formation (170). The acetyl groups are formed from all the oxidizable fuels in the body, including CHO, lipids, and amino acids from protein (PRO). One complete turn of the TCA cycle will generate two molecules of CO2, one ATP equivalent, three NADH, and one reduced Flavin Adenine Dinucleotide ($FADH_2$). The reduced forms of NADH and $FADH_2$ carry hydrogens to the ETC, which provides the necessary molecules for ATP production. It is important to keep in mind that each glucose molecule elicits two pyruvate molecules, thus, the listed products of the TCA cycle will be doubled.

Going back to glycolysis, the 3-C pyruvate molecule produced will subsequently be converted via pyruvate dehydrogenase (PDH) into: a 2-C acetyl-CoA, reduced nicotinamide adenine dinucleotide (NADH), and releasing CO_2. Once again, glycolysis is based on rates for pyruvate metabolism is dependent upon both ATP supply and demand. Acetyl-CoA will then enter the tricarboxylic acid (TCA) cycle, while the NADH produced will enter the electron transport chain (ETC) for ATP production. Advantages of pyruvate decarboxylation include more ATP produced per unit glucose, and reduced

lactate, H^+, and ammonia accumulation (170). On the other hand, a noteworthy disadvantage of having to use pyruvate oxidation is a slower rate of ATP production.

1.3.6 Electron Transport Chain (ETC)

The ETC is a respiratory chain consisting of four protein-lipid complexes located within the inner membrane of the mitochondria for the process of ATP production (170). Various metabolic pathways produce NADH and $FADH_2$, and these products transfer the high-energy potential across the inner mitochondrial membrane to promote ATP production. The process of ATP production stems from ADP being phosphorylated via an electrical gradient created by the pumping of H^+ across the inner mitochondrial wall and cristae through the ATPases. Each of the four complexes will produce ATP, with the final acceptor of electrons being oxygen. This is the reason why it is called aerobic (with oxygen) glycolysis because oxygen becomes the final acceptor of electrons. The energy transfers via the ETC include the phosphorylation of ADP into ATP, which then produces H_2O and releases heat. The net ATP produced per molecule of NADH will be three ATP, while the net ATP produced per molecule of $FADH_2$ will be two ATP.

1.3.7 β-Oxidation

Triacylglycerol (FAT) provides a substantial amount of fuel during exercise, and this conversion into ATP integrates many metabolic pathways (29). This breakdown is termed beta-oxidation (β-oxidation). Once a fatty acid is mobilized, the process begins by cleaving the fatty acid molecule into 2-C acetyl fragments. As this happens, a NADH and $FADH_2$ are also produced. The acetyl fragments can then be utilized by the TCA cycle, while the NADH and $FADH_2$ can be utilized by the ETC. Much like aerobic glycolysis,

one strong advantage of β-oxidation is the enormous amount of ATP produced (~130 ATP per 16-C palmitate; which must be multiplied by three). Conversely, the disadvantage is the amount of time required to receive the pay-off of the high ATP production. As described by Brooks et al. (2005) (29), the sequence for lipid catabolism for ATP production is as follows:

1) Mobilization – the breakdown of adipose and intramuscular triglyceride
2) Circulation – the transport of free fatty acids (FFAs) from adipose to muscle; albumin is usually the carrier
3) Uptake – the entry of FFAs into muscle from blood
4) Activation – raising the energy level of fatty acids preparatory to catabolism
5) Translocation – the entry of activated fatty acids into mitochondria
6) β-oxidation – the production of acetyl-CoA from activated fatty acids and the production of reducing equivalents (NADH and $FADH_2$)
7) Mitochondrial oxidation – TCA cycle and ETC activity

1.3.8 Amino Acid Bioenergetics

Our principal source of amino acids come from ingested PRO (170). Following digestion and absorption, amino acids enter what is known as the amino acid pool. Cells take up amino acids from this pool to synthesize new PROs. Additionally, amino acids can be degraded to synthesize new PROs or be release to the amino acid pool. Out of all the primary food sources used by mammals for energy, PRO is the only molecule to contain nitrogen. This allows for the potential of ammonia production. Amino acids are composed of four components: 1) an amine group (NH_2), 2) a carboxyl group ($COOH^-$), 3) a unique side "R" chain, and 4) a hydrogen. This unique composition makes up the 20

different amino acids, which when arranged into a particular order make up the long polypeptides known as PRO. Protein can contribute to bioenergetics by supplying the carbon backbone(s) to produce pyruvate, TCA cycle intermediates, or acetyl-CoA (and derivatives) (29). Proteins are generally used as a fuel source during time of fasting, starvation, and/or prolonged exercise when glycogen and blood glucose levels are depleted. During exercise, glucagon, glucocorticoids, and catecholamines work together to catabolize PROs for energy production. In contrast, some of the fates of excess PRO intake are: 1) conversion into triacylglycerol, 2) degradation into ammonia and urea by the liver, and/or 3) degradation into ammonia by the intestinal flora by urease. It is important to intake the proper amount of PRO for activity, for the breakdown of PRO into glucose is a costly process. For example, gluconeogenesis from PRO requires six ATP to convert pyruvate into glucose.

Three important amino acids that are involved in bioenergetics are leucine, valine, and isoleucine, also known as the branched chain amino acids (BCAA's). A large percentage of dietary PROs come from BCAA's (`20-30%), and during exercise, BCAA's provide the primary amino acids needed for the synthesis of other substrates. Specifically, BCAA's can be deaminated to their respective keto acids with alpha ketoglutarate accepting the ammonia group and forming glutamate (75). From here, glutamate can further be converted into TCA cycle intermediates. A further explanation of ammonia formation via BCAA's will be addressed in subsequent sections.

1.3.9 Ammonia Production

One such proposed theory that is being revisited is the formation of ammonia (NH_3). Tashiro in 1922 was the first to investigate whether there is a link between the

production of ammonia and fatigue among isolated nerve fibers after electrical stimulation (166). Under normal conditions, ammonia is an important metabolic end product, as well as an intermediate of several biochemical pathways in the body. The appearance of ammonia in the systemic circulation stems from a number of sources such as the gut, muscle, kidney, or brain. At rest, the majority of systemic ammonia is released from the gut or gastrointestinal tract, where it is delivered to the liver to be incorporated into either urea via ureagenesis or glutamine. Skeletal muscle within humans accounts for about 40% of total body mass, therefore, has a large potential capacity for the production and/or uptake and metabolism of ammonia. However, studies within humans have shown that ammonia uptake by skeletal muscle at rest may be close to zero (135). On the other hand, during times of exercise when production far exceeds the capacity for removal, ammonia production may contribute significantly to the onset of fatigue (14). It is important to remember that during exercise, blood flow is shunted away from the viscera and directed to working muscles and brain. If ammonia formation is too great, then problems will arise because blood flow is shunted away from the liver and kidneys, which are the primary organs for ammonia excretion. Instead of the body excreting ammonia via urea, it will stay within circulation as blood flow travels to working muscles. With this, another problem with high blood ammonia concentrations arises as blood circulates through the brain. Ammonia is able to cross the blood brain barrier, therefore, setting up for further implications within the central nervous system (181). All in all, high production of ammonia can contribute to a host of issues if it isn't properly disposed.

Parnas and colleagues in the 1920s were the first to report on ammonia production within skeletal muscle, specifically, the relationship between ammonia production and anaerobic work (137). It has been established that there exists an intensity dependent relationship between plasma ammonia concentration and exercise (11, Buono, 1984 #964). As exercise intensities rapidly increases above 60% of VO_{2max}, ammonia production tends to rapidly increase as well. The mechanisms behind this increase in ammonia have been identified as part of the PNC. Within this cycle, AMP is converted to IMP and an ammonium ion (NH_4^+) via the enzyme AMP deaminase. This enzyme is highly present in FF muscle fiber types, which is beneficial for the help of removing the AMP buildup and ridding the system of ammonia. However, the downfall is as AMP deaminase forms ammonia, it also at the same time removes an adenine nucleotide from the available pool of substrates for ATP formation. This step is irreversible, thus, ensuring fatigue if the body needs energy and there are no available substrates for ATP formation. Since the majority of sports incorporate FF muscle fiber types, as well as high repeated stimulation of these fiber types, it can be assumed that high concentrations of AMP deaminase within these fiber types may be playing a role with the onset of fatigue. If there are high concentrations of substrates within a given area, as well as high concentration of enzymes that work on that substrate, then logically it can be assumed that the enzymes will be working to form new products. Since ammonia production increases alongside intensity, then this assumption may be correct regarding AMP deaminase.

During exercise, the body will continue to use ATP as needed. As ATP is hydrolyzed for energy, an accumulation of ADP will be present, which will be available

for phosphorylation. If the rate of ATP hydrolysis exceeds the rate of ADP phosphorylation, then the ATP content of the muscle fiber can become depleted. However, this decrease in ATP does not necessarily appear as a stoichiometric increase in ADP concentration (172). The reason for this is due in part to myokinase operating to produce an ATP and AMP. This is where the PNC comes into play, for this cycle begins to form IMP and NH_4^+ via a hydrolysis of AMP. The benefit of this system is the formation of new ATP for energy, however, the downside is the removal of an adenine nucleotide. As stated earlier, the common thought to fatigue deals with substrate utilization, however, this may not be the case due to the cell may be forming the proper amount of ATP. The problem may arise with the subsequence production of AMP, which will follow with ammonia formation. The obvious goal of any athlete enduring their training program would be to maximize ATP formation while decreasing the removal of AMP. It should be noted that the activity of AMP deaminase differs between fiber types (172). Within rats, slow-twitch muscle does not show large decreases in adenine nucleotide content and corresponding increases in IMP during brief, intense exercise. Conversely, fast twitch red and fast twitch white muscle both are capable of exhibiting extremely high rates of AMP deamination during very intense exercise (172).

1.4 Inhibition of Actomyosin Interaction

1.4.1 Introduction

Investigations employing biochemical techniques have used single fibers, which are living, chemically skinned, and mechanically peeled (107). These *in vitro* assays have provided considerable insights into how the thin actin and the thick myosin filaments

interact to generate force, velocity, and power (6, 67). The cross-bridge cycle is shown in (107).

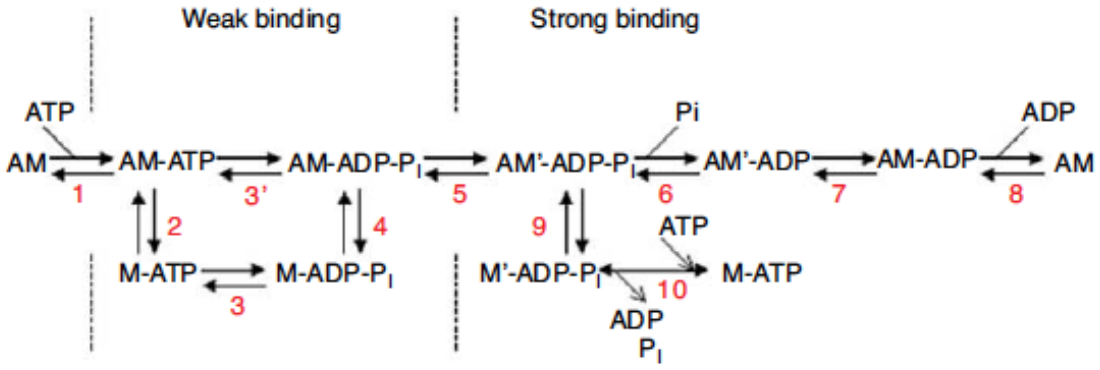

Figure 2 Schmatic model of actomyosin ATP hydrolysis reaction during contraction in skeletal muscle, where A is actin and M is the myosin head (myosin S1). Scheme is adapted from current models of ATP hydrolysis, and shows the conventional path (steps 1-8), and the unconventional branched path (steps 9 and 10). [Figure is a modification of the scheme reprinted, with permission (223), p. 23.]

The key steps related to fatigue are: step 5 the transition from the low- to the high-force state of the actin-myosin (AM) cross bridge; and step 8, the dissociation of ADP from the cross bridge (107). The former step 5 is associated with the release of P_i and H^+ ions. This site is thought to be the rate-limiting site for the rate of force development (*dP/dt*), and the major site of inhibition of force with fatigue during high-intensity exercise (45, 66). The latter step is associated with the release of ADP, which in loaded contractions is thought to limit cross-bridge cycle speed, thus, affecting fiber velocity. For over 40 years it has been known that a high rate of ATP utilization accelerates the creatine kinase (CK) reaction, thus, leads to a large decline in cell PCr. Along with the high ATP turnover and increased metabolism via intense contractile activity, $[H^{+}]$ also increases. This is especially true in fast glycolytic (FG) fibers, where intracellular pH may drop from 7.0 to 6.2.

1.4.2 Inorganic Phosphate and Muscular Contractile Properties

Originally, it was thought that in both slow and fast fibers a high P_i and H^+ directly depressed force by reducing the force of the high-force states of the cross-bridge. This then reduced the transition from the low- to the high-force state (123). Within the last few years, investigators have found that increases in P_i to decrease isometric force and fiber stiffness, which proportionally indicates that the force per cross-bridge remained constant and that the decline in force was entirely explained by a reduced transition to the strong binding state (38). Although both P_i and H^+ affect this transition, the mechanisms appear to be different. High P_i depresses force but increases the rate constant of tension development (rate of force development) (k_{tr}) following a rapid slack and re-extension of a fiber. This suggests that Pi accelerates the reversal of step 5 (67, 176). Conversely, a low pH in the presence of Ca^{2+} depressed force but had no effect on k_{tr} (123). This elevated [H^+] depresses the number of high-force bridges by inhibiting the forward rate constant for the transition from the weak to the strong binding states of the cross-bridge (123).

During fatiguing exercises and increases in P_i, fast but not slow fibers elicited a decline in efficiency, with isometric force and ATPase being depressed by 60 and 13%, respectively (111). These authors described that P_i buildup allows the force-generating state (AM'-ADP-P_i) to detach before the release of hydrolysis products. Within step 9, the product (M'-ADP- P_i) is structurally and kinetically different from the M-ADP- P_i state that is in rapid equilibrium with the weakly bound AM-ADP- P_i state. Linari and colleagues suggested that the M'-ADP-P_i state completes the normal structural change associated with the power stroke in the attached myosin head and the ATPase cycle by an

essentially irreversible release of P_i and ADP (111). In contrast to fast fibers, increased P_i in slow fibers has been shown to depress isometric force and ATPase proportionally (111). However, although there have been reported differences in fiber types, the extent to which fiber-type differences in efficiency within humans remains unknown.

It has been hypothesized that an early loss of force is mediated by an increase in P_i (176). This idea has been supported by the observation in stimulated mouse fibers lacking CK, which failed to show an increase in P_i or the early phase of loss of force (46, 47). During high stimulation (i.e., contractile) rates, the initial decline in force would be mediated by the combined accumulation of P_i and H^+. Additionally, the effects of these ions on the myofilaments have been shown to be additive and involves a direct inhibition of force, and a reduced Ca^{2+} sensitivity (134, 141). In the early stages of fatigue, the amplitude of intracellular $Ca2^+$ is high, however, the reduced Ca^{2+} sensitivity has little to no effect. As fatigue continues, there is a subsequent decline in the amount of Ca^{2+} released from the sarcoplasmic reticulum (SR), thus, reducing the intracellular Ca^{2+} content. This has been seen by a rightward shift in the pCa-force relationship, with one factor contributing to this decline in force being thick filament directly being affected by a reduced number of high-force cross-bridges (67, 123). Therefore, it is apparent that fatigue-inducing effects of P_i and H^+ become more important when the amplitude of the Ca^{2+} transient is depressed.

1.4.3 ADP and Muscular Contractile Properties

Micromolar increases in cell ADP has been known to occur with intense contractile activity, which depresses fiber velocity (6). To determine changes in velocity, studies utilized skinned fibers that were exposed to micromolar ADP, and results showed

that velocity decreased while force increased (107). This increase in force was presumably due to more cross-bridges in the high force states (AM'-ADP-P_i, AM'-ADP, and AM-ADP states in **FIG2**). Another important role increased ADP plays in eliciting fatigue appears to be related to inhibition of the SR Ca2+ pump, which results in disturbances in Excitation-Contraction Coupling (ECC) rather than affecting the cross-bridge (116-118). What hasn't been determined extensively is the combined effects *in vivo* of low pH, high Pi, and elevated [ADP] on calcium sensitivity and peak power in individual slow and fast fibers. The effects of these biochemical products *in vitro* have been investigated at the molecular level in order to assess the sliding velocity of the actin filaments where the regulatory proteins tropomyosin and troponin on myosin have been lacking (49, 77). It appears that both high H^+ (low pH) and ADP appear to slow filament unloaded sliding velocity by increasing the affinity of ADP for the myosin head. This then in turn slows the rate of ADP release. One main concern is that the said conclusion was done *in vitro*, thus, making it difficult to elucidate the finding to intact fibers. Therefore, future studies should try to incorporate a means to investigate the effects of low pH, high Pi, and elevated [ADP] within intact muscle fibers.

1.4.4 Muscle Fatigue and Lactic Acid Accumulation

Lactic acid plays an important part in metabolic biochemistry and human physiology, thus, a short history of lactic acid is warranted. Lactic acid was first discovered in 1780 by the Swedish chemist Carl Wilhelm Scheele (96). Scheele was investigating samples of sour milk when he discovered lactic acid, which led to trivial name of the molecule ("lactic," of or relating to milk). It wasn't until 1869 that lactic acid was found to exist in multiple optical isomers (D- and L-isomers), however, only the L-

isomer has biological metabolic activity. During the initial stages of lactic acid research, fermentation was the main direction of early scientific inquiry into the biochemistry of lactic acid production. It wasn't until the 1920s when two researchers studied lactic acid within skeletal muscle biochemistry. Otto Meyerhoff and Archibald V. Hill in 1922 won the Nobel Prize for their work on the energetics of CHO catabolism in skeletal muscle (95, 146). Specifically, Meyerhoff elucidated most of the glycolytic pathway and demonstrated that lactic acid was produced as a side reaction to glycolysis in the absence of oxygen. Hill quantified the energy release form glucose conversion to lactic acid and proposed that glucose oxidation in times of limited oxygen availability can supply a rapid and high amount of energy to fuel muscle contraction. Additionally, this glucose oxidation can take place when the energetic demands of muscle contraction exceed that from oxidation involving oxygen (146).

The work of Meyerhoff and Hill seemed to cement the acceptance into exercise biochemists and physiologists of lactic acid production being the primary cause of muscle acidosis. Unfortunately, not much has changed within muscular fatigue since the 1920s. On the surface, it is clear why previous researchers believed that lactic acid caused fatigue, for lactic acid production does increase alongside an increase in exercise intensity. For example, Margaria and colleagues demonstrated that changes in blood pH are concomitant with lactic acid concentration (120). In a more recent investigation, Sahlin and colleagues measured muscle pH and the sum of lactate and pyruvate during exercise and recovery from different intensities of exhaustive exercise (153). The results of this investigation revealed a strikingly linear relationship between the two variables. At the time, the sum of lactate and pyruvate were interpreted as metabolic acids. This

early investigation displaying a linear relationship between muscle pH and by-products was interpreted as strong indirect evidence for a cause-effect relationship between lactate/pyruvate production and acidosis. There have been further investigations that have accepted this cause-effect interpretation between increases in "lactic acid" production and decreases in blood/muscle pH (13, 86, 104, 165, 178).

Going from rest to high-intensity exercise increase energy consumption within skeletal muscle by up to 100-fold, therefore, a large fraction of ATP required will stem from anaerobic metabolism due the aerobic system not being able to supply the energy needed for work (178). As stated throughout the paper, metabolism is based on rates, and when the rate of anaerobic metabolism increases, so does lactate production increase due to the inability of pyruvate being converted into acetyl-CoA for TCA cycle functions. This has led many physiologists from the 1960s to the 1990s to believe that lactic acid was the main source of H^+ production (31, 51, 74, 180). In contrast, research during that era also showed that the main source of H^+ production came from ATP hydrolysis coupled with glycolysis, which of course would decrease both muscle and blood pH. More specifically, investigators have reported that lactate production actually consumes proton during times of acidosis (171). Going back to reaction1 in the energy systems section, protons are given off during ATP hydrolysis from the H_2O molecule, and during glycolysis, two protons are given off when staring with glucose and one proton is given off when starting with glycogen. Lactate may in fact actually help to remove these protons, thus, helping to reduce acidosis.

According to a review by Robegs et al., from a biochemical standpoint, the cellular production of lactate is beneficial for several reasons (146). First, as the LDH

reaction occurs, cytosolic NAD^+ is produced to support the NAD^+ substrate demand of the glyceraldehyde 3-phosphaste dehydrogenase reaction. This substrate production helps to maintain the cytosolic redox potential (NAD^+/NADH), and supports the substrate flow through glycolysis. In turn, this helps to allow ATP production from glycolysis. Another important aspect LDH reaction performs is by acting a buffer against cellular proton accumulation (acidosis) (146). This does so by when every pyruvate molecule is converted into lactate and NAD^+, a proton is consumed. Specifically, two electrons and a proton are removed from NADH during the LDH reaction. In order to support the two electrons, one more proton must be added to convert pyruvate to lactate. Therefore, based upon this biochemistry, the LDH reaction is alkalinizing to the cell, not acidifying. This is how lactate may in fact act as a buffer to cell. Red blood cells are a good example of how lactate production is beneficial for the efficiency of a cell. Considering that red blood cells are devoid of mitochondria, they rely primarily on glycolysis for ATP regeneration by using glucose as the original glycolytic substrate. Going back to the glucose reaction, two protons are yielded from glycolysis and this proton yield is balanced when pyruvate is converted into lactate. Additionally, the cytosolic redox is maintained within red blood cells by the NAD^+ produced from the LDH reaction. It can be concluded that lactate production is essential to prevent an acidosis and maintain cellular NAD^+.

In summary, the basis for the cause-effect relationship between lactate production and acidosis has been based upon indirect evidence of a rise in lactate accompanies a rise in exercise intensity. The problem is however that there is no experimental evidence that reveals a cause-effect relationship of lactate production and acidosis. Literature

supporting this cause-effect relationship is based entirely on correlations, and it must be kept in mind that association is not causation. Therefore, for fatigue research to be impactful, it is imperative to break away from the notion that lactate production is the cause to acidosis, thus, leading to fatigue.

1.4.5 Ammonia Formation from AMP, Aspartate, & BCAA Deamination

It is commonly believed that the main source, and perhaps the only source, of ammonia is from AMP deamination (reaction 3)(75). This is especially apparent when exercise is brief (a few minutes or less) and intense (90% VO_{2max} or greater). The key enzyme in this reaction is AMP deaminase, and the activity of this enzyme appears to increase when there are rises in AMP, ADP, H^+, and decreases in CP. The fundamentals of this reaction have been document consistently within the literature (76, 115, 172, 174). Additionally, it has been reported that during intense exercise AMP deaminase shifts to a bound form on myosin, which may be a critical factor in regulation (148, 149). It is unclear what promotes this binding, and if this even important within humans. A second source of ammonia comes from when the PNC is working in its entirety (75). Many tend to reduce the PNC down to just AMP deaminase, which it is one step and often referred to as the initial step. However, if the PNC is cycling it is difficult to determine a start and finish. With that being said, during the PNC when AMP is deaminated to IMP and ammonia, the IMP is then processed by the "reaminating arm" of the cycle. Going back to reactions 1, 2, and 3 under the PNC section, once IMP is formed it then reacts with aspartate to form adenylosuccinate. From here subsequent steps lead to the formation of AMP, which can then produce another IMP and ammonia. This process is not fully understood, but IMP, AMP, and P_i all inhibit this process. Therefore, it is difficult to

accept that during exercise this step is the primary source of ammonia production. Authors have argued that if PNC cycling exists during exercise, then it is in fibers that have already fatigued and are in the process of recovering while exercise is still continuing (97, 172). In contrast, others have proposed that cycling occurs in humans, but only during moderate exercise (27). The evidence on cycling during exercise is circumstantial, for the authors failed to consider BCAA deamination being the producer of ammonia.

The third process, and more plausible than process two, is where skeletal muscle produces ammonia by the deamination of BCAA's (75). Unlike the second process of ammonia production, this process takes place in the mitochondria. Once again, BCAA's are made up of leucine, isoleucine, and valine. During this process, each BCAA can be deaminated into their respective keto acid with alpha ketoglutarate accepting the ammonia group and forming glutamate. The following reactions will help to illustrate said process:

1) BCAA + αKG => Glutarate (Glu) + BCKA (KIC, KIV, KMV)
   KIC – alpha ketoisocaproate; KIV – alpha ketoisovalerate; KMV – alpha keto 3- methylvalerate

2) BCKA + NAD^+ + CoA => acyl CoA derivatives + NADH + CO_2

3) Acyl CoA derivatives => acetoacetate , acetyl CoA, succinyl CoA

The process is a near equilibrium reaction, meaning that the keto acids can either be released and transported to the liver for further oxidation, or they can be catabolized to TCA intermediates in the muscle mitochondria or oxidized (reactions 2 & 3) (75). Although the AMP deamination reaction is the primary source of ammonia during short duration exercise, it appears ammonia formation from BCAA deamination may be the

dominant process during prolonged, steady state exercise. The enzyme involved within this reaction (branched chain keto acid dehydrogenase – BCKAD) exists in both the active and inactive form. The muscle form of BCKAD is inactive during rest, but increases in activity during prolonged exercise (150, 174). This increase in activity is presumably due to the increase in BCAA within the mitochondria (75).

1.5 Summary

It can be concluded that both muscular and metabolic fatigue involves multiple factors, so to pinpoint a singular factor as the main cause of fatigue is difficult. As with many other issues within exercise physiology, especially within clinical populations, there is no one cause to whatever is trying to be investigated. In order to get an idea of what are the main mechanisms leading to fatigue, investigators need to think outside the box a little bit more. It can be seen from the lactate section above that not much has been done since the 1920s in regard to metabolic fatigue. All exercise physiologists have to stand on since that time period is that lactate being the main cause of fatigue. However, it can also be seen from the above sections that the biochemistry of lactate does not support the cause of acidosis hypothesis. What is starting to be discussed more and more in relation to fatigue is how the nervous system acts during athletic competition or exercise. Unfortunately, not much is known regarding the nervous system under fatiguing conditions. Additionally, what also isn't understood is if metabolic by-products produced during activity have any affect upon the nervous system. Therefore, it is the purpose of this review to explore what is known regarding both pre- and post-synaptic neuromuscular transmission failure. Also, it will be discussed if metabolic by-products produced during activity impact the nervous system to cause further fatigue.

CHAPTER 2. PRESYNAPTIC SITES OF NEUROMUSCULAR TRASMISSION FAILURE

2.1 Introduction

The MU is the primary structure that conveys output from the CNS to the PNS by conveying sensory and descending neural inputs into forces to generate movement, thus, is known as a neuromechanical transducer (89). Since the MU is the final common pathway, its activity will determine the magnitude and direction of the force exerted by a muscle. As stated earlier, the MU has two primary components: the motorneuron (MN) and the muscle fibers that the axon innervates. Generally speaking, any structure before the neuromuscular junction (NMJ) is considered presynaptic, while anything after the NMJ is post-synaptic. The neuromuscular junctions (NMJ) between the axon terminals and muscle fibers within skeletal muscle are large, secure synapse. However, for the sake of this review, muscle fiber recruitment will be grouped in with presynaptic discussion. The reason for this is the close relationship between recruitment order and firing frequency, that is, the motor neuron and its muscles that it innervates function as a single entity that represents the quantal element in the control of movement (89).

The main problem with the majority of muscular fatigue research is the lack of application to sport or exercise. More specifically, the majority of research tends to utilize small muscle groups such as the thenar muscle or tibialis anterior (TA), which provide little to overall body movements that occur during sport or exercise. Additionally, protocols tend to look at fatigue during isometric contractions. Unfortunately, this is unproductive when trying to equate what is happening during full body movements because sports and/or exercise is not generally done under an isometric

condition. This section will provide what is known regarding fatigue at the NMJ presynaptic level.

2.1 Fatigability within the Motor Unit

2.1.1 Fatigue within Contractile Properties

The MU that comprise a single muscle can exhibit a range of contractile properties, and are due to the variation in muscle fiber number, properties, and arrangement (89). Van Cutsem and colleagues conducted a comprehensive survey of motor unit properties in the TA by using spike-triggered averaging (173). This technique was utilized to estimate the contractile properties of 528 motor units from ten subjects performing voluntary contractions. Recruitment thresholds ranged from 1% to 90% of MVC torque, with 50% of the MUs having recruitment thresholds less than 20% MVC torque. The literature is consistent with MUs displaying a significant lower twitch torque and recruitment thresholds following MVCs. In the 1970s, Edwards and colleagues began reporting that one key characteristic found within fatigued muscle is a slow-relaxation time (58). Others have reported similar conclusions of slower relaxation times (33). These conclusions have been thought to occur via some form of metabolic changes taking place following exercise. Jones and colleagues stimulated the adductor pollicis under a fatiguing protocol, and showed that both maximum power and relaxation rate declined (102). The difficult task of many fatigue protocols is the use of intraneural stimulation to evoke responses at a range of stimulus frequencies. Macefield and colleagues were able to determine the contractile properties for 13 MUs from three muscles that extend the toes (extensor digitorum brevis, extensor digitorum ongus, and extensor hallucis longus)

(119). Each muscle unit was stimulated with short trains (1.0-5.0 s) of constant frequency (2-100 Hz). There were differences found between all three muscles in respect to twitch contraction and half relaxation times, and when compared to motor units in human thenar muscles, those of the toe were stronger but slower. Lastly, both acute and chronic responses have been shown to occur within MU twitch characteristics. For example, twitch amplitude can be increased by preceding activity (17); muscle temperature (62) muscle pain (63), and may be modified by the sympathetic nervous system (SNS) (144, 145).

A major discrepancy that is found amongst fatigue research is the low application to real life, that is, those working to reduce fatigue in either a sport or exercise setting find it difficult to use the majority of the research. As stated earlier, a large amount of research has utilized MUs from small muscle groups, including the thenar muscle or TA. The main MUs from the investigations listed above came from a TA, and they were tested under a MVC conditions. When trying to equate to dynamic movements, which represent sport and exercise, it is futile to attempt to correlate what is happening during large gross movements based upon a small muscle group investigated under an isometric contraction. Additionally, using intraneural stimulation may provide valuable data, but trying to acquire this type of data amongst athletes is next to impossible. Therefore, the common protocol techniques found within muscular fatigue research may provide significant data, but the results are meaningless if they can't be transferred over to exercise/sport realm.

2.1.2 Recruitment During Voluntary Contractions

Adrian and Bronk (5) were pioneers within muscle physiology for determining that the force exerted by a muscle depends on the number of MUs recruited into action and the rates at which these MUs discharge action potentials (53). It is well understood that MUs are recruited in an orderly sequence (121) that depends on differences in MN size (91, 93, 94). Original investigations into this area were conducted within cat hind-limb MUs, and human investigations have stayed consistent with earlier investigations by showing that MUs are recruited in order of increasing peak twitch force (37, 39, 54, 168). Additionally, MUs with higher recruitment thresholds have shown to have a faster contraction time compared to those with lower recruitment thresholds (125). These properties have been expressed in relation to MVCs, therefore, the recruitment threshold of a MU will indicate the relative size of a MU in the population that contributes to the muscle force. Therefore, as fatigue begins to occur, the lower threshold MUs will reduce the threshold of higher (or larger) MUs. Furthermore, during fatiguing contractions, the magnitude and direction of the change in recruitment threshold is related to the proportion of the task the MU is active. This occurs even when the contraction involves only low threshold MUs (64). Lastly, the upper limit of MU recruitment is certainly less than MVC force (89). This upper limit can differ between muscles. For example, MUs in the adductor pollicis are recruited at forces less than 30% MVC and none are recruited at forces greater than 50% MVC (108, 110). Conversely, MUs in the bicep brachii and TA are recruited up to approximately 90% MVC force (110, 173).

2.1.3 Resistance Training Fatigue Amongst Exercise Variables

Resistance training investigations have begun to appear more and more within the literature, and the manifestation of potentiation and fatigue is dependent on the volume, load, and velocity of the exercise stimulus (40). Authors have stated that high volume stimuli typically elicits low frequency fatigue, whereas high load stimuli elicits high frequency fatigue (18, 41, 56). Studies investigating exercise fatigue have used long duration (82-84), high volume (80, 81, 85), and high load (79) conditions. Both maximal static and dynamic protocols have been utilized, and under these conditions, neuromuscular force production decreases. Additionally, the force-time curve undergoes a change in shape indicating a decrease in explosive strength (82, 83). Hakkinen has shown that there is a relationship existing such that as muscle activation changes, so does muscular force production change (80). This relationship indicates that changes in contractile properties with resistance exercise are a result in PNS and not CNS responses. This main discrepancy the above articles are that they are not typical of training programs used within athletics. The volume and load used by Hakkinen is excessive (20 sets x 1 rep @ 100% or 5 sets x 10 reps with maximum weight), which would never occur within a normal weight room. Additionally, Hakkinen fails to address for velocity specific parameters (68) as of which, most training programs incorporate a mixture of volume, load, and velocity parameters (40, 41). The incorporation of load and volume is called high intensity training, and this training involves lifting moderate to heavy resistance in a rapid fashion. When this occurs, power production and/or RFD are high (127).

2.1.4 Variability in Recruitment Order

Despite our understanding of recruitment order occurring in an order consistent with the size principle, it is possible that MUs are recruited selectively while other MUs with lower thresholds are inhibited (89). This notion though would require an amount of control over the inputs received by each MN as to exceed the capacity of the brain to accommodate all the possible combinations (92). There is also evidence that the order in which the MUs in a muscle are recruited can differ when the muscle contributes to different actions (32, 152). For example, the first dorsal interosseus muscle produces most of the abduction force exerted by the index finger, however, it is one of several muscles that flex the index finger about the metacarpophalangeal joint. Desmedt and Gidaux (52) found that 8% of the 142 MU pairs in the first dorsal interosseus consistently changed recruitment order between the two tasks. This has then been confirmed during static (169) and dynamic contractions (168). Lastly, recruitment order can be influenced by the manipulation of the feedback received by the MN pool. One investigation found that digital nerve stimulation of the index finger increased the recruitment threshold force of low-threshold MUs in the first dorsal interosseus and decreased it for high-threshold MUs (71). The interpretation form Garnett and Stephens article can indicate a preferential distribution of excitatory cutaneous input to high-threshold MUs.

2.1.5 Modulation of Firing Frequency During Voluntary Contractions

Just like MU recruitment, motor unit firing frequency depends primarily upon the level of force and the speed of action (126). In order to maintain a constant force during fatiguing contractions, there must be an increase in MU recruitment or some modulation

of firing frequency. Studies have reported a decline in MU firing rates as muscles becomes fatigued (22, 39, 43). It is understood that MU firing rates will decline during isometric contractions of moderate length (<30 s) and moderate to maximal effort MU firing rates will decline. On the other hand, the firing behavior of MUs during prolonged contractions remains controversial. For example, MU firing rates for the vastus lasteralis (VL) have been studied during prolonged submaximal isometric muscle actions (2, 3). Subjects were required to hold a force level corresponding to 50% of their MVC for 50 seconds, and the results showed that MU firing rates first increased but then decreased. Additionally, the authors reported that the recruitment threshold of MUs declined throughout the contraction series (2). The recruitment threshold force is typically measured as the force at which action potentials begin to discharge repetitively during a slow, ramp increase in muscle force. Additionally, recordings indicate the amount of discharge rate modulation that is exhibited by the detected MUs to produce the prescribed rate of change in force. Studies have noted a decrease in firing rates during submaximal fatiguing contractions for the majority of MUs in the following muscles: thenar, 45-90% MVC (72); biceps brachii, 11-60% MVC (70); and triceps brachii, 20% MVC (98). Some investigations have noted an increase in average firing rates towards the end of an experiment, however, this can be speculated to represent the recruitment of faster type II muscle fibers in order to achieve twitch fusion (19).

2.1.6 Mechanisms Underlying Rate Modulation

In addition to the magnitude of discharge rate modulation, recordings of MU discharge times have been used to infer details about the synaptic input and the state of the MN during voluntary contractions (89). Some examples of the above include the

quantification of correlated discharges, variability in discharge times, double discharges, and indices of persistent inward currents (PICs). There are two primary measures for correlated discharges, which include short-term MU synchronization and common drive (129). Motor unit synchronization is represented when there is a greater-than-chance number of coincidental discharge times that produce a central peak in the cross-correlation histogram. This is thought to be caused either by branched common input to the spinal MNs or by presynaptic synchronization due to common input to efferent fibers from the motor cortex. Variability in discharge times is typically expressed as the coefficient of variation for interspike interval. This will vary as a function of the difference between the recruitment threshold force of the MU and the force exerted by the muscle (64). Motor units recruited during a sustained contraction can exhibit high or low variability in discharge times due to factors such as the difference between the target force and recruitment threshold force, the compliance of the load, and the age of the individual.

Double discharges involve the generation of a second action potential when the MN is still within a state of depolarization or delayed depolarization (89). Generally, these double discharges occur during tasks when the muscle force changes rapidly, rather than during sustained contractions (42, 162). Although it is believed that double discharges can be increased with training, the functional significance still remains uncertain. It was once thought that the dendrites of MNs were passive conduits for synaptic inputs, however, it is known that they have voltage-dependent channels that provide the capacity to generate a very strong persistent inward current (PIC) (90). The

amplitude of a PIC is proportional to the level of neuro-modulatory input form the brain, which is modulated by serotonin and norepinephrine.

2.2 Neuromuscular Transmission Failure

2.2.1 Resting Membrane Potential

Motoneurons, like most cells, contain leak channels that primarily pass potassium (K^+), which maintains the resting potential at about -70 mV (89). This resting potential is typically seen with *in vitro* preparations where synaptic input is restricted, however, tonic synaptic input can alter the resting potential, which will substantially depolarize resting potential. Therefore, the resting potential should not be thought of as a steady parameter (89). The uncertainty of knowing the resting potential within humans makes for one of the greatest difficulties in understanding MU function. Sodium (Na^+) and calcium (Ca^{2+}) channels both contribute to action potential generation as well are active just sub threshold to spike initiation.

2.2.2 Presynaptic Sites of Neuromuscular Transmission Failure

Axons and presynaptic terminals are both presynaptic sites where neuromuscular transmission failure (NTF) can occur (157). When looking at the axon, NTF would result from a failure of axonal propagation of an action potential. Axonal propagation failure would present itself at the muscle fiber by an absence of evoked end-plate potential (EPP). As for the presynaptic terminal, NTF can stem from an inadequate neurotransmitter release. Failure at the presynaptic terminal would appear within a muscle fiber by a reduction in EPP amplitude and ineffective synaptic transmission. A

depression of EPP amplitude could also reflect postsynaptic mechanisms of NTF, which will be discussed in further sections below.

2.2.3 Failure of Axonal Propagation of Action Potentials

It has been known since the 1930's that action potentials fail to propagate along each branch of a motor nerve (16). Studies have shown that prolonged repetitive stimulation of the rat phrenic nerve resulted in significant incidence of action potential propagation failure, and this was attributed to a failure of propagation at axonal branch points (157). It was also noted that the incidence of presynaptic NTF was greater at higher rates of stimulation. In another study conducted by Smith (1980) within crayfish abductor muscle, it was reported that prolonged stimulation of the axon led to action potential propagation failure at axonal bifurcations (160). Blockage was found to first occur in the more peripheral terminal arborization of the axon and then progressively spread centrally to regions where axonal calibers were larger. Henceforth, these axonal bifurcations may be the sites where a failure of action potential generation and neurotransmission may occur.

Prolonged depolarization of the axonal membrane and a decrease in the inward Na^+ current may additionally accompany failure in axonal action potential propagation (87). Hyperpolarization of the axon may help reverse axonal propagation failure, or the axon can be placed in a solution with low external K^+ levels. It has been suggested that the above observations are due to a prolonged refractory period of the axon. This may be responsible for the failure of action potential generation and the consequent propagation failure at these axonal branch points. Therefore, axonal conduction block may be a result of alterations in perineural Na^+ and K^+ concentrations with repetitive axonal stimulation.

Additionally, studies have supported the above suggestions regarding [K^+] and the development of conduction failure by showing that during repetitive stimulation K^+ accumulates in the space surrounding the axon (4, 161) }. It is interesting to note that shifts in ion concentrations are more likely to occur in regions with smaller axonal sizes, that is, along the many axonal bifurcations. This is due to the surface-to-volume ratio is increased in these smaller axons, which will result in a more pronounced change in membrane potential due to ionic concentration changes (160).

2.2.4 Mechanisms of Failure at the Presynaptic Terminal

Neurotransmission failure at the presynaptic terminal can result from a reduction in the release of acetylcholine (ACh). The release of ACh into the synaptic cleft is done when Ca^{2+} enters the nerve terminal via voltage-dependent channels and attaches to the synaptic vesicles of the nerve terminal membrane. In 1954, del Castillo and Katz reduced extracellular Ca^{2+} at the frog NMJ, and demonstrated that Ca^{2+} influx is critical for neuromuscular transmission (50). Additionally, it has been discussed that calmodulin and other second messengers are affected during repetitive stimulation; however, a definitive explanation still remains unclear. Nevertheless, the release of ACh at the NMJ by the quantal (vesicular) may be reduced by either a decrease in Ca^{2+} influx through voltage-gated Ca^{2+} channels or the sensitivity of Ca^{2+} within the nerve terminal may be reduced that leads to vesicular release (157).

It has been recognized that during repetitive nerve stimulation of curarized nerve-muscle preparations, the change in EPP amplitude is dynamic (157). Initially, there is a facilitation of EPP amplitude, which is then followed by depression. Specifically, repetitive stimulation will elicit an accumulation of intracellular Ca^{2+} at presynaptic

terminals, and this may form the basis for the initial facilitation of EPP amplitude, i.e., an increased probability of quantal release and increased quantal content. The depression in EPP amplitude with repetitive stimulation has been attributed to either a reduction in the number of quanta of ACh being released by the presynaptic nerve terminal, or to a reduction in quantal size (50); (159). Furthermore, during repetitive stimulation, a reduction in the number of vesicles released by the presynaptic terminal may be attributed to a reduction in Ca^{2+} influx, a decrease in Ca^{2+} sensitivity of excitation-secretion coupling, or a depletion of the number of vesicles available for release (157). There may also be an accumulation of extracellular K^{+} during repetitive stimulation due to limited diffusion possibilities, and as a results, the terminal membrane would depolarize thereby initially increasing Ca^{2+} influx. Lastly, an increase in intracellular Ca^{2+} may also increase K^{+} conductance via Ca^{2+} dependent K^{+} channels. This makes the terminal membrane refractory to further depolarization, thus, the net effect would be a reduction in Ca^{2+} influx and reduced neurotransmitter release.

2.2.5 Branch Point Failure

The literature is almost vacant in regards to branch point failure, and this is interesting because the probability of a blockade of action potentials at axonal branch points may be an important factor influencing the differences in neurotransmission properties of different types of MUs (157). Branch point failure is simply defined as any failure along the neural bifurcations. Generally speaking, most neuronal axons of MNs have branches of varying lengths, and when a neuron is being stimulated at high frequencies, some of the longer branches cannot repolarize as fast as the rest of the axon. Thus, when the neuron is activated again, some of the branches can't depolarize because

they haven't been able to repolarize. Henceforth, failure occurs at the point of branching. Branch point failure can begin to develop at the onset of exercise, and this fatigue can be manifested by a failure of either a single MU or multiple MUs' by interfering with a muscle cells ability to contract.

Despite small changes in resistivity and/or capacitance, axonal conductance can be limited to only a band of stimulation frequencies, with varying probabilities of conduction block at different stimulation frequencies (157). Additionally, within smaller branches, axonal conduction failure may be facilitated at higher frequencies of stimulation compared to longer branches (163, 164). Neurotransmission failure may be enhanced due to this interaction between the susceptibility of action potential propagation failure and axonal geometry. This failure could have detrimental effects during competition despite possibly only occurring within a portion of muscle fibers in a MU. Therefore, a blockade of action potentials at axonal branch points should be seen as an important factor influencing NTF, particularly within different types of MUs. Type FF MUs are more susceptible to NTF due to they contain more muscle fibers within the unit, thus, a larger innervation ratio. Based upon the geometry of type FF muscle MUs, it can be speculated that axons innervating type FF MUs with larger innervation ratios will have a greater number of axonal branches, therefore, a greater probability of axonal conduction blockage. Sandercock and colleagues found action potential failure to be more prevalent in type FF MUs when compared to other MU types, and the incidence such presynaptic NTF was dependent on the frequency of stimulation (155). Lastly, any conduction block of only some muscle unit fibers during repetitive stimulation would reduce the effective innervation ratio of said MUs, thus, leading to a reduction in mechanical force.

2.3 Summary

The MU is a highly complex structure, thus, any small changes away from the normal operation can lead to detrimental consequences. It can be seen from the above section that any small change can lead to a decrease in force/power output, ultimately leading to fatigue. Many tend to think of fatigue as the point when exercise ceases, however, fatigue begins to set in once exercise begins. From the above section, it can be seen that during times of fatigue, MU recruitment and firing frequency will begin to change in an attempt to match the desired force needed to accomplish a task. Both recruitment and firing frequency have been studied extensively, however, branch point failure is relatively unknown, even amongst muscle physiologists. Therefore, future research should attempt to better understand the severity of branch point failure, with hopes to alleviate any potential blockage of action potentials. If branch point failure is a main culprit to a loss in force/power, then preventing this failure from occurring will only help to enhance both athletic competition and exercise in general.

CHAPTER 3. POSTSYNAPTIC SITES OF NEUROMUSCULAR TRASMISSION FAILURE

3.1 Introduction

Conversely to the presynaptic area of a MU, anything that follows the NMJ is considered the postsynaptic area. Within the postsynaptic area, fatigue can occur at either the motor endplate due to desensitization of the cholinergic receptor (AChrR) and/or at the sarcolemma due to a reduction in excitability (157). Investigations of postsynaptic failure dates back to the 1950s where Krnjevic and Miledi provided evidence that NTF

within rat diaphragm muscle at the NMJ was due a combination of a decrement in EPP amplitude and a reduction in sarcolemma excitability (109). Therefore, this led to a failure in the generation of action potentials in muscle fibers. Muscle contraction depends on electrical excitation of the muscle fiber, henceforth, if the muscle fiber is properly polarized, then neuromuscular transmission initiates an AP. Subsequently, this AP will propagate very rapidly along the muscle fiber surface (sarcolemma) in both directions and then much more slowly throughout the t-tubular system (T system). This synchronization of activating all parts of the muscle fiber via the rapid sarcolemma propagation is necessary in order to produce a useful contraction. Lastly, it is necessary to have AP propagation in the T system because passive spread of surface excitation into the T system is not enough to properly activate the voltage sensors and trigger Ca^{2+} release and contraction (6). This section will discuss the above mechanisms related that relate to proper AP propagation once at the postsynaptic area of the NMJ.

3.2 Excitability and Extracellular K^+ Accumulation

3.2.1 Desensitization of the Cholinergic Receptor

In 1957 Katz and Thesleff found that there was a reduction in endplate conductance due to a desensitization of the AChR following a prolonged exposure of the NMJ to ACh (106). Desensitization resembles the end plate under times of no activity by involving a slow transition of AChR channels to non-conducting states. Additionally, this desensitization is primarily due to the continued presence of ACh, and recovery of this disruption can only be brought about through the removal of the stimulation (65). In above sections, it was discussed that quantal release during repetitive stimulation would

result in a decrease in EPP amplitude, however, a decrease in amplitude may reflect this desensitization of the AChR. Especially at higher frequencies of stimulation, this desensitization would result from a continued accumulation of ACh in the synaptic cleft, and this would be compounded by both an inadequate diffusion of the transmitter (ACh) out of the synaptic cleft and an incomplete hydrolysis of the ACh by acetylcholinesterase (106). Lastly, it has been suggested that cyclic AMP may be involved in the phosphorylation of the AChR (157). Changes in cAMP levels during repetitive stimulation may lead to AChR desensitization and reduced EPP amplitudes.

3.2.2 Ion Channel Types and Distribution

Rapidly activating and inactivating voltage-dependent channels carry the sodium current within adult muscle (105), with the density of these Na^+ channels being higher in fast-twitch than slow-twitch muscles (147). Additionally, there is approximately a threefold higher amount of Na^+ channels located near the NMJ than at the ends of the fiber, and is thought to provide a safety factor for action potential imitation (147). The total number of Na^+ channels on the sarcolemma is similar to that in the T system, however, the nearly fivefold larger membrane area of the T system means that the Na^+ channel density in the T tubules is only about 20% of the surface density (55). The larger surface area enables for fast conduction and increases the likelihood of initiating an action potential in each t tubule. On the other hand, the relatively small tubular Na^+ current is normally adequate for propagation (6). The most common potassium (K^+) channel on the sarcolemma is the ATP-sensitive K^+ channel, and these channels are also found in the T system but at a lower density (131). Additionally, there are also Ca^{2+}-

activated large conductance K^+ channels at approximately equal density in the sarcolemma and T system (131).

3.2.3 Sarcolemma resting potential

It has been suggested that preventing cell activation by altering sarcolemma function can induce muscle fatigue (66). This theory believes that the K^+ efflux and inhibition of the Na^+-K^+ pump (or its inability to keep pace with K^+ efflux and Na^+ influx) causes the cell to depolarize, reduced action potential amplitude, and in some cells, complete inactivation. Cell depolarization has been suggested to provide a safety mechanism to protect the cell against ATP depletion and Ca^{2+} accumulation (56). Specifically, when the uncoupling of activation occurs at the first step rather than latter such as at the cross-bridge cycle, then ATP-utilizing steps will be prevented along with increases in intracellular Ca^{2+}. Armstrong and colleagues have proposed that the latter might activate Ca^{2+} sensitive proteases and phospholipases, which would lead to sarcolemma and intracellular organelle disruption (9). Additionally, it has been speculated that the membrane mechanism of fatigue would allow contractions at reduced rates and forces while preventing catastrophic changes in cellular homeostasis that might lead to cell damage (112, 113). It is not established that this change affects the propagation of the sarcolemma action potential into the t-tubules or subsequent steps in E-C coupling (66).

3.2.4 Reduction in Sarcolemma Excitability

Sarcolemma excitability may be reduced due in part to repetitive activation of the NMJ, and Edwards has further categorized fatigue into high- and low-frequency fatigue

(57). High-frequency fatigue is characterized by a transient, rapidly recovering loss of force after high frequencies of stimulation, and low-frequency fatigue is characterized by prolonged loss in force. Additionally, Edwards suggested that high-frequency fatigue was due partly to a failure of action potential propagation by the sarcolemma (57). This would result from an accumulation of extracellular K^+, membrane depolarization, and Na^+ channel inactivation leading to a reduction in Na+ conductance (21, 57) Conversely, the influence of K^+ on sarcolemma excitability during repetitive stimulation of muscle remains controversial. Despite this controversy, Juel found that high-frequency fatigue of the mouse soleus and extensor digitorium longus muscle was associated with a decrease in action potential propagation velocity (103). This decrease was induced by extracellular K^+, however, was nearly independent of moderate change in the Na^+ gradient. Additionally, SjØgaard suggested that an accumulation of K^+ occurred following repetitive stimulation, and she attributed the increase in extracellular K^+ the opening of Ca^{2+}-dependent K^+ channels (158).

On the other hand, there are studies that suggest that changes in extracellular K^+ concentrations are not large enough to change sarcolemma excitability (157). Metzger and Fitts found no change in resting membrane potential of muscle fibers following either high- or low-frequency stimulation, which argues against the accumulation of extracellular K^+ (122). The authors argued that the decline in amplitude of the sarcolemma action potentials during repetitive stimulation was most likely due to changes in extracellular Na^+. What may play a role in muscle fatigue at the sarcolemma are pH levels. Renaud found that extracellular pH inhibits tetanic force recovery by acting on the outer surface of the sarcolemma (143). A few years later Brody and

colleagues reported that changing extracellular pH initially results in decreased conduction velocity along the muscle membrane (28). This decrease was shown via a leftward shift in the median frequency of the EMG, however, with stimulation, the rate of decay of conduction velocity was less than the rate of decay of the median frequency. The authors suggested that this represents a complex interaction between pH and sarcolemma excitability (28).

3.2 Factors Preventing Loss of Excitation During Normal Exercise

3.3.1 Motor Unit Recruitment

The CNS can vary the MUs activated during submaximal force production *in vivo*, and this varying is done so to spread the workload across the pool of MUs (61). This obviously will help reduce the requirements on a given MU. During times of activity, less fatigable MUs will be utilized at lower workloads, and the most fatigable MUs performing the highest power movements will be recruited only during very demanding tasks (19). The recruitment of additional MUs during activity explains why EMG recordings increase, which is necessary to maintain the desired force output. Additionally, discharge rates of originally active MUs can decrease during prolonged submaximal contractions. This occurs despite that the overall excitatory drive to the MN poll increases as fatigue develops (70).

3.3.2 Activation at Optimal Firing Rate

Fast-twitch and slow twitch MUs are generally activated at the firing rate most appropriate to the contractile properties (6). This is done to give a fused tetanus of close to maximum force, which in turns keeps the number of Amps used to excite a muscle

fiber to the minimum (22). If MUs are stimulated at higher than optimal firing rates, whether from imposed nerve or muscle stimulation, then this will cause the muscle fibers to fatigue more rapidly than during voluntary contractions (6).

3.3.3 Decreasing Firing Rate

During submaximal contractions, MNs will decrease their firing rates, and this reduction is well matched to the slowing of relaxation that occurs with muscle activation (20). Basically, firing rates will remain at a sufficient enough rate to elicit the greatest tetanic force possible at a given point in time. Additionally, impulse trains *in vivo* typically begin with groups of closely spaced impulses (doublets), and these appear to enable force production with less associated fatigue (23). This may be partly due to the force production occurring with the closely spaced impulses, which will give more effective force production for a given number of Aps (6).

3.3.4 Sarcolemma AP Changes

Repeated activation can elicit some unwarranted changes amongst the sarcolemma, including slowing the propagation of the surface AP (103), and/or reduce its size and increase its duration (12). However, it appears the proposed changes to the surface AP do not appear to contribute to fatigue (12). The AP evidently is able to sufficiently initiate an AP within the T system due in part to a large safety factor in the process. Essentially, sarcolemma changes and the accompanying AP can lead to intermittent failure in a train of APs, however, the accompanying slowing of fusion frequency means that AP failure does not evidently contribute to decreased force (6).

3.3.5 Na -K^+ Pumping

The Na^+-K^+ pump plays a vital role lowering [K^+], particularly in the T system (44). Muscle activation, raised intracellular [Na^+], β-adrenergic stimulation, and raised temperature all help to stimulate Na^+-K^+ pump activity, and this can be increased to more than 20-fold above its resting rate (132, 136, 138). For example, within rat fast-twitch muscle, ~50% of all α_2-isoform of the pump are in the T system and are estimated to be able to clear K^+ at ~4 mM/s (133). Additionally, pump activity is electrogenic and can hyperpolarize a muscle fiber a further ~8 mV (44).

3.4 Summary

Although many *in vitro* and in *vivo* studies show that a loss of muscle excitability can occur with imposed stimulation, it appears that this may not appear to be the cause of fatigue in exercising humans. The reason why is because many compensatory processes act together to prevent changes in [K^+] and fiber depolarization or to reduce their deleterious effects. At the postsynaptic membrane, NTF may be more a result of both AChR desensitization and sarcolemma excitability during repetitive stimulation. Obviously, failure at the postsynaptic junction may adapt under a variety of conditions, and the contribution of each might vary depending on fiber type.

CHAPTER 4. CONCLUSION

4.1 Practical Implications

It is difficult to equate the majority of fatigue research to an athletic and/or exercise environment due to low application the research offers. The majority of the

research to date isn't necessarily bad, however, the majority of research is not necessarily applicable to large, gross movements. One issue that arises is that investigations utilize small muscles that are relatively unimportant within large movements, that is, generally not seen as a prime mover within an athletic competition. For example, a lot of the research has investigated the fatigability of the thenar muscle under isometric contractions. It must be stated that in order to understand any mechanism within the body, a certain level of research must be established. However, trying to equate research of a small muscle like the thenar is extremely difficult when trying to understand fatigue within a MU at a gross level, such as during sport or exercise. What is interesting is that after nearly 90 years of research of the MU, mechanisms have yet to be fully elucidated. Using muscles like the thenar can be beneficial to get some understanding of how MUs behave under fatiguing conditions, but eventually investigating larger, prime movers must be undertaken in order to get an idea of how the body actually reacts to fatiguing conditions.

Another issue that arises with MU investigations is the utilization of isometric contractions. Along with investigations utilizing small muscle groups, these types of contractions within a protocol are not necessarily wrong to use, however, muscles experience all three types of contractions during natural movements. Therefore, it makes it very difficult to determine how MUs will react under a fatiguing condition that is seen during normal movements that incorporate both concentric and eccentric actions. On the other hand, trying to investigate MU activity within sport is a difficult task, which is one reason as to why not much has been done within this realm. It is fairly easy to get recordings of a thenar muscle performing an isometric contraction. But, trying to see how

larger muscles like the quadriceps react during a sprint is much more difficult. Nonetheless, for the benefit of sport performance based research, it would be in the best interest to start looking at fatigue within gross movements.

In addition to fatigue research involving the MU, trying to see how metabolic by-products influence fatigue is another difficult task to accomplish. Over 90 years ago lactate was thought to be the cause of fatigue, however, it is now starting to be shown that it associated with fatigue and not the cause. The problem is that conventional wisdom still believes it to be one of the main causes of fatigue. It is understandable why fatigue from metabolism has not progressed as much as it should have because acquiring samples either during or directly after an event is difficult. The issue arises when the biochemistry doesn't support what conventional wisdom has been saying for over 90 years. Nonetheless, the majority of metabolic by-products that are produced are caustic in nature, thus, it would be plausible that these by-products may inflect a change in the way other tissues function. Take ammonia for example. Ammonia is used to clean floors, therefore, why would it be beneficial for cells when produced in high amounts? Nonetheless, the way metabolic by-products impact other tissues still remains controversial. Additionally, acquiring blood samples will show different recordings than that at the muscular level. Not many individuals will allow a muscle biopsy to be taken in general, let alone during an activity.

Branch point failure seems to be one plausible source of fatigue. The problem with this notion is that it is a theory with little supporting evidence. How to fully study branch point failure still remains a mystery due to the difficultly of reaching axonal bifurcations, especially during an activity. On the surface though, branch point failure

does seem to make sense. In theory, if axonal bifurcations are being fatigued in any way, then that will ultimately lead to a diminished force output. As stated, force is modulated during voluntary actions by a combination of MU recruitment and changes in MU activation frequency. Thus, branch point failure could result in fewer MUs being recruited or a decrease in firing frequency, both of which are unfavorable during an activity. Also, more MUs could be recruited or firing frequency could be increased in other MNs due to blockage in hopes to match a desired force output. If this happens, then force will be decreased and unwarranted performance during either exercise or sport will occur. Nonetheless, no matter the changes, the most preferable situation would be for axonal bifurcations to not experience any failure. Since this theory still remains, more needs to be done in order to understand how MNs react under fatiguing conditions.

4.2 Future Research

Despite the difficulties, more needs to be done in order to understand the mechanisms leading to fatigue during both sport and exercise. In order for fatigue research to advance, more needs to be done to see how metabolic by-products influence other tissues, especially the MN. What is interesting is that our culture is possessed by sport and exercise, however, there is little interest in wanting to see what limits individuals from performing at the highest level possible. Additionally, understanding the mechanisms behind fatigue could possibly help with preventing injuries. This remains to be fully explained, however, future research would hopefully aid athletes to be able to perform at their most optimal level.

One way to study fatigue is to incorporate protocols that are utilized by strength coaches. Going off the previous section, many protocols used are something that would

never be seen in a weight room. For example, some protocols have looked at resistance training protocols by using over 10 sets of 5 repetitions. Anyone that has worked in a weight room knows that this is a type of training coaches never use in their programs. Additionally, these same protocols will lower the weight as set progress to maintain force output. When this happens, how can one equate their findings to what is actually happening within the body? If researchers are lowering weight each set to maintain force output, then did fatigue actually occur? Most strength programs will have their athletes increase weight and decrease reps as sets progress. Therefore, to understand fatigue, protocols should mimic that which is seen in most strength & conditioning weight rooms. Future research should aim to investigate muscles that play the major roles during movements, such as the quadriceps, hamstrings, or glutes. As stated, a lot of neuromuscular research focuses on the thenar muscle due to accessibility and ease of use. Unfortunately, the muscle mechanics of the thenar muscle are different from that of larger muscles. It is likely that the muscle fibers within the thenar muscle runs from origin to insertion, while the quadriceps muscle fibers are going to be more complex. Large muscles like the quadriceps will multiple layers of fibers that don't run the entire length of the muscle. This added complexity will only change up muscle mechanics when relating to neuromuscular fatigue. Therefore, future research should direct itself to understanding how muscles that are primary movers during an activity are affected while under fatiguing conditions.

In conclusion, fatigue research needs to start redirecting itself to focusing on more applicable investigations, that is, protocols that better mimic what is seen in competition or the weight room. There is no perfect study that will perfectly mimic what is seen

during sport, however, more can be done to start understanding how the body reacts during times of competition. As stated earlier, a better understanding fatigue will only aid in allowing athletes to perform at their highest level possible. Additionally, this may also help reduce the number of injuries sustained. This is all why more needs to be done with neuromuscular and metabolic factors of fatigue.

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APPENDIX 2. IRB/CONSENT FORMS

ACTION ON PROTOCOL APPROVAL REQUEST

Institutional Review Board
Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu | lsu.edu/irb

TO: Arnold Nelson
Kinesiology

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: October 27, 2014

RE: IRB# 3552

TITLE: The Impact of Weight Lifting Upon Neurotransmission Failure within Trained Males and Females

New Protocol/Modification/Continuation: New Protocol

Review type: Full ____ Expedited X **Review date:** 10/16/2014

Risk Factor: Minimal ___X___ Uncertain _____ Greater Than Minimal_____

Approved_____X_____ **Disapproved**_____

Approval Date: 10/16/2014 **Approval Expiration Date:** 10/15/2015

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 36

LSU Proposal Number (if applicable): _____

Protocol Matches Scope of Work in Grant proposal: (if applicable) _____

By: Dennis Landin, Chairman _____

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants, including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. SPECIAL NOTE:

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb*

Consent Form

1. Study Title:
The impact of weight lifting upon neurotransmission failure within trained males and females

2. Performance Sites:
Louisiana State University, Department of Kinesiology

3. Contacts:
Dr Arnold Nelson wk: 2225-578-3114
Tyler Farney 719-251-9320

4. Purpose of the Study:
The study will investigate the impact that weight training upon muscle mechanical properties

5. Subjects:
A. Inclusion Criteria
Any female or male between the ages of 18 and 35 currently enrolled in an LSU weight training class.

B. Exclusion Criteria
Any female or male who has a history of knee or back problems of any type. Anyone who answers "yes" to any question on the PAR-Q form.

C. Maximum number of subjects: 36

6. Study Procedures:
Experimental Overview
Seven days of testing will be conducted during this protocol, with two different weight lifting exercises being investigated (power clean and calf raise). The first day will consist of acquiring a 5-rep maximum (5RM) for both power clean (PC) and calf raise (CR). As for the other 6 days, each participant will perform each exercise over 3 days. On each of the 3 days for both exercise, participants will perform each exercise at a set number of repetitions (1, 3, or 5), which will be administered in a random order. H-reflex will be recorded pre- and post-lifting sessions via stimulating the femoral nerve and recording at the gastrocnemius, and M-wave will be acquired via EMG recordings of the same muscle.
Training Protocol
The training protocol will consist of 6 training sessions involving the power clean (PC) and calf raise (CR), with 3 sessions per exercise. Participants will perform both exercises with each training session consisting of a set number of repetitions – 1, 3, or 5. Both exercise and daily repetitions will be in a randomize order.

Power Clean and Calf Raise 5RM weight lifting exercise
For both the 5RM and all training sessions, the PC and CR will be performed with an Olympic barbell and bumper plates. Prior to all sessions, participants will begin with a five-minute warm-up via a stationary cycle ergometer. Following a standard bike warm-up, participants will perform an un-weighted barbell warm-up that will go through the full range of motion that will be occurring during the PC and CR. Next, each participant will begin by performing the designated exercise with the prescribed repetitions at 50% and 75% of their 5RM. Finally, participants will perform the prescribed repetitions at their 5RM.

M-wave and H-reflex

EMG of the gastrocnemius will be recorded from 1 second before to 1 second after the exercise. H-reflex of the gastrocnemius will be recorded via stimulating femoral nerve and recording at the gastrocnemius. This will be done both right before and right after each participant performs the final set.

7. Benefits: No personal benefits will be gained from participation, other than the knowledge that the individual has helped researchers gain more insight into the workings of the human body during exercise.

8. Risks/Discomforts: Participation in the weight lifting may result in muscle soreness 24-72 hours after completion of the activities. This muscle soreness can lead to a feeling of moderate pain and discomfort for the 24-72 hours. Also some people may feel a slight pricking sensation during the H-reflex test.

9. Measures taken to reduce risk: The weight lifting exercises have a "warm-up" built into them to help alleviate muscle soreness. This "warm-up", however, will not totally eliminate the chance of muscle soreness, especially for those persons who do not use their muscle regularly. Also, the students will have participated in at least six months of weight training and this is usually enough training to prevent muscle soreness during a 5RM test.

10. Right to Refuse: All volunteers may terminate their participation at anytime and without warning. The individual just needs to stop the activity and leave the testing room. No attempt will be made by the research personnel to stop the volunteer. In fact, the research personal will do all in their power to help the volunteer leave at any time.

11. Privacy: The results of this study will be tabulated in an anonymous manner. This means that the researchers will be unable to give you your results, as they will not be able to identify yours from the rest

12. Financial Information: The project will not effect the participants finances in either a positive or a negative manner.

13. Withdrawal: Withdrawal from the study can occur at anytime without any fear of punitive action.

14. Removal: If the researcher has trouble scheduling the volunteer to come into the weight room, that volunteer will be dropped from the list of volunteers. The volunteer has the right to change their minds about participation at anytime before or during the study.

Part 5: Signatures:

The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have questions about subjects' rights or other concerns, I can contact Robert C. Mathews, Chairman, LSU Institutional Review Board, (225)578_8692. I agree to participate in the study described above and acknowledge the researchers' obligation to provide me with a copy of this consent form if signed by me.'

Printed Name of Participant	Date
Signature of Participant	
Signature of Person Administering Informed Consent	Date

ACTION ON PROTOCOL APPROVAL REQUEST

Institutional Review Board
Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu | lsu.edu/irb

TO: Arnold Nelson
Kinesiology

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: August 17, 2015

RE: **IRB#** 3628

TITLE: The effects of high-intensity exercise on isometric strength parameters

New Protocol/Modification/Continuation: New Protocol

Review type: Full __X__ Expedited ____ **Review date:** 8/3/2015

Risk Factor: Minimal _____ Uncertain __X__ Greater Than Minimal _____

Approved ____X____ **Disapproved** _____

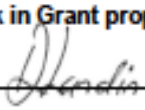
Approval Date: 8/14/2015 **Approval Expiration Date:** 8/13/2016

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 40

LSU Proposal Number (if applicable):

Protocol Matches Scope of Work in Grant proposal: (if applicable)_____

By: Dennis Landin, Chairman 

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants, including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. SPECIAL NOTE:

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb*

Consent Form

1. Study Title:

The effects of high-intensity exercise on isometric strength parameters.

2. Performance Sites:

LSU Biomechanics Lab

3. Contacts:

Dr. Arnold Nelson (Work 578-3114/ Hm. 766-4621)
Tyler Farney (Cell. 719-251-9320)
Dr Michael MacLellan (Work 578-3314)

4. Purpose of the Study:

The study will investigate the acute effects of high-intensity exercise on isometric strength.

5. Subjects:

a. Inclusion Criteria

Healthy males and females ages 18-40 who exercise no less than 30 minutes 3 day/week for the last 6 months.

b. Exclusion Criteria

Anyone with a history or current cardiovascular disease and/or individuals that answer "yes" to any question on the PAR-Q will be excluded from the study.

c. Maximum number of subjects: 40

6. **Study Procedures:**

Experimental Overview

One day of testing will be done to investigate the muscle contractile characteristics changes of healthy, exercise trained individuals. Each subject will perform one high-intensity exercise protocol consisting of barbell thrusters, squat jumps, walking lunges, and forward jumps. A pre and post-exercise measurement to determine changes of muscle activation, force production, and rate of force development (RFD). Upon agreement of participating in this study, each subject will undergo a familiarization session.

High Intensity Exercise Protocol

Each subject will perform one high-intensity exercise protocol consisting of four exercises: barbell thrusters, squat jumps, walking lunges, and forward jumps. Subjects will go through each exercise four times for a set determined number of repetitions. Ample rest time will be allotted to ensure completion.. Ample rest time will be allotted to ensure completion. The barbell thruster will be performed by first completing a front squat, and then as the subject stands from the squat, they will push the bar overhead, ending with the bar balanced over your heels. The jump squat will be performed by first

doing a regular squat, followed by then jumping up explosively. When the subject lands, they will lower their body back into the squat position to complete one rep. The lunge jumps will be performed by stepping forward with one leg while simultaneously lifting up onto the ball of the back foot. Subjects will then jump and switch legs, ending with the opposite leg in the forward position. The forward jumps will be performed by jumping forward over twelve inch hurdles.

Electromyography (EMG) Protocol

Small surface electrodes will be placed on the skin to record muscle activity. The electrodes used to collect muscle activation will include Motion Lab Systems Inc. designed pre-amplifiers that have stainless steel contacts for the skin as well as the traditional silver-silver chloride gel electrodes. These electrodes will be placed on the bellies of the specific muscles with the location determined using SENIAM guidelines or by palpation. Prior to the placement of the electrodes, the specific sites will be cleaned with rubbing alcohol and shaved with a disposable razor. The electrodes will initially be affixed using hypoallergenic tape, then reinforced using a larger latex-free, self-adhering, cohesive wrap. Once setup is complete, the experiment will begin. Recordings of the quadriceps muscle will be acquired with both pre and post-measurements.

Isometric Strength Protocol

A force platform will be used to record vertical ground reaction force (VGRF) data to determine mean and peak force (Newtons), and RFD After an initial warm up, subjects will pull on an immovable barbell from the mid-thigh pull position for 5 seconds in 3 separate trials. Each 5 second trial will be separated by a rest period of 3 minutes. The barbell will be secured with safety pins in a resistance training rack over the force platform to record vertical ground reaction force (VGRF) data (Newtons). To ensure maximal force output, subjects will be encouraged to pull the bar explosively with maximal effort for the full 5 seconds.

7. Benefits: No personal benefits will be gained through participation in this study, other than the knowledge that the individual has helped researchers gain more insight about how a single exercise session can alter muscle contractile properties.

8. Risks/Discomforts: Exercise does entail the possibility of injury to the muscle. Additionally, there might be a slight risk of skin irritation due to the tape used to attach electrodes. If injury shall occur, volunteers will be directed to the LSU Student Health Center. Any medical issues that come about from this study will be taken care of via volunteers' own insurance coverage.

9. Measures Taken to Reduce Risk: The loads in this experiment should be well within the capabilities of the population but the possibility of injury is still there. In order to reduce this risk, all strength protocols will be supervised by graduate students or faculty with proof of human research subjects training (IRB certificate). Furthermore, supervisors will all have the proper credentials/certifications (e.g. CSCS) indicating training in monitoring weight lifting. In addition all supervisors and subjects will undergo a familiarization period for the exercises and isometric mid-thigh pull. Supervisors are in charge of equipment, instructing the subjects in correct technique of movements related to the study, encouraging the subjects to exercise with correct technique while trying to maximize force and power, and stopping subjects who fail to adhere to correct technique associated with the exercises and mid-thigh pull.

10. Right to Refuse: All volunteers may terminate their participation at any time and without warning. No attempt will be made by the research personnel to stop the volunteer.

11. Privacy: Data collection and results will be kept private, and will be tabulated in an anonymous manner. All information will be stored in a filing cabinet, with the room being able to be locked when researchers are not present.

12. Financial Information: Volunteers will not receive any finances for participating in the study.

13. Withdrawal: Withdrawal from the study can occur at any time without any fear of punitive action.

14. Removal: If the researcher has trouble scheduling the volunteer to come into the testing room, that volunteer will be dropped from the list of volunteers. The volunteer has the right to change their minds about participation at any time before or during the study.

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15. Signatures:

The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have questions about subjects' rights or other concerns, I can contact Dennis Landin, Institutional Review Board, (225) 578-8692, irb@lsu.edu, www.lsu.edu/irb. I agree to participate in the study described above and acknowledge the investigator's obligation to provide me with a signed copy of the consent form.

Subject Signature:_____ Date:_____
The study subject has indicated to me that he/she is unable to read. I certify that I have read this consent form to the subject and explained that by completing the signature line above, the subject has agreed to participate.

Signature of Reader: _____ Date:_____

ACTION ON PROTOCOL APPROVAL REQUEST

Institutional Review Board
Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu | lsu.edu/irb

TO: Arnold Nelson
Kinesiology

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: October 16, 2015

RE: **IRB#** 3628

TITLE: The effects of high-intensity exercise on isometric strength parameters

New Protocol/Modification/Continuation: Modification

Brief Modification Description: Add a finger prick and give participants aspartate

Review type: Full ___ Expedited __X__ **Review date:** 10/8/2015

Risk Factor: Minimal _____ Uncertain ____X____ Greater Than Minimal_____

Approved___X____ **Disapproved** _____

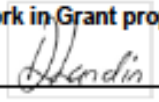
Approval Date: 10/16/2015 **Approval Expiration Date:** 8/13/2016

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 40

LSU Proposal Number (if applicable):

Protocol Matches Scope of Work in Grant proposal: (if applicable) _____

By: Dennis Landin, Chairman 

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. **SPECIAL NOTE:**

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb*

ACTION ON PROTOCOL APPROVAL REQUEST

Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu | lsu.edu/irb

TO: Arnold Nelson
Kinesiology

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: January 19, 2016

RE: **IRB#** 3628

TITLE: The effects of high-intensity exercise on isometric strength parameters

New Protocol/Modification/Continuation: Modification

Brief Modification Description: Give participants sodium bicarbonate via baking soda prior to the protocol.

Review type: Full ___ Expedited __X__ **Review date:** 1/19/2016

Risk Factor: Minimal _____ Uncertain ____X____ Greater Than Minimal_____

Approved___X___ **Disapproved** _____

Approval Date: 1/19/2016 **Approval Expiration Date:** 8/13/2016

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 40

LSU Proposal Number (if applicable):

Protocol Matches Scope of Work in Grant proposal: (if applicable) _____

By: Dennis Landin, Chairman _____

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
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6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. **SPECIAL NOTE:**

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb*

ACTION ON PROTOCOL APPROVAL REQUEST

Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu | lsu.edu/irb

TO: Arnold Nelson
Kinesiology

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: January 19, 2016

RE: **IRB#** 3628

TITLE: The effects of high-intensity exercise on isometric strength parameters

New Protocol/Modification/Continuation: Modification

Brief Modification Description: Add trial that contains aspartate and sodium bicarbonate together, test lactate levels, 4 days of testing for the exercise, and one more day of familiarization.

Review type: Full ___ Expedited __X__ **Review date:** 1/19/2016

Risk Factor: Minimal _____ Uncertain ____X____ Greater Than Minimal_____

Approved___X___ **Disapproved** _____

Approval Date: 1/19/2016 **Approval Expiration Date:** 8/13/2016

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 40

LSU Proposal Number (if applicable):

Protocol Matches Scope of Work in Grant proposal: (if applicable) _____

By: Dennis Landin, Chairman _____

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
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4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. **SPECIAL NOTE:**

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb*

ACTION ON PROTOCOL APPROVAL REQUEST

Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu | lsu.edu/irb

TO: Arnold Nelson
Kinesiology

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: February 5, 2016

RE: **IRB#** 3628

TITLE: The effects of high-intensity exercise on isometric strength parameters

New Protocol/Modification/Continuation: Modification

Brief Modification Description: Collect 10mL of blood via venipuncture

Review type: Full ___ Expedited _X_ **Review date:** 2/4/2016

Risk Factor: Minimal ___X___ Uncertain _____ Greater Than Minimal_____

Approved___X___ **Disapproved** _____

Approval Date: 2/4/2016 **Approval Expiration Date:** 8/3/2016

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 40

LSU Proposal Number (if applicable):

Protocol Matches Scope of Work in Grant proposal: (if applicable) _____

By: Dennis Landin, Chairman _____

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. **SPECIAL NOTE:**

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb*

Consent Form

1. Study Title:

The effects of high-intensity exercise on isometric strength parameters.

2. Performance Sites:

LSU Biomechanics Lab

3. Contacts:

Dr. Arnold Nelson (Work 578-3114/ Hm. 766-4621) anelso@lsu.edu
Tyler Farney (Cell. 719-251-9320) tfarne1@lsu.edu
Dr Michael MacLellan (Work 578-3314) michaelm@lsu.edu
Dr. Neil Johannsen (578-5314) njohan1@lsu.edu

4. Purpose of the Study:

The study will investigate the acute effects of high-intensity exercise on isometric strength.

5. Subjects:

a. Inclusion Criteria

Healthy males and females ages 18-40 who exercise no less than 30 minutes 3 day/week for the last 6 months.

b. Exclusion Criteria

Anyone with a history or current cardiovascular disease and/or individuals that answer "yes" to any question on the PAR-Q will be excluded from the study.

c. Maximum number of subjects: 40

6. **Study Procedures:**

Experimental Overview

A randomized crossover design will be used to test the acute effect of aspartate and sodium bicarbonate. Each participant will be tested four times after ingesting 20g of sports drink powder dissolved in water containing 12.5g of aspartate, 20g of sports drink powder dissolved in water containing .3 g/kg of sodium bicarbonate, 20g of sports drink powder dissolved in water with a combination of 12.5g of aspartate and 0.3g/kg of sodium bicarbonate, or a placebo containing 20g sports drink powder dissolved in water. Each subject will consume all the conditions 23 hrs prior to testing, and then again one hour prior to testing. The experimental design will be a double-blind in addition to placebo controlled. A one-week 'wash-out' period will separate each treatment.

High Intensity Exercise Protocol

Each subject will report to the lab five times to perform one high-intensity exercise protocol consisting of four exercises: barbell thrusters, squat jumps, walking lunges, and

forward jumps. The first session will consist of a familiarization trial consisting of the four exercises. Following the familiarization trial, the remaining four sessions will begin all separated by one week. Each session will consist of a pre- and post-exercise blood draw & finger prick. Subjects will go through each exercise four times for a set determined number of repetitions. The barbell thruster will be performed by first completing a front squat, and then as the subject stands from the squat, they will push the bar overhead, ending with the bar balanced over your heels. The jump squat will be performed by first doing a regular squat, followed by then jumping up explosively. When the subject lands, they will lower their body back into the squat position to complete one rep. The lunge jumps will be performed by stepping forward with one leg while simultaneously lifting up onto the ball of the back foot. Subjects will then jump and switch legs, ending with the opposite leg in the forward position. The forward jumps will be performed by jumping forward over twelve inch hurdles.

Blood Draws and Finger Prick

Each exercise session will consist of a pre- and post-exercise blood draw & finger prick. Upon arrival to the lab, a standard venipuncture blood draw of 10 mL will be acquired by Dr. Johannsen. Dr. Johannsen has been approved to collect blood through the LSU IRB. Additionally, a finger prick testing will be done immediately pre-exercise to determine lactate levels. Each finger prick will collect a max of 40 μL of blood via a capillary tube. Following completion of each exercise protocol on all testing days, a post blood draw and finger prick will be collected. A total of 2 blood draws and finger pricks will be collected for each testing day.

Electromyography (EMG) Protocol

Small surface electrodes will be placed on the skin to record muscle activity. The electrodes used to collect muscle activation will include Motion Lab Systems Inc. designed pre-amplifiers that have stainless steel contacts for the skin as well as the traditional silver-silver chloride gel electrodes. These electrodes will be placed on the bellies of the specific muscles with the location determined using SENIAM guidelines or by palpation. Prior to the placement of the electrodes, the specific sites will be cleaned with rubbing alcohol and shaved with a disposable razor. The electrodes will initially be affixed using hypoallergenic tape, then reinforced using a larger latex-free, self-adhering, cohesive wrap. Once setup is complete, the experiment will begin. Recordings of the quadriceps muscle will be acquired with both pre and post-measurements.

Isometric Strength Protocol

A force platform will be used to record vertical ground reaction force (VGRF) data to determine mean and peak force (Newtons), and RFD After an initial warm up, subjects will pull on an immovable barbell from the mid-thigh pull position for 5 seconds in 3 separate trials. Each 5 second trial will be separated by a rest period of 3 minutes. The barbell will be secured with safety pins in a resistance training rack over the force platform to record vertical ground reaction force (VGRF) data (Newtons). To ensure maximal force output, subjects will be encouraged to pull the bar explosively with maximal effort for the full 5 seconds.

7. Benefits: No personal benefits will be gained through participation in this study, other than the knowledge that the individual has helped researchers gain more insight about how a single exercise session can alter muscle contractile properties.

8. Risks/Discomforts: Exercise does entail the possibility of injury to the muscle. For the blood draw and finger prick, there is a possibility of pain, bruising, and/or infection at the site of the needle insertion. Additionally, there might be a slight risk of skin irritation due to the tape used to attach electrodes. Aseptic (sterile) technique will be enforced to minimize the risk. Dr. Johannsen will perform all blood draws, and has been trained to perform insertions under the supervision of a licensed physician. If injury shall occur, volunteers will be directed to the LSU Student Health Center. Any medical issues that come about from this study will be taken care of via volunteers' own insurance coverage.

9. Measures Taken to Reduce Risk: The loads in this experiment should be well within the capabilities of the population but the possibility of injury is still there. In order to reduce this risk, all strength protocols will be supervised by graduate students or faculty with proof of human research subjects training (IRB certificate). Furthermore, supervisors will all have the proper credentials/certifications (e.g. CSCS) indicating training in monitoring weight lifting. In addition, all supervisors and subjects will undergo a familiarization period for the exercises and isometric mid-thigh pull. Supervisors are in charge of equipment, instructing the subjects in correct technique of movements related to the study, encouraging the subjects to exercise with correct technique while trying to maximize force and power, and stopping subjects who fail to adhere to correct technique associated with the exercises and mid-thigh pull. To prevent any infection from the finger prick, the finger will first be cleaned with a 62% alcohol gel. A single use disposable safety lancet will be used for each prick.

10. Right to Refuse: All volunteers may terminate their participation at any time and without warning. No attempt will be made by the research personnel to stop the volunteer.

11. Privacy: Data collection and results will be kept private, and will be tabulated in an anonymous manner. All information will be stored in a filing cabinet, with the room being able to be locked when researchers are not present.

12. Financial Information: Volunteers will not receive any finances for participating in the study.

13. Withdrawal: Withdrawal from the study can occur at any time without any fear of punitive action.

14. Removal: If the researcher has trouble scheduling the volunteer to come into the testing room, that volunteer will be dropped from the list of volunteers. The volunteer has the right to change their minds about participation at any time before or during the study.

15. Signatures:

The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have questions about subjects' rights or other concerns, I can contact Dennis Landin, Institutional Review Board, (225) 578-8692, irb@lsu.edu, www.lsu.edu/irb. I agree to participate in the study described above and acknowledge the investigator's obligation to provide me with a signed copy of the consent form.

Subject Signature:_____ Date:_____
The study subject has indicated to me that he/she is unable to read. I certify that I have read this consent form to the subject and explained that by completing the signature line above, the subject has agreed to participate.

Signature of Reader: _____Date:_____

APPENDIX 3. PARTICIPANT QUESTIONNAIRE

PAR-Q FORM

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their physician before they start becoming more physically active. **Please complete this form as accurately and completely as possible.**

Please mark YES or No to the following: YES NO

Has your doctor ever said that you have a heart condition and recommended only medically supervised physical activity? Yes/No

Do you frequently have pains in your chest when you perform physical activity? Yes/No

Have you had chest pain when you were not doing physical activity? Yes/No

Have you had a stroke? Yes/No

Do you lose your balance due to dizziness or do you ever lose consciousness? Yes/No

Do you have a bone, joint or any other health problem that causes you pain or limitations that must be addressed when developing an exercise program (i.e. diabetes, osteoporosis, high blood pressure, high cholesterol, arthritis, anorexia, bulimia, anemia, epilepsy, respiratory ailments, back problems, etc.)? Yes/No

Are you pregnant now or have given birth within the last 6 months? Yes/No

Do you have asthma or exercise induced asthma? Yes/No

Do you have low blood sugar levels (hypoglycemia)? Yes/No

Do you have diabetes? Yes/No

Have you had a recent surgery? Yes/No

If you have marked YES to any of the above, please elaborate below:

_____

_____

_____

Do you take any medications, either prescription or non-prescription, on a regular basis? Yes/No

What is the medication for?

_____

_____

_____

I have read, understood, and completed the questionnaire. Any questions I had were answered to my full satisfaction.

Print Name: _____ Signature: _____

Date: _____

VITA

Tyler Farney was born and raised in Salida, Colorado. He graduated from Pueblo South High school in 2003, and following graduation, attended Colorado State University (CSU). Tyler graduated in 2008 from CSU with a bachelor's degree in Health and Exercise Science. After completing his undergraduate degree, Tyler pursued his master's degree at The University of Memphis, and graduated in 2011 with a degree in Exercise and Sport Science. While at The University of Memphis, Tyler worked for Dr. Richard Bloomer in the Cardiorespiratory/Metabolic Laboratory studying oxidative stress and free radical formation. After his master's degree, Tyler pursued his doctoral studies in Kinesiology at Louisiana State University (LSU) under Dr. Arnold Nelson. At LSU he worked in the Exercise Biochemistry Laboratory studying the underlying mechanisms responsible for the biochemical and physiological adaptations of skeletal muscle and nervous system.

Tyler has worked as a strength and conditioning/ Olympic weightlifting coach under Gayle Hatch. Additionally, Tyler worked as a strength and conditioning coach for Memphis Tiger Football and LSU Baseball. While with LSU Baseball, the team made the College World Series in 2015. Tyler is currently certified with his Certified Strength and Conditioning Specialist® (CSCS®) through the NSCA, and holds a Club Coach certification through USA Weightlifting (USAW). He is a member of the American College of Sports Medicine (ACSM), NSCA, and USAW. Tyler anticipates to graduate form LSU in August of 2016 with a Doctor of Philosophy in Kinesiology. He accepted a position as an Assistant Professor with the Department of Health and Kinesiology at Texas A&M University-Kingsville.