Decreased Motor Unit Firing Rate in the Potentiated Tibialis Anterior in Humans

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ABSTRACT

With repeated activity, force production, rate of force production, and relaxation time are impaired. These are characteristics of a fatigued muscle (Vandenboom, 2004). However, brief bouts of near maximal to maximal activity results in the increased ability of the muscle to generate force, termed post activation potentiation (PAP)(Vandervoort et al., 1983). The purpose of the present study was to characterize motor unit firing rate (MUFR) in the unfatigued, potentiated tibialis anterior (TA). Using a quadrifilar needle electrode, MUFR was measured during a 5s 50% MVC in which the TA was either potentiated or unpotentiated; monopolar electrodes measured surface parameters. A 10s MVC was used to potentiate the muscle. Firing rate decreased significantly from 20.15±2.90pps to 18.27±2.99pps, while mean power frequency decreased significantly from 60.13±7.75 Hz to 53.62±8.56 Hz. No change in root mean square (RMS) was observed. Therefore, in the present study, MUFR decreases in response to a potentiated TA.

ACKNOWLEDGEMENT

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Dad, thank you so much for helping me through everything, I would be nothing without you two; and Ashley, your perpetual understanding and ability to remind me of the silver lining in life will never be forgotten and will always be repaid. You mean the world to me.

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CHAPTER I

EXTENDED ABSTRACT

Introduction

The degree to which skeletal muscle can be activated is largely history dependent. With repeated activity, force production, the rate that force is produced, as well how quickly the muscle can relax are all impaired. These are the characteristics of a fatigued muscle (Vandenboom, 2004). Skeletal muscle fatigue, although extremely well studied and easy to identify is still not clearly understood. Muscle force is also modulated by post activation potentiation (PAP). Post activation potentiation is the result of brief bouts of near maximal to maximal activity leading to the increased ability of the muscle to generate force (Vandervoort et al., 1983). Therefore, the capacity of a muscle to generate force is a fine balance between fatigue and PAP, both of which are defined by past activity.

The focus of this study was to observe and document the reaction of the central nervous system to a muscle in a potentiated state by measuring motor unit firing rate. Firing rates have been shown to both decrease (Klein et al., 2001), and increase (Suzuki et al., 1988) in a potentiated muscle.

Methodology

Ten university aged subjects free from neurological disorders were recruited to participate. Both a monopolar and intramuscular electrode configuration were used on the tibialis anterior (TA). Participation consisted of 3 days in the lab. Day 1 was used to familiarize the subject to the experimental procedure and apparatus. Day 2, Mmax was determined; this voltage was then used to elicit twitches for the remainder of the day. Peak potentiation was characterized on day 2 by recording twitches for 5 minutes (2, 5,

15, 30, 60, 120, 180, 300 s) post conditioning contraction (CC). The CC used was a 10s MVC. Firing rates were recorded with a quadrifilar needle electrode placed directly below the monopolar electrode in the potentiated and unpotentiated muscle throughout day 3. Peak twitch force, firing rate, Root-mean-square (RMS), Mean-power-frequency (MPF), and peak to peak M-wave amplitude (PP) were recorded and analyzed.

Results

The CC (10s MVC) on day 2 resulted in a potentiated twitch force that was maximally elevated 2.40 ±0.58 times the baseline twitch 2 s post CC. Force increased from 18.36±5.43 N to 42.92±13.60 N. Day 3 twitch force was potentiated 2 s post CC 161% from 18.21±5.73 N to 47.60±28.37 N, values similar to day 2. Motor unit firing rate decreased 10.8% from 20.15±2.90 pulses per second (pps) in the unpotentiated TA to 18.27±2.99 pps in the potentiated TA. Mean power frequency also decreased from the potentiated condition to the unpotentiated condition from 60.13±7.75 Hz to 53.62±8.56 Hz, respectively. Neither RMS, nor PP M-wave amplitude changed significantly pre to post CC.

Conclusions

Results reveal that motor unit firing rate and MPF in the potentiated tibialis anterior both decrease when compared to the unpotentiated condition. RMS and PP did not change indicating the 10s MVC did little to fatigue the muscle. It is suggested that in order to maintain the required constant force output, firing rates have to adjust in a downward direction to offset a myofilament that is more sensitive to Ca^{2+} and can thus more easily generate force.

STATEMENT OF THE PROBLEM

Introduction

Muscle fibers and the motoneurons that innervate them together are referred to as a motor unit. Motor unit firing rate and recruitment modulate muscle activation and thus force production. Motor unit firing rate decreases in response to sub maximal isometric contractions lasting between 8-15 seconds (De Luca et al., 1996) while during isometric contractions leading to fatigue, a triphasic response has been reported. Adam & De Luca (2005) defined the triphasic response as a decrease in firing rate early in the fatigue protocol, followed by an increase which was accompanied by the recruitment of additional motor units (Adam & De Luca, 2005). The initial decrease in firing rate has been attributed to potentiation but evidence to support this claim is scarce. Literature attempting to explain the role of potentiation in regulating motor unit firing rate is lacking with existing studies reporting conflicting results (Klein et al. 2001; Vandervoort et al. 1983; Suzuki et al., 1988). Klein et al. (2001) measured motor unit firing rate in the triceps brachii following sub-maximal conditioning contractions and observed a decrease in firing rate in 33 of 35 motor units. Conversely, Suzuki et al. (1988) reported increased motor unit firing rates following a sub-maximal conditioning contraction. Therefore, the effect of potentiation on motor unit firing rate is unclear.

Force potentiation in skeletal muscle refers to the increased isometric twitch force which is observed following either low frequency or high frequency stimulation of skeletal muscle. Potentiation of skeletal muscle has been hypothesized to be a modulating component of muscular fatigue and the molecular mechanisms responsible for it have received considerable attention over the last twenty to thirty years (Gallagher et al. 1997; MacIntosh & Willis, 2000; Moore & Persechini, 1990; Rassier et al. 1997, Tubman et al.

1996). The majority of the cited work reporting concrete and reproducible findings related to twitch force potentiation have been carried out in the animal model while the role of potentiation in the human model has been less clearly defined.

Activation of skeletal muscle, whether in animals or humans, is history dependent, meaning past activity will determine future functioning. Fatigue can be defined as an exercise induced decrease in the ability to generate maximal voluntary force or power in a muscle or muscle group (Taylor & Gandevia, 2008). Muscles that have undergone long lasting fatiguing contractions will elicit a decrease in tetanic force output as well as a slowing of shortening velocity (Allen et al., 2002). Brief muscle activity however, has been shown to immediately potentiate muscle twitch force. It is the balance between these two effects that is responsible for muscle force generation (Stuart et al., 1987; Houston & Grange, 1989; Houston & Grange, 1990; Grange & Houston, 1991). Herein lays one of the most difficult aspects for researchers interested in potentiation. To potentiate a muscle it must be primed with a conditioning contraction strong enough to catalyze the chemical events necessary to elicit this phenomenon; but if muscle activation is the balance between potentiation and fatigue the conditioning contraction must not be so intense as to fatigue the muscle.

The following is a brief description of the chemical cascade that leads to potentiation in skeletal muscle. Muscle is signaled to contract, for instance during the conditioning contraction, Ca^{2+} is released into the myoplasm where it binds to calmodulin. The Ca^{2+}/calmodulin complex activates myosin light chain kinase, a kinase responsible for phosphorylating the regulatory light chain (R-LC) on the myosin head of the thick filament. A phosphorylated R-LC slightly changes the structure of the thick

filament leaving it in a state that is more sensitive to sub-optimal levels of intracellular Ca^{2+} (Persechini et al., 1985; Metzger et al. 1989, Sweeney & Stull., 1990). The myofilament now requires less Ca^{2+} for a given amount of force leading to the hypothesis that potentiated skeletal muscle may be able to maintain that force with less neural drive, manifested experimentally as a decrease in motor unit firing rate.

Statement of Purpose

The purpose of this study was to examine motor unit firing behavior in the tibialis anterior of healthy subjects in the potentiated state. With the use of quadrifilar needle electrodes the characterization of motor unit firing rate in the TA following a potentiation protocol was determined. Previous accounts of firing rates, specifically in the potentiated TA are scarce with published literature inconsistent in their findings. Firing rates have been reported to both increase (Suzuki et al., 1988) and decrease (Klein et al., 2001) following a conditioning contraction. A study by Adam & De Luca (2005) observed a decrease in firing rates in the early stages of a fatigue protocol and discussed that it may have been due to potentiation although their primary objective was not to potentiate the muscle in question. We aimed to clarify the mixed results published to this date by systematically measuring motor unit firing rates in the potentiated TA and discussing the potential mechanism of this physiological adaptation.

Hypothesis

It is hypothesized that a 10 s maximal voluntary contraction will suffice to potentiate the tibialis anterior and that motor unit firing rate to the potentiated TA will be

slower when compared to the unpotentiated TA. Motor unit firing rate was measured during an isometric contraction at 50% MVC for 5 seconds.

Significance of the Study

The ability of skeletal muscle to generate force is reliant upon neurological drive as well as the capacity of the contractile unit to harness that drive to produce physical movement (Gandevia, 2001). At the level of the contractile unit, force can be altered by either potentiating or fatiguing the muscle, the former results in an increased twitch force while the latter results in decreased twitch force (Thomas et al. 2006). Neurologically, force can be graded by either modulating motor unit firing rate or by recruiting or derecruiting motor units (Fuglevand et al. 1993). By examining the response of the nervous system to a potentiated tibialis anterior we can begin to ascertain the mechanisms responsible for the change.

Twitch force potentiation has been studied extensively (Gallagher et al. 1997; MacIntosh & Willis, 2000; Moore & Persechini, 1990; Rassier et al. 1997, Tubman et al. 1996) and the phenomenon itself is quite remarkable, however, the importance and role in muscle physiology in the human model is currently unclear. Many authors have reported the effects of potentiation on human performance with mixed results (Baudry & Duchateau, 2007; Chatzopoulos et al. 2007; Gossen & Sale, 2000; Magnus et al. 2006; Robbins & Docherty, 2005) leading us to believe that perhaps this topic may not be performance related.

The significance of this study is to clarify the effect of potentiation on motor unit firing rate in the tibialis anterior. This information will facilitate the ability of the scientific community to continue the exploration into the mystery as to why our muscles

have the capacity to potentiate. How does the nervous system respond to potentiation and could this alter energy expenditure to some degree? The basic science undertaken in the present study will hopefully serve to aid in answering some of these questions.

Basic Assumptions

1. The electromyographic signal recorded at the skin surface by a monopolar electrode represents activity from motor units within the contracting tibialis anterior,
2. Dorsiflexion force is produced mainly by the tibialis anterior with minimal contribution from other dorsiflexors such as extensor digitorum longus and extensor hallicus longus,
3. Maximal voluntary contractions produced by the subject are a reflection of their true maximum effort.
4. The quadrifilar needle electrode records myoelectric activity from muscle fibres within close proximity to its recording surface, it is assumed that these signals are representative of activity in the entire tibialis anterior.

Glossary

The following glossary includes definitions of terms as they pertain to the present study. It is understood that variations of these definitions may exist.

Post Activation Potentiation (PAP). Twitch force elicited post conditioning contraction is greater than twitch force elicited pre conditioning contraction. This is a reflection of the muscle being in a potentiated state. The conditioning contraction used in the present study was a 10s MVC.

Conditioning Contraction (CC). A contraction used to potentiate the muscle being investigated. Twitches are elicited pre and post conditioning contraction. The CC serves to phosphorylate the regulatory light chain on the myosin head, the biochemical reaction that is most likely responsible for twitch potentiation.

Ca^{2+}/Calmodulin-Dependent Myosin Light Chain Kinase (MLCK). The kinase responsible for phosphorylating the regulatory light chain on the myosin head. During muscle contraction Ca^{2+} enters the myoplasm and binds to calmodulin. This Ca^{2+}/Calmodulin complex then activates myosin light chain kinase to phosphorylate myosin.

Muscular Fatigue/Peripheral Fatigue. Contractile impairment caused by repeated muscular activity. Repeated activity leads to a decreased ability of the muscle to generate force. The rate of force development as well as the rate of relaxation is compromised. These characteristics of fatigue are reversible with sufficient recovery time.

Central Fatigue. Exercise related changes occurring proximal to the motor axons and which lead to failure of voluntary activation of all motor units and thus a decrease in maximal force production. Central fatigue is most often measured via the interpolated twitch technique wherein a supramaximal twitch is elicited during an MVC and any observed increment in force is indicative of motor units that could not be voluntarily activated.

Neuromuscular Fatigue. Neuromuscular fatigue can be defined as the inability of a motor unit to maintain a given force output. To compensate, the motor unit must increase its firing rate or additional motor units will be recruited to maintain force.

Motor Unit. The alpha motor neuron as well as all the muscle fibres it innervates is termed a motor unit.

Motor Unit Firing Rate. The number of action potentials sent per second by a motoneuron to the muscle fibre it innervates. Motor unit firing rate can be altered to control force output, referred to as rate coding.

Motor Unit Recruitment. Motor units are recruited in an orderly fashion from low threshold to high threshold. Threshold is based on axon size with smaller axons innervating slower more fatigue resistant muscle fibres recruited first. Larger axons innervating fast twitch muscle fibres which are more prone to fatigue are recruited later in a contraction.

Quadrifillar Needle Electrode. A 25 gauge stainless steel cannula that houses 4 wire electrodes inserts into the muscle belly to record motor unit activity. From the 4 wires, 3 channels of myographic activity can be recorded giving a clear view of action potentials from various motor units.

CHAPTER II

LITERATURE REVIEW

Neurological Control of Movement

Skeletal muscle is responsible for converting the energy we consume in food to mechanical force. This force when applied by muscles across joints results in movement of the skeleton. Coordinating skeletal movement is extremely complex and involves both incoming and outgoing signals through the nervous system. Skeletal muscle is muscle which can be controlled voluntarily and the degree to which this movement can be smoothly coordinated relies on sensory (afferent) input and motor (efferent) output. Muscle fibers are innervated by alpha motoneurons originating from the spinal cord. The alpha motoneuron and the muscle fibers it innervates is called a motor unit (Gandevia, 2001).

Contraction of a muscle relies on input from the pool of motoneurons which innervates it (Mendell, 2005). Although a motor unit consists of one motoneuron and all the muscle fibers it innervates the number of motoneurons required to activate an entire muscle varies greatly from muscle to muscle. For instance muscles which require extremely precise fine motor control such as muscles around the eye have a motoneuron to muscle fiber ratio that is low, approximately 1:15. Whereas the tibialis anterior, a larger muscle requiring less precise control, has a ratio closer to 1:2000, meaning that for each motoneuron 2000 muscle fibers are innervated (Cope & Sokoloff, 1999).

Innervating muscle fibers begins with the conduction of nerve impulses down the motoneuron. Conduction of nerve impulses through a neuron is similar to the conduction of an action potential down the axon. The cell membrane of a neuron at rest has a negative electrical potential of about -70mM due to the semi-permeability of the

membrane to K^+ as well as the maintenance of the electrical difference by Na^+/K^+ pumps. The higher concentration of positive ions outside the cell compared to inside the cell is responsible for the negative resting membrane potential. Sensory input signals the neuron to either hyperpolarize or depolarize. Hyperpolarization will result in the membrane potential becoming more negative and is thus termed 'inhibitory postsynaptic potential' or IPSP while sensory input resulting in the membrane potential becoming more positive is termed 'excitatory postsynaptic potential'. The summation of IPSPs and EPSPs determines whether an action potential will be generated. Assuming afferent input depolarizes the membrane beyond threshold, 15-20 mV, an action potential will follow (Leonard, 1998).

How are Motoneurons Recruited?

Given the extreme complexity of the nervous system which controls voluntary movement it is astounding to consider that one of the original principles developed to explain coordinated movement has held true. In 1965 a series of papers were published outlining the details of orderly recruitment of motoneurons during contraction. A conclusion was reached that motoneurons were recruited based on their size with smaller neurons being recruited earlier and more frequently than larger neurons, this is referred to as 'Henneman's Size Principle' (Mendell, 2005).

In 1965 Elwood Henneman's lab published five papers that eventually led to them proposing that motoneurons were recruited according to their size. The first two papers focused on describing motor unit properties in the soleus and the medial gastrocnemius. The major findings from these papers were that based on conduction velocity small axons supplied motor units generating less tetanic tension than those associated with large

axons. Another important conclusion was that only those axons supplying motor units capable of generating large forces were fatigable when stimulated at high rates. These important motor unit properties would soon lead to the development of the size principle.

Henneman next investigated recruitment properties during muscle stretch in the triceps surae in decerebrate cats. Results revealed that small spikes recorded from ventral roots were recruited before large ones and that the large ones were interpreted as impulses in axons of larger diameter. Spike size was highly correlated to axon size, and recruitment threshold or excitability was significantly correlated to the size of impulses from their axons (Mendell, 2005). It was finally reported that the order of motoneuron recruitment determined the ability of that neuron to resist fatigue. Motoneurons recruited earlier in a contraction fired more often than those recruited later in the contraction and were more resistant to fatigue. Motoneurons that have higher recruitment thresholds and start to fire later in the sustained contraction are more likely to fatigue. The size principle states that motoneurons are recruited from low to high axonal conduction velocity and motor units are recruited from slow to fast, weak to strong, and fatigue resistant to fatigue susceptible during isometric contractions (Cope & Sokoloff, 1999).

In the forty years since Henneman first introduced the size principle much progress has been made in the field of neurophysiology. Of particular interest to this paper is the use of intramuscular quadrifilar needle electrodes to identify and record single motor unit action potentials in muscles contracting at various force levels. A more recent paper by Akaboshi et al. (2000) used intramuscular electrodes to investigate whether the size principle holds up when MUAP parameters were recorded using this sophisticated and precise methodology. Five MUAP parameters were measured in the

first dorsal interosseous, biceps brachii, rectus femoris, and tibialis anterior during contractions at 50% of MVC. In each muscle, the amplitude, duration, area, and size index all correlated positively with recruitment threshold allowing the authors to conclude that the size principle applies during recordings using intramuscular quadrifilar needle electrodes (Akaboshi et al., 2000).

Contractile Level Muscle Regulation

Muscle contraction is regulated primarily by the interaction of Ca^{2+} with the regulatory proteins of the thin filament. Upon action potential induced muscle membrane depolarization the sarcoplasmic reticulum (SR) releases Ca^{2+} into the myoplasm where it attaches to troponin C (TnC). The movement of tropomyosin (Tm) which exposes myosin binding sites is one of two ways in which thin filament activation occurs. By exposing the active sites myosin can now weakly bind to the thin filament. Myosin binding to actin is the second way the thin filament is activated. This binding is thought to further move Tm into the grooves of the F-actin double helix exposing more binding sites on actin. The two methods of thin filament activation, Ca^{2+} binding to TnC, and further activation by attached cross-bridges, work in conjunction and are considered to be cooperative in nature (Gordon et al. 2001; Fuchs, 1995).

In the 1993 paper by McKillop and Geeves the three-state model of thin filament activation was proposed which describes the effect of Ca^{2+} and cross-bridge attachment on Tm positioning and thus thin filament activation (McKillop & Geeves, 1993). According to the three-state model the thin filament can exist in either a blocked, closed, or open state. The transition from one state to the next is dependent on the myoplasmic Ca^{2+} concentration as well as the presence of weakly and strongly bound cross-bridges.

With low myoplasmic [Ca^{2+}], the amount of Ca^{2+} inside the muscle cell, Tm blocks the myosin binding sites on actin preventing cross-bridge attachment, this is the blocked state. As Ca^{2+} concentration increases prior to muscle contraction it binds to TnC allowing Tm to roll over the actin molecules exposing myosin binding sites available for some strong and weakly bound cross-bridges, this is the closed state. The strongly bound cross-bridges are able to produce some tension while further pushing Tm over, exposing additional strong binding sites. This pushing or holding of Tm so that more myosin can bind is the open state, the state in which the thin filament is considered to be fully activated and peak tension can be generated (Fuchs, 1995; Mckillop & Geeves, 1993).

A brief influx of Ca^{2+} ,and thus low myoplasmic Ca^{2+} concentration, from a single action potential results in a transient increase in force known as a twitch. In skeletal muscle the duration of the twitch ranges from 10 miliseconds to 250 milliseconds depending on the muscle, while the duration of an action potential is only approximately 5 milliseconds (Vander et al. 2001). The single muscle fibre twitch does not produce enough force to be physiologically useful. However the difference between twitch time and action potential time means that with a high enough stimulation frequency to the muscle, twitches can summate to produce a tetanus. As the frequency of action potentials increases, more Ca^{+} is released into the myoplasm which activates the thin filament exposing a greater number of binding sites for myosin. At higher frequencies twitches begin to fuse, summating their forces to produce a maximal contraction (Mela et al. 2001).

Muscle Fatigue (Peripheral Fatigue)

Whether participating in short bouts of high intensity movements or activities requiring repetitive low frequency muscle contractions over a long period of time a decrease in performance will be observed at the level of the muscle, sometimes referred to as peripheral fatigue. Muscular fatigue is characterized by the inability of the muscle to generate maximum tension, an observed reduction in shortening velocity, as well as a slowing of relaxation. The mechanisms responsible for these impairments have been reviewed extensively and will be touched upon here only briefly.

The hydrolysis of ATP to ADP, Pi and H^+ provides the energy necessary to power muscle contraction, therefore muscle fatigue could be the result of the depletion of ATP stores or the accumulation of its hydrolysis products. Allen et al. (2002) used the firefly luciferin/luciferase reaction to monitor intracellular ATP concentration in single mouse skeletal muscle fibres. Their lab concluded that although ATP concentration does decrease with fatigue it does not decrease enough to be considered the major cause of contractile dysfunction (Allen et al., 2002). The accumulation of ADP, Pi, and H^+ that accompanies ATP hydrolysis have all been shown to contribute to muscle fatigue, referred to as end product inhibition (EPI). The most common EPI fatigue theory among athletes and trainers alike is that the accumulation of lactic acid in the muscle causes a decrease in performance. Although concentration of H^+ increases approximately 10 fold in the fatigued muscle it is now generally accepted that at physiological temperatures this is not one of the main causes of contractile impairment (Vandenboom, 2004; Allen, 2004). Changing intracellular acidosis by altering extracellular CO_2 in intact fibres has been utilized to investigate the effect of acidosis on muscle cell function. Tetanic force

from mouse single fibers at three different temperatures was determined. With a 1 pH unit change in intracellular pH, tetanic force was depressed by approximately 47% at 12°C whereas at 32°C force was reduced by only approximately 11% revealing the minimal effect muscle acidosis has on tetanic force at physiological temperatures in mouse skeletal muscle (Allen, 2004).

In a rested fast twitch muscle [ADP] is maintained at around 10μM due to the creatine kinase (CK) reaction in which ADP and CP are converted to ATP and creatine. Under fatiguing conditions this concentration can increase several fold to between 200μM and 3.0 mM due to the inability of the CK reaction to keep up with the regeneration of ATP resulting in large amounts of ADP accumulating (Macdonald & Stephenson, 2004). During the cross-bridge cycle the release of ADP following the power stroke and the subsequent binding of ATP to the myosin head leads to the detachment of myosin from actin allowing the muscle to relax. An increase in ADP concentration in the myoplasm during fatigue leads to a longer time to relaxation as well as a slight increase in force. An elevated [ADP] leads to competitive inhibition at the ATP binding domain on the myosin head as ADP remains bound for a longer period of time. The cross-bridge cannot detach leading to a rigor state which would explain the small increase in force as well as the longer time to relaxation and thus the impairment to $V_{max,}$, or maximum shortening velocity. (Vandenboom, 2004; Allen, 2004).

Elevations in myoplasmic [P_i],inorganic phosphate, that occur as a result of repeated muscle contraction affect both the myofilament component of the cross-bridge cycle as well as the Ca^{2+} available for release from the SR which both contribute to fatigue. The release of Pi from the myosin head during cross-bridge cycling is necessary

to complete the power stroke and thus generate force. An accumulation of Pi is thought to either inhibit the ability of P_i to release from the nucleotide binding pocket or as Pi releases, excess Pi rebinds so quickly the power stroke cannot generate force and may actually be reversed (Vandenboom, 2004). Increased $[P_i]$ results in a decrease in F_{max} with no decrease in V_{max} observed, with the decrease in F_{max} being more pronounced in fast twitch fibers.

Over the course of a fatigue protocol a reduction in myoplasmic Ca^{2+} ($Ca^{2+}{}_i$) is observed which is coupled with a reduction in Ca^{2+} activated force (Kabbara & Allen, 1999). Kabbara & Allen (1999) conducted a study to reveal the mechanisms responsible for this decrease in $[Ca^{2+}]_i$. They hypothesized that the increase in Pi observed during fatigue resulted in the movement of Pi into the SR where it would precipitate with Ca^{2+} forming CaPi which inhibits the ability of the SR to pump Ca^{2+} into the myoplasm. To test this hypothesis either caffeine or 4-chloro-*m*-cresol (4-CmC), both agents known to increase the $[Ca^{2+}]_i$ by opening the SR Ca^{2+} release channels, were applied to muscles before as well as after a fatigue protocol to measure the amount of rapidly releasable Ca^{2+} available in the SR (Kabbara & Allen, 1999). Results indicated that the amplitude of tetanic $[Ca^{2+}]_i$ was reduced to 50% of the control during fatigue while the amplitude of the 4-CmC-induced $[Ca^{2+}]_i$ was also reduced to about half of the control. These results suggest the amount of Ca^{2+} available for release in the SR is reduced during fatigue (Kabbara & Allen, 1999). Twenty minutes of rest restored the 4-CmC-induced $[Ca^{2+}]_i$ to control levels. To confirm the recovery in $[Ca^{2+}]_i$ was indeed due to the CaPi dissolving as oppose to simply the pumping of more Ca^{2+} from the extracellular space, the same recovery experiment was performed with all extracellular Ca^{2+} replaced by Mg^{2+}. All

results were similar to the previous experiments, a gradual decrease in $[Ca^{2+}]_i$ followed by a much reduced 4-CmC-induced $[Ca^{2+}]_i$ which was rectified by a 20 minute rest period suggesting that recovery was due to CaPi dissolving in the SR (Kabbara & Allen, 1999). Therefore, elevated levels of Pi in the myoplasm during fatigue may result in the precipitation of Pi with Ca^{2+} in the SR thus reducing the amount of Ca^{2+} available in the myoplasm to activate the thin filament leading to a decrease in F_{max}.

Force Potentiation in Skeletal Muscle

The contractile response of skeletal muscle is history dependent. Following voluntary contraction or repeated stimulation of the muscle at low or high frequency, twitch force is greater than before stimulation. In the case of low frequency stimulation the staircase effect is observed ,whereas potentiation observed following a tetanus or high frequency stimulation is termed post tetanic potentiation (PTP) or postactivation potentiation (PAP) when referring to the human model (Zhi et al., 2005). The mechanism and reasoning behind this phenomenon is thought to modulate fatigue as twitch potentiation occurs as a result of the increased sensitivity of the thick filament to sub-optimal levels of myoplasmic Ca^{2+}, conditions which are observed during muscle fatigue (Zhi et al., 2005; Vandenboom & Houston, 1996).

How is Muscle Twitch Force Potentiated?

Skeletal muscle potentiation is largely mediated by the phosphorylation of the myosin regulatory light chain by the Ca^{2+}/calmodulin-dependent myosin light chain kinase (Zhi et al., 2005; Vandenboom & Houston, 1996; Grange et al., 1993). Membrane

depolarization signals the SR to release Ca^{2+} into the myoplasm. This Ca^{2+} plays two regulatory functions for muscle contraction. As mentioned previously Ca^{2+} binds to the thin filament at TnC to encourage the sliding of Tm away from the myosin binding sites allowing myosin to weakly bind. The second regulatory function Ca^{2+} plays involves the thick filament. Calcium binds to calmodulin in the myoplasm, the Ca^{2+}/calmodulin complex now binds and activates myosin light chain kinase (MLCK), the kinase responsible for phosphorylating the regulatory light chain on the myosin head (Zhi et al., 2005; Vandenboom & Houston, 1996). The negatively charged phosphate ion now bound to the myosin head is thought to cause a conformational change in cross-bridge positioning because of the already negatively charged myosin tail to which the cross-bridge is attached. This conformational change results in the head being able to more easily attach to the thin filament in a force generating state (Grange et al. 1993). When the muscle is in the relaxed state Ca^{2+} is sequestered into the SR deactivating MLCK. Myosin light chain phosphatase will now dephosphorylate the regulatory light chain returning it to its non force generating position. The net activation between the kinase and phosphatase will determine the degree to which myosin is phosphorylated, note that MLCK is easily and rapidly activated while the phosphatase takes a while to be activated making phosphorylation of the light chains likely upon Ca^{2+} influx even at suboptimal levels.

Structural and Functional Response of Myosin to Phosphorylation

Phosphorylation of the regulatory light chain on the myosin head by MLCK results in the leftward shift of the force-pCa curve displaying the ability of the muscle to generate maximal force at impaired calcium levels (Figure 1) (Grange et al., 1993; Levine et al., 1998; Yang et al., 1998; Sweeney et al. 1993)

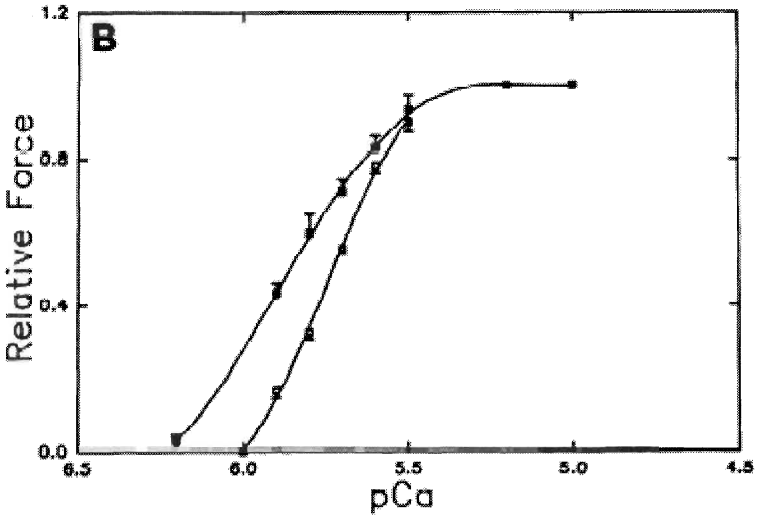


Figure 1. Force PCa relationship. Before (open symbols), and after (closed symbols) myosin regulatory light chain phosphorylation. Sweeney, H.L., Bowman, B.F., & Stull, J.T. (1993). Myosin light chain phosphorylation in vertebrate striated muscle: regulation and function. *The American Journal of Physiology*. 264: 1085-1095, Figure 3, page 1091. Phosphorylation shifts the curve to the left indicating that more force can be generated at a given Ca^{2+} concentration.

This leftward shift of force/PCa curve can be attributed to the movement of the myosin heads away from the thick filament upon RLC phosphorylation (Figure 2). In the relaxed state the thick filament is arranged in an ordered, helical structure with myosin heads closer to the surface of the thick filament. The activation of MLCK by Ca^{2+}/calmodulin, phosphorylates serine on the regulatory light chain changing the charge compliment of its N-terminal region; the phosphorylatable serine is adjacent to many positively charged residues. This change in charge interaction between the N-terminus and the thick filament backbone is thought to be the mechanism for the myosin heads moving away from the thick filament (Levine et al., 1998; Yang et al., 1998). The phosphorylated filaments appear to be disordered whereas the relaxed, unphosphorylated filaments appear ordered and structured. This disordered arrangement results in the cross-bridges being in closer proximity to the thin filament binding sites thus requiring less Ca^{2+} to be fully activated.

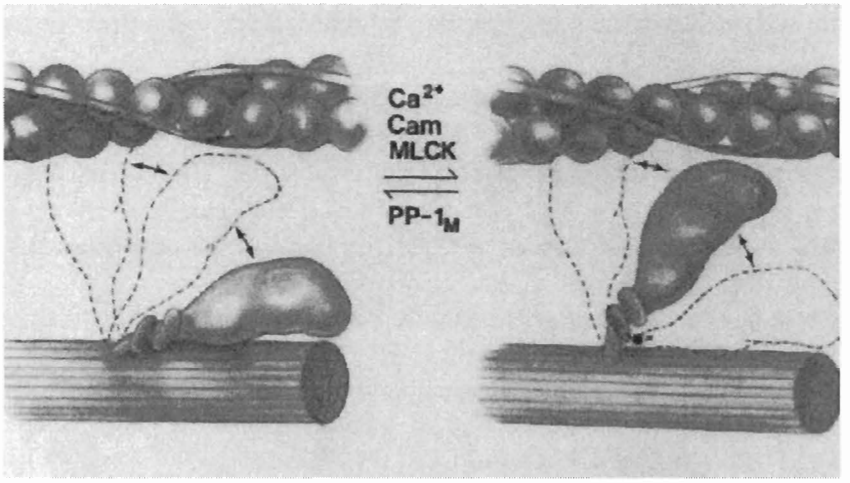


Figure 2. Proposed mechanism of myosin phosphorylation by Ca^{2+} dependent myosin light chain kinase. MLCK phosphorylates the myosin head altering the cross-bridge position and allowing the thick filament to increase the rate at which it can bind to actin. As Ca^{2+} leaves the myoplasm, a phosphotase ($PP\text{-}1_M$), dephosphorylates the light chain returning the myosin head to its resting state Sweeney, H.L., Bowman, B.F., & Stull, J.T. (1993). Myosin light chain phosphorylation in vertebrate striated muscle: regulation and function. *The American Journal of Physiology.* 264: 1085-1095, Figure 4, page 1092.

Yang et al. in 1998 designed a study to investigate whether reduced lattice spacing between the thick and thin filament, by either stretching the muscle or by applying osmotic compression, would produce a similar leftward shift in the force-pCa relationship. Results showed that the leftward shift in the force-pCa relationship was similar between fibres displaying decreased lattice spacing when compared to phosphorylated fibres revealing similar mechanisms for both. Interestingly, there was no additive effect on calcium sensitivity when compressed fibres were phosphorylated (Yang et al., 1998).

Skinned Fibres: Contractile Characteristics

Using skinned muscle fibres allows for exquisite control over the external and thus internal environment of the muscle preparation facilitating the ability of researchers to make concrete conclusions following experiments. In 1985 Persechini, Stull, and Cooke set out to further define the role the phosphorylated myosin light chain plays in regulating muscle contraction. Evidence had been accumulating suggesting that following a conditioning stimulus twitch potentiation was observed and that it was temporally correlated to myosin light chain phosphorylation. The temporal correlation, although convincing, did not rule out the possible effect of increasing intracellular Ca^{2+} levels on twitch potentiation. Persechini et al. (1985) designed an experiment to clarify some of the questions. Skinned fibers from the rabbit psoas muscle were prepared and suspended in solution at 25°C. The chemically skinned fibers allow for a permeable membrane with the structural integrity of the myofilament lattice intact. Parameters such as force and velocity can still be measured. With a permeable membrane calcium concentrations of the cell can be controlled to help determine the mechanism of force potentiation. By altering the concentrations of intracellular Ca^{2+} results revealed that phosphorylation of the myosin light chain by MLCK increased isometric tension in response to low levels, 0.6μM, of Ca^{2+}. The addition of a phosphatase returned force to control levels while subsequently returning phosphorylated light chains to their unphosphorylated states. There was no apparent effect of phosphorylation on isometric tension when the fibers were exposed to maximally activating levels, 10μM, of Ca^{2+}. Maximum shortening velocity was unaffected by myosin light chain phosphorylation indicating cross-bridge detachment rate is also unaltered (Persechini et al., 1985).

One of the possible mechanisms for the leftward shift of the force-pCa curve is thought to be the change in rate of tension redevelopment (k_{tr}) caused by phosphorylation of the regulatory light chain (Sweeney et al., 1993; Metzger et al., 1989). Phosphorylation of the RLC results in an increase in isometric force as well as an increase in the rate of tension redevelopment; although the increase in force is most pronounced at low levels of intracellular Ca^{2+} whereas the increase in the rate of tension redevelopment is observed at higher levels of Ca^{2+} (Sweeney et al., 1993; Metzger et al., 1989; Sweeney & Stull, 1990). This observation in skinned rabbit psoas muscle can be explained by reducing a muscle contraction to two simple states, f_{app} represents the rate constant for the transition from the non-force generating state to the force- generating state, while g_{app} is the opposite, force-generating to non-force generating state (Sweeney & Stull, 1990). Based on this two-state model Sweeney and Stull concluded that RLC phosphorylation affected f_{app}, the rate at which cross-bridges enter the force generating state while g_{app} was unaffected (Sweeney & Stull, 1990). The conclusion that g_{app} is unaffected by phosphorylation can be made as maximum unloaded shortening velocity does not change with RLC phosphorylation. At low levels of Ca^{2+} the increase in isometric force production is a result of an increase of cross-bridges entering the force-generating state, f_{app}, not an increase in the number of cross-bridges formed. k_{tr} is simply the sum of f_{app} and g_{app} and thus the effect of RLC phosphorylation on this rate constant is Ca^{2+} specific. At high levels of $[Ca^{2+}{}_i]$, Ca^{2+} is bound to TnC activating the thin filament and resulting in a larger amount of cross-bridges in the force generating state, f_{app} compared to g_{app}. Therefore, when f_{app} is larger than g_{app}, even a small fractional increase in f_{app} results in a significant increase in k_{tr}, which is precisely that which is observed when skinned fibers

are phosphorylated at intermediate levels of intracellular Ca^{2+} with k_{tr} measured (Sweeney & Stull, 1990).

Post-Activation Potentiation (PAP) in the Human Model

Force potentiation in skeletal muscle has been thoroughly demonstrated in the animal model with results clearly showing increased twitch force following a conditioning stimulus (Vandenboom & Houston , 1996a; Vandenboom & Houston , 1996b). The effect of PAP on human performance has been more difficult to harness as recent literature reviews in this area have reported (Robbins, 2005).

Whether or not potentiation occurs in humans is not the question. Grange and Houston in the late 80's and early 90's published many convincing papers in which they elicited potentiation in the knee extensor muscles of healthy subjects. One of the purposes of these papers was to clarify whether or not the mechanisms behind twitch potentiation in humans was similar to that of animals.

Stuart et al. (1987) conducted a study where two separate experiments were performed. In the first experiment the effects of a 10s MVC on twitch tension and P-light chain phosphate content at two muscle lengths was investigated. For experiment two, maximal unloaded knee extension velocity was determined at rest or following a 10s MVC. P-light chain phosphorylation was also measured in the second experiment. Results from both experiments clearly show that the 10s MVC phosphorylated the P-light chains by 90-100%, and the light chains remained phosphorylated for at least 60 s post MVC. Twitch tension was potentiated in experiment one at both the short and long muscle lengths for up to 2 minutes after the MVC while potentiation was significantly

more pronounced at the shorter length (knee angle of 135° versus 90°). Peak twitch tension occurred immediately after the MVC (2 s) for both lengths. Maximal velocities of unloaded knee extension at rest and after the MVC were not significantly different although there was an increasing trend. This study confirms that a 10 s MVC is sufficient to potentiate twitch force in the knee extensors and results in the phosphorylation of the P-light chains for up to 60 s following the conditioning stimulus (Stuart et al. 1987).

Houston & Grange (1989) investigated twitch potentiation in 18 subjects focusing on the differing effects on twitch force, P-light chain phosphorylation, as well as the accumulation of muscle metabolites following a 10 s MVC versus a 60 s MVC. Using the 10 s MVC as a conditioning stimulus leads to only a slight decrease in voluntary force output. While a 60 s MVC has been associated with many indices of fatigue such as a decrease in force, slowing of mean motor neuron discharge rate as well as an increase in Pi and hydrogen ion concentrations (Houston & Grange, 1989).

Potentiation peaked at 2 s following the 10 s MVC and was 1.53 times greater than at rest. Twitch tension immediately after the 60 s MVC was 0.66 of the tension at rest, 3-8 minutes following the MVC tension potentiated to a maximum of 1.21 times the resting value. Muscle metabolite displacement following the 10 s MVC was modest with ATP, PCr, and lactate levels returning close to resting levels by 1 min post MVC. However, the 60 s MVC resulted in a significant 20% decrease in ATP concentration, PCr concentration decreased by 84% while muscle lactate was elevated by a factor of 15. Six minutes later only muscle lactate levels were still greater than at rest. P-light chain content was significantly elevated following both the 10 and 60s MVC. At 10 minutes following the 10 s MVC P-light chain content was still elevated while the 60 s MVC

samples had returned to resting levels. These results show that a 10 s MVC potentiates twitch force while minimizing the displacement of muscle metabolites associated with the onset of fatigue (Houston & Grange, 1989).

Houston and Grange continued to study the effect of a 60 s MVC on muscle metabolite displacement, and P-light chain phosphorylation in knee extensor muscles and reported findings consistent with their earlier papers (Houston & Grange, 1990; Grange & Houston, 1991). ATP and PCr concentrations were significantly lowered while lactate levels were significantly enhanced following the MVC. P-light chain phosphorylation was increased immediately following the conditioning stimulus and remained elevated for 4 minutes Twitch torque was depressed 2 sec after the MVC but was potentiated by 25% by 4 minutes of recovery.

These findings by Houston and Grange confirm that twitch potentiation can be elicited in human subjects. They also confirm that post-activation potentiation is the result of two opposing processes, one that enhances force output while the other, fatigue, reduces force output.

The underlying problem in harnessing this physiological occurrence and deciphering its significance in muscle function is finding the balance between displaying potentiation while minimizing fatigue.

Could Potentiation Protect Against Neuromuscular Fatigue

Neuromuscular fatigue can be defined as the inability of a motor unit to maintain a given force output. To compensate, the motor unit must increase its firing rate or additional motor units will be recruited to maintain force (de Ruiter et al., 2005). Post-activation potentiation increases the amount of force a muscle fiber can generate, therefore to maintain a set force target with the muscle in a potentiated state motor unit firing rates would have to decrease. A decrease in motor unit firing rate could protect against fatigue (Sale, 2004). For example, characteristics of fatigue such as neuromuscular transmission failure, muscle action potential propagation failure, and excitation-contraction coupling impairment are increased as nerve impulse firing increases. Thus, decreased nerve impulse firing would lead to less fatigue.

Sustained muscular contractions can also lead to impaired central drive to motoneurons. Impaired central drive, commonly referred to as central fatigue, is the inability to voluntarily activate the muscle, resulting in a decrease in voluntary force. Central fatigue can be measured by superimposing a twitch during a maximal contraction. Any increment in force observed as a result of the evoked nerve stimulation signifies there were motor units that were either not recruited or not firing fast enough and that central processes proximal to the site of motor axon stimulation are impaired (Taylor & Gandevia, 2007). Post activation potentiation increases the amount of force for a given motor unit firing rate. This should relieve the need to maintain a high level of excitation to the motoneuron leading to the maintenance of functional central drive, and thus the ability to voluntarily sustain a given force level (Sale, 2004).

Motor Unit Firing Characteristics during Fatiguing Isometric Contractions

The characterization of motor unit firing rates during sustained as well as brief intermittent isometric contractions has been investigated extensively with varying results. Identifying and following single motor units throughout the contraction protocol can prove to be difficult and may account for the opposing findings in the current literature.

Intramuscular quadrifilar needle electrodes are inserted into the muscle and are used to detect three channels of myoelectric activity from the motor unit. The three channels can be decomposed in order to acquire unique motor unit action potentials. Firing times can be obtained with an accuracy of 100% when this technique is used (Mambrito & De Luca, 1983).

The firing rates of motor units are generally studied either during brief isometric contractions (less than 30 s) or during isometric contractions to fatigue (15-45 min.). Contraction intensity varies considerably between reports. Although results in this area are quite divergent it has been established and accepted that during brief moderate to high intensity contractions firing rates tend to decrease (De Luca et al., 1996).

Studies that have followed or attempted to follow single motor units during fatigue protocols to exhaustion have reported findings that consistently conflict one another. Carpentier et al. (2001) upon fatiguing the first dorsal interosseous at a contraction level of 50% MVC observed a decrease in mean firing rate (Carpentier et al., 2001), while another group of researchers studying the biceps brachii during contractions to exhaustion at 20% MVC reported an initial decrease in mean firing rate which was followed by an increase in firing rate. The recruitment of additional motor units toward the end of the protocol was also observed (Dorfman et al., 1990). Motor unit discharge

rates in the triceps brachii during a fatigue protocol displayed increasing, decreasing, or constant firing rates (Miller et al., 1996).

Adam & De Luca (2005), in an attempt to clarify motor unit discharge rate during fatiguing isometric contractions systematically investigated the knee extensor muscle of the thigh. Firing rate was measured by quadrifilar-fine wire needle electrodes during repeated isometric contractions of the vastus lateralis at 20% MVC held for 50s separated by a brief 6s rest. Electrically evoked tetanic (50 Hz) force was measured during the 6s rest. Twitch force was measured in only two of the five subjects. The average number of contractions completed was 8.2±2.0, which translates into an average time to fatigue of 6.83±1.67 minutes. The triphasic response observed by Dorfman et al. (1990) in the biceps brachii was reproduced such that an initial decrease in mean firing rate was reported from 15-40s of the first contraction, which was followed by an increase in motor-unit discharge rate during subsequent contractions. Additional motor-units were recruited toward the end of the fatigue protocol in order to maintain constant force output. The initial decrease in firing rate coincided with a brief increase in twitch and tetanic force that was induced by electrical stimulation. Tetanic force was potentiated by 101% in one subject and 106% in another; twitch force was potentiated in only one subject by 111% (Adam & De Luca, 2005).

The findings presented by Adam and De Luca (2005) bring clarity to the differing reports in the literature that firing rates may decrease, remain constant, or increase as all three of these phases were observed during their fatigue protocol. Previous reports more than likely measured and reported on each of these phases at different locations along the fatigue continuum which lead to opposing results.

Motor Unit Firing Characteristics During Brief Isometric Contractions

The decreasing trend of motor-unit firing rates during brief constant force isometric contractions has been well established (De Luca et al., 2006; Bigland-Ritchie et al., 1983; Woods et al., 1987; Klein et al., 2001). De Luca et al. (1996) investigated the mean firing rates of motor-units in the tibialis anterior and the first dorsal interosseus during the first 8-15 s of constant force isometric contractions at 30, 50, and 80% MVC. Subjects were required to maintain a force plateau at 30, 50, or 80% MVC for 8-15 s depending on the given force level. Mean firing rates decreased both in the TA and the FDI at each contraction level. Percent decreases were not explicitly reported. Motor units with higher recruitment thresholds and lower firing rates were found to decrease to a greater extent when compared to lower threshold motor units. In addition, increasing contraction levels displayed the greatest decrease in motor-unit firing rates. These results support earlier findings by Bigland-Ritchie et al. (1983) who measured firing rates in the human adductor pollicis muscle during constant force maximal contractions held for 40-120 s. A decrease in mean firing rate was observed most prominently throughout the first 60 s. Woods et al. (1987) observed a 30% decrease in firing rates in the quadriceps during maximal effort contractions.

The question of how the force output remains constant when the firing rate of the motor-units decrease is not fully understood. Three possible mechanisms could be responsible for maintaining force output: 1) in extended fatiguing contractions additional motor-units could be recruited to maintain force, 2) an increase in agonist activity and/or decrease in antagonist activity to compensate for the reduced force, or

3) alterations in the contractile properties of the muscle enabling it to increase force at a given firing rate.

De Luca et al (1996) investigated these possible mechanisms and concluded that twitch potentiation which is a result of altered contractile properties was most likely responsible for the maintenance of force. In the contractions studied by De Luca et al. (1996) new motor units were not recruited while already active motor-units were decreasing their firing rates. Furthermore, during contractions at 80% MVC in the FDI all motor units had already been recruited, making the recruitment of additional units to compensate for the decrease in firing rate implausible. Agonist/antagonist muscle interaction was also monitored by surface EMG. An increase in agonist muscle activity can maintain force in the muscle being investigated while firing rates decrease, just as reduced activity from antagonists can help maintain force output. Neither of these scenarios was reported as agonists around the wrist joint actually displayed similar decreases in firing rate as the FDI and antagonist activity did not change (De Luca et al. 1996).

Twitch force potentiation therefore may be the mechanism behind maintaining isometric force in the face of declining motor unit firing rates during brief contractions. As previously mentioned and consistently reported, muscle force production is history dependent. Houston and Grange (1991) and Vandervoort et al. (1983) both reported that a 10 s MVC is sufficient to produce potentiation in skeletal muscle. Therefore it is likely that the contractions elicited by De Luca et al. (1996) between 30-80% MVC for 8-15s could cause P-light chain phosphorylation and thus render the contractile apparatus in a potentiated state. To control for the potentiated state motor unit firing rates decrease to

maintain constant force output. De Luca et al. (1996) point out that the twitch force potentiation theory is consistent with their 1996 findings. Firing rates were found to decrease at a faster rate in the FDI than in the TA, the FDI has more fast twitch muscle fibers so it would potentiate more quickly leading to a decrease in firing rates to keep force constant. Firing rates at higher force levels, such as 80% MVC, would be faster leading to rapid potentiation of the muscle fibers. Motor-unit firing rates would then have to decrease if constant force was to be maintained.

Motor Unit Behavior in the Potentiated Muscle

Muscle twitch potentiation has been proposed as the means to maintaining constant isometric force in the presence of decreased motor unit firing rate during brief contractions but few studies exist that purposely potentiate muscle to investigate this theory.

Klein et al. (2001) investigated the effect a CC of 75% MVC intended to potentiate the triceps brachii on motor unit discharge rate. Discharge rates were measured during isometric contractions held at 10, 20, or 30 % MVC at 10s, 2, 6, and 11 min post CC. Results show that a 5 s conditioning contraction at 75% MVC was sufficient to potentiate twitch force by 1.3-2.0 fold 10s post CC and the half-relaxation time decreased to 89.0 +/- 11.8%. Contraction time was unchanged. Discharge rates in 33 of 35 motor units declined significantly by 1-6 Hz 10s post CC supporting the theory that muscles in a potentiated state experience lower motor unit firing rates. The decline in motor unit discharge rates was similar across force levels. The increase in twitch force post CC was significantly correlated to the decrease in motor unit firing rate ($r= -0.74$, $P<0.01$).

Additionally, RMS amplitude was unchanged following the CC implying fatigue was not a major contributor to the decline in discharge rate. (Klein et al., 2001).

An earlier study by Suzuki et al. (1988) reported that motor unit discharge rate in the biceps brachii increased following a 5 s conditioning stimulus at either 25 or 50% MVC. However, discharge rates were measured during isometric contractions held at only 1-2% MVC which may account for the divergent findings (Suzuki et al., 1988).

It is clear that more work is needed in this area to confirm motor unit firing behavior during twitch potentiation.

CHAPTER III

METHODS

Subjects

Healthy, right leg dominant individuals with no known history of neuromuscular illness were recruited from Brock University for this study. Participants signed an informed consent document (REB #02-283) that complies with Brock University Research Ethics Board (Appendix A). All testing occurred within a Faraday cage housed in the Electromyographic Kinesiology Laboratory in Welch Hall, room 21. Involvement consisted of three visits to the EMG Kinesiology Laboratory on three separate days. The first day was used to familiarize the participants with the protocol for finding maximal voluntary contractions (MVC). Participants were also introduced to the protocol for eliciting M-waves in the tibialis anterior. Maximal voluntary contraction was determined on both day 2 and day 3. The needle electrode was inserted only on day 3. Anthropometrics, peak post-activation potentiation (PAP) as well as fatigue in response to the MVCs were recorded on day 2. Motor unit firing rate in the TA was measured in both the potentiated state as well as the control state (unpotentiated) on day 3.

Electrode Placement and Stimulation

Subjects entered the lab and were asked to lie down on the gurny while the investigator located motor points in the tibialis anterior. Several motor points were stimulated and marked with the point most proximally located in the TA being used for surface electrode placement. A constant-current (150 mA) source was used to find the motor point using the lowest possible voltage. The cathode and anode electrodes were connected in series with an isolation unit (Grass Telefactor SUI8, Astro-Med, Inc., West Warwick, RI) and a stimulator (Grass Telefactor S88, Astro-Med Inc., West Warwick, RI) to deliver a square-wave pulse, 1 msec in duration at a rate of 10 pulses per second (pps) (Calder et al., 2005). A self-adhesive anode (Pals Plus, 5.0 cm, Axelgaard, Fallbrook, CA) was secured on the gastrocnemius while a stainless-steel cathode probe (3 mm) was used to systematically explore the skin surface of the tibialis anterior until a barely perceptible muscle twitch was observed beneath the skin (Roy et al., 1986). Once the location of the motor point was determined, the stimulus intensity was increased to produce a clearly visible, localized muscle twitch at 1 pps.

The skin was shaved, mildly abraded (NuPrep®, Weaver and Company, Aurora, CO), and cleaned with isopropyl alcohol. The recording monopolar electrode was prepared with two sided tape and electrolyte gel (Signa Gel®, Parker Laboratories, Inc., Fairfield, NJ) before it was placed over the belly of the TA where the motor point was located; an electrode was also placed over the extensor retinaculum as a reference reading. The medial malleolus was used for the location of the ground (CF5000, Axelgaard, Fallbrook, CA). Once the electrodes had been secured to the skin surface, the impedance was assessed (Grass EZM Electrode Impedance Meter, Astro-Med Inc.,

Warwick, RI) to ensure that it was lower than 10 kΩ and no further preparation was necessary. The tibialis anterior was electrically stimulated from the common peroneal nerve located just posterior to the fibular head.

Recording Motor Unit Firing Rate

An intramuscular quadrifilar needle electrode was inserted into the TA distal to the monopolar electrode to measure individual motor units and their firing rates. Needle electrodes were only used on day 3 as firing rates were not measured during the familiarization and baseline measure days.

The needle electrode to measures multiple independent channels of EMG activity. Within the 25 gauge cannula there are four insulated platinum-iradium wires that are arranged so that activity from several motor units can be detected. Each wire is 50μm in diameter. There is a portion of the needle that is open where the four wires are exposed and it is through this 'window' myoelectric activity is collected. Three different channels of EMG activity can be collected from the four wires; these raw analogue signals are converted and stored digitally to be decomposed into their constituent motor unit action potentials. The motor unit signals were amplified and band pass filtered between 1 kHz and 10kHz. Signals were sampled at 25.6 kHz with a 16 bit A/D converter (NI-DAQmx PCI-6251, National Instruments, Austin, TX), and stored on a computer for offline analysis using Dasylab software (Data Acquisition System Laboratory, Dasytec, USA, Inc, Amherst, NH). All motor unit signals were digitally up-sampled to 51.2 kHz using a custom written program in MatLab (Mathworks, Inc., Natlick, MA) prior to identification.

The purpose of collecting activity from three independent channels is so individual motor units can be more accurately identified. The three channels display action potentials from several different motor units from three different geometrical perspectives making it possible to discriminate between and follow motor units throughout the protocol. Digitally stored myoelectric activity is decomposed to identify individual motor units and their firing characteristics. Decomposition algorithms are used to identify action potentials and match them to motor units via the template matching, template updating, and superpostitions techniques (Mambrito & De Luca, 1983). Each file was manually inspected by a trained operator to resolve misidentifications and temporal superposition of multiple motor units. This methodology has been employed elsewhere (Knight & Kamen, 2004; Kamen et al. 1995).

Force

Subjects were seated in an upright custom made experimental chair designed to measure force output of the tibialis anterior during dorsiflexion. A load-cell (JR3 Inc., Woodland, CA) was secured beneath a foot-plate so that the ankle joint was placed at 110° (slight plantar flexion). Hip and knee angles were at 90° with a restraining plate over the distal aspect of the quadriceps to decrease motion in the transverse plane while enabling motion in the longitudinal plane. A steel bar was tightened proximally to the metatarsals to enable force generation. Padding under the bar was used to increase comfort and decrease any pain in the upper foot.

Targets representing either 100% or 50% MVC were displayed, depending on the portion of the testing day being carried out, as a horizontal line on an oscilloscope

(Hitachi, VC-6525). The target was at the intended % of MVC and the horizontal lines represented ±2.5% of this voltage level.

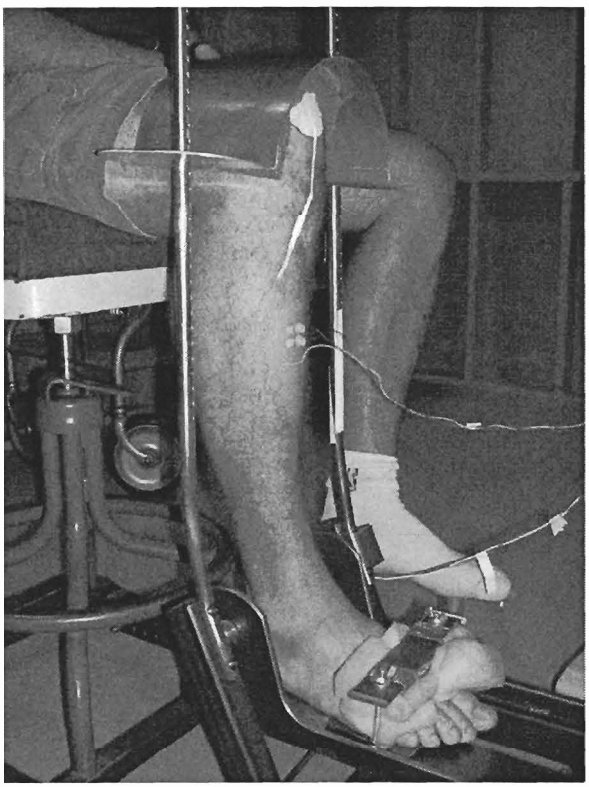


Figure 3.Testing Apparatus and Position.

Data Recording

The sEMG signals were band-pass filtered between 3 and 300 Hz and amplified (Grass P511, Astro-Med Inc., West Warwick, RI) to maximize its resolution on the 16 bit analogue-to-digital converter (NI PCI-6052E, National Instruments, Austin, TX). The force and sEMG signals were sent to the connector block (BNC-2110, National Instruments, Austin, TX) associated with the analogue-to-digital conversion board. All signals were sampled at 25.6 kHz using a computer-based data acquisition system (DASYLab, DASYTEC National Instruments, Amherst, NH). The data was stored on a Celeron PC for off-line processing (Dell, Round Rock, TX).

Day 1: Familiarization to MVC Protocol

A separate session was dedicated to familiarizing the subjects to the protocol used to find MVC. A target was presented as a horizontal line on an oscilloscope (Hitachi, VC-6525) placed in front of the subject. It was important that the MVC used throughout the study was as close to a true maximum as possible in order to potentiate the TA. Large strength gains have been observed without training due to subjects familiarizing themselves to a novel movement. Knight & Kamen (2001) studied the effect a six week strength training program had on quadriceps muscles in old and young subjects. Prior to starting the program two baseline measures were taken with eight days separating them. Results showed that the fastest jump in strength occurred during the eight days between baseline measures. Calder & Gabriel (2007) investigated the effect timing of the familiarizations had on subjects maintaining the skill. They concluded that sessions could be done on the same day, and it was determined that strength gains were maintained up to 3 months after the initial session.

The familiarization day allowed participants to practice maximally contracting their TA and ensured that on day 2 and day 3 as close to a true value for MVC as possible would be recorded. A total of 3 maximal contractions with 3 min rest separating each contraction were performed. Subjects were then allowed to leave.

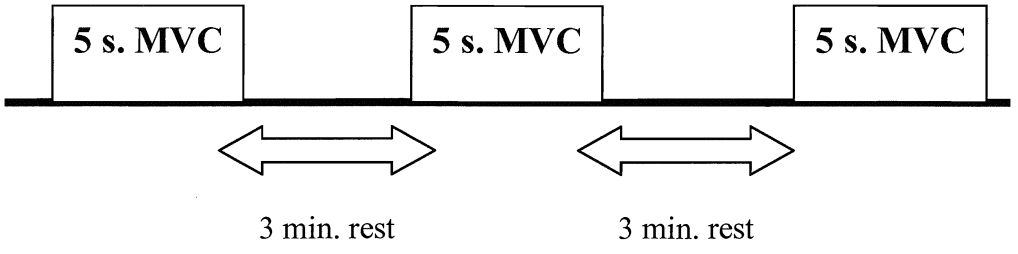


Figure 4.Schematic representation of the day 1 familiarization protocol.

Day 2: Determine M-wave max, MVC, PAP, and Central Fatigue

Subjects arrived for day 2 and were prepared for the protocol as outlined in the apparatus section; anthropometrics (height (m), weight (kg), lower leg length (cm), leg circumference (cm), and foot length (cm)) were then recorded. BMI was calculated using height and weight (Table 1). Day 2 began with the peroneal nerve being located and stimulated until an M-wave with a stimulation artifact that returned to baseline was observed. At this point the voltage was increased until the amplitude of the M-wave reached a plateau. When the experimenter was satisfied that a Mmax had been reached, based on the above criteria, three initial twitches were elicited and recorded. These twitches were used as the baseline twitches to which all other twitches were compared. In order to avoid any potentiating effects that may have occurred while finding M max the subject rested for at least 5 minutes prior to the recording of the baseline twitches. Determining MVC was also performed after the baseline twitches were recorded to avoid the same potentiating effect. To determine the force level for maximal voluntary contractions, subjects maximally contracted 3 times for 5 seconds each (Figure 5). The rest time that followed each contraction was a minimum of 3 minutes. The contraction that elicited the greatest force level was used as the MVC for the rest of day 2. A force level equal to 50% MVC was calculated based on that value. Subjects then rested for 10 minutes in order to recover from any fatigue associated with the MVC's. The rest period was also necessary to allow potentiation to dissipate.

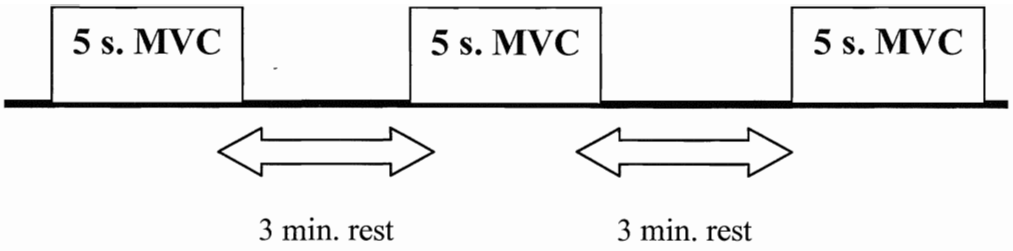


Figure 5.Protocol for determining MVC on day 2. Baseline twitches were recorded prior to the MVC protocol to avoid any potentiating effect.

Post-tetanic potentiation was quantified following a conditioning contraction at 100% MVC for 10 seconds. Subjects were asked to maximally contract for 10 seconds with encouragement from the experimenter. After 10 seconds the subject relaxed and twitches were stimulated at the common peroneal nerve for 5 min. Twitches were elicited 2s, 5s, 15s, 30s, 1min, 2min, 3min, and 5 min post MVC to observe when twitch torque would be maximal as well as when twitch torque would return to baseline (Figure 6). Subjects then rested for at least 10 min to allow potentiation to fully dissipate.

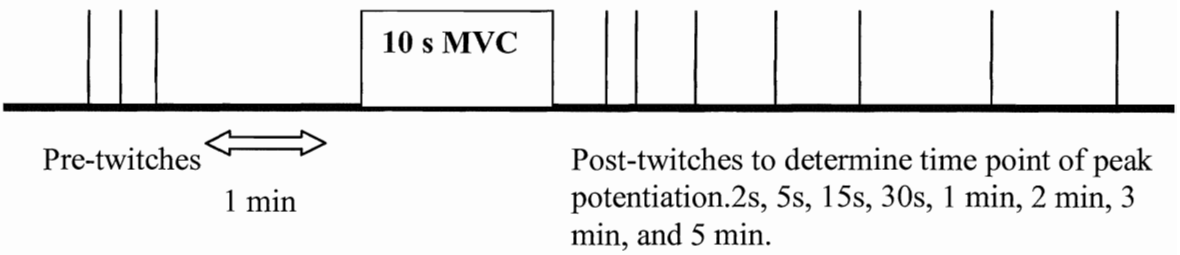


Figure 6. Protocol for tracking PAP following the 10 s condition contraction.

The purpose of the next series of experiments in the protocol was to observe whether having the subjects contract for 10s maximally, and 5s at 50% of maximum would affect central activation. To do this the interpolated twitch technique was applied.

The subject was asked to maximally contract and when a clear plateau in force was evident a supramaximal twitch was delivered. The voltage was set at 1.1 times that of the voltage used to elicit M max to ensure complete activation. Immediately following the supramaximal twitch the subject relaxed and another twitch was elicited in the resting state. Five minutes of rest was then allowed.

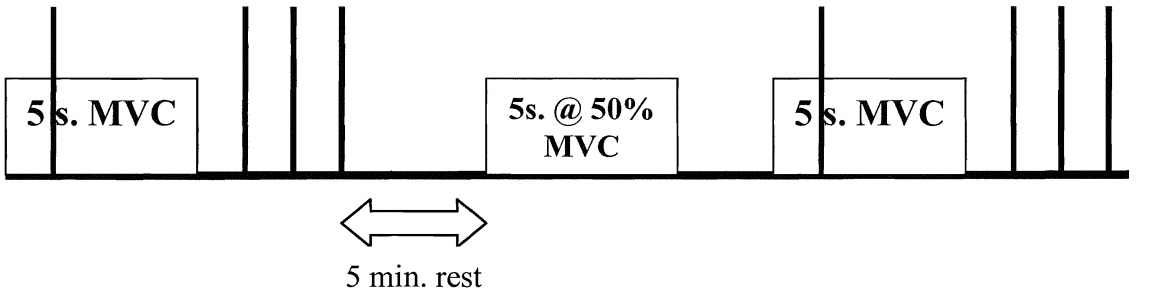


Figure 7. Interpolated Twitch Protocol for 50% MVC.

After the five minute rest period the subject then contracted for 5 seconds at 50% MVC. Immediately following the 5s 50% contraction another interpolated protocol was carried out (Figure 7). The results of the interpolated twitch following the 5 second 50% MVC were compared to the results prior to the 5 second 50% MVC to determine the effect on central activation to the muscle. The same protocol was then followed with a 10 second MVC. Each subject rested for 10 minutes between protocols (Figure 8).

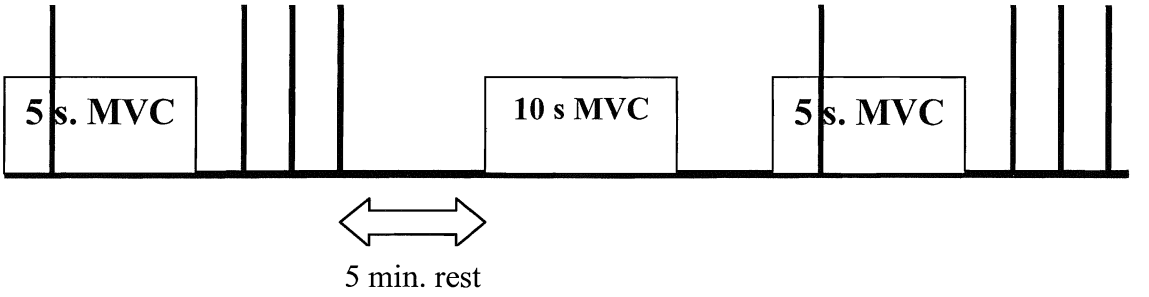


Figure 8. Interpolated Twitch Protocol for 10s MVC

Day 3: Measure MUFR in the potentiated as well as unpotentiated tibialis anterior

Subject were prepared as they were for day 1 and 2, however on day 3 a quadrifilar needle electrode was inserted just below the monopolar electrode to measure motor unit firing rate. The depth and location of the needle was adjusted until the audio component of the signal was acceptable, and clear action potentials were visible on the oscilloscope. At this point the needle was secured and left in the same location unless pain was felt by the subject or the signal was distorted in any way.

Mmax was found and baseline twitches were recorded. Subjects were asked to perform 2-3 MVCs, the highest MVC was used as 100% for the remainder of day 3. The force value for the MVCs performed during day 2 were not used to ensure that any reduction in force caused by the insertion of the needle electrode would be accounted for. After 10 minutes of rest pre twitches were recorded. These twitches were followed by a 5 second contraction at 50% MVC. Immediately after this contraction 3 post twitches were elicited (Figure 9). Motor unit firing rate was measured during the 5 second contraction. This was the control condition in which the muscle was not potentiated.

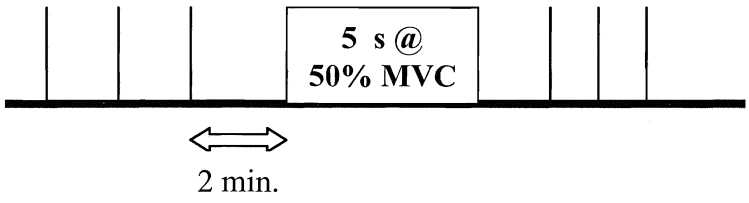
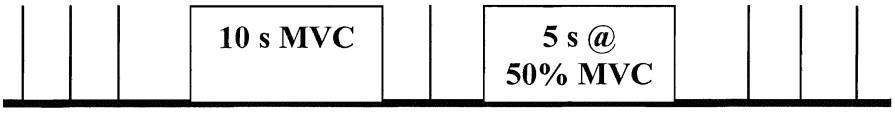


Figure 9. Control protocol for measuring motor unit firing rate in the unpotentiated tibialis anterior.

After 10 minutes of rest, 3 pre twitches were recorded followed by a conditioning contraction of 10 seconds at 100% MVC which was immediately followed by a 5 second contraction at 50% MVC (Figure 10). Firing rate was measured during the 5 second contraction. A twitch was recorded during the brief period of time between the two contractions to verify the conditioning contraction had the intended effect. 3 twitches were also recorded after the protocol.



Pre twitches	Potentiating intervention	Twitch to verify potentiation	Motor unit firing rate measured in potentiated state	Post twitches to verify potentiation during measurement

Figure 10. Protocol for measuring motor unit firing rate in the potentiated TA.

After a minimum of 10 minutes rest motor unit firing rate was again recorded in the control condition to verify that any changes seen in the potentiated TA was a result of the intervention (conditioning contraction) and not simply a time dependent change, figure 8. At this point the experiment was complete.

Statistical Analysis

An ANOVA using planned comparisons was used to compare means between the firing rates of motor units in the control versus potentiated tibialis anterior as well as all other myoelectric and torque data ie: twitch force, RMS, MPF, and PP amplitude of the

m-wave. An n=10 was used to protect against type II error. Data are reported as means ± standard deviation (SD). Significance level was set at 0.05.

CHAPTER IV

RESULTS

Subject Characteristics

All subjects (n=10) were university aged males and females. Anthropometrics were recorded during day 2 of testing while the subject rested seated in the experimental chair. Characteristics of interest were as follows: Age 22.9 ±1.85, height 1.78±0.10m, weight 79.32±12.92Kg, BMI 24.81±2.32 Kg/m^2 , lower leg length 54.10±5.34cm, lower leg circumference 39.15±3.64cm, and foot length 26.10±2.77cm.

Quantifying Potentiation

A 10 second MVC was used as the CC (Vandervoort et al. 1983). To measure the potentiation caused by this CC twitches were elicited in the tibialis anterior before and for 5 minutes after the contraction. Average baseline twitch force pre conditioning contraction was 18.36±5.43 N. Twitch force recorded 2 seconds following the 10 s MVC was potentiated 2.40 times (42.92±13.6N) (P<0.05). Force levels for all subjects peaked at the 2 second twitch and steadily decayed throughout the remaining 5 minutes (Figure 11). Twitch force remained significantly elevated at all time points including the 5 minute recording, which remained elevated at 22.19±9.4N (P<0.05). Twitch force decreased by 48% from the 2 second recording to the 5 minute recording.

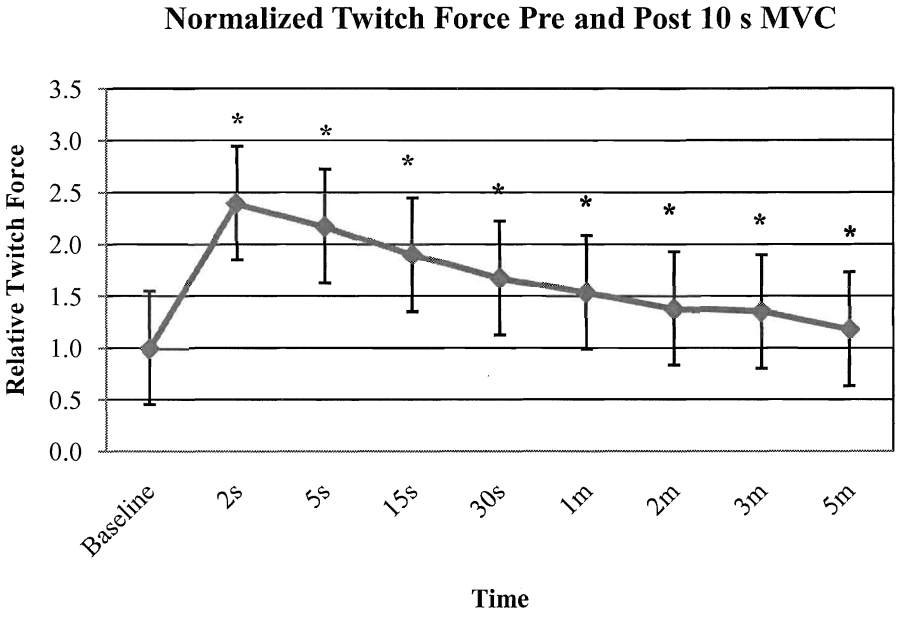


Figure 11. Decay in potentiation 2 s, 5 s, 15 s, 30 s, 60 s, 120 s, and 300 s post CC. Baseline measure was taken 1 min before the CC. * denotes significance set at the 0.05 level. Values represent means ± SD. At the 5 minute mark twitch force was still elevated by 1.18 (±0.18) times.

Day 3 Twitch Force

Twitch force from twitches elicited pre and post 50% MVC during the control experiments did not differ significantly ($P<0.05$) During the experiment in which the conditioning contraction was used to potentiate the muscle pre and post twitches were analyzed to verify the contraction had its indended effect. Mean voluntary force through out the 10s conditioning contraction decreased 10.85 ± 4.86% (223.45±59.81-199.19±55.97 N) (Table 2) . Twitches recorded immediately post conditioning contraction were significantly potentiated from 18.21±5.73N pre 10 second MVC to 47.60±28.37N post MVC, an increase of 161% (Table 1). Normalized twitch force is

depicted in Figure 12. These values are similar to those presented for Day 2. The standard deviation for the potentiated twitch force for day 3 was substantial and was due to one subject increasing twitch force 6 fold. In keeping with one of the objectives of the study, namely to potentiate the tibialis anterior, data for this subject was included in the analysis.

Table 1- Summary of Major Results. Values are means ± standard deviations for twitch force (N), Motor Unit Firing Rate (pps), Mean Power Frequency (Hz), Root Mean Square (mV), and Peak to peak amplitude of the max M-wave (mV). All values are presented for both the potentiated and unpotentiated condition.

	Unpotentiated (n=10) Mean±SD	Potentiated (n=10) Mean±SD
Twitch Force (N)	18.21±5.73*	47.60±28.37*
Firing Rate (pps)	20.15±2.90*	18.27±2.99*
Mean Power Frequency (Hz)	60.13±7.75*	53.62±8.56*
RMS (mV)	0.28±0.10	0.33±0.12
$PP_{m\text{-}wave}$	11.23±2.04	10.45±2.11

* denotes significance at the 0.05 level

Table 2- MVC Force During Conditioning Contraction. Mean Force ± SD in Newtons (N) at the start and end of the maximal conditioning contraction (CC), as well as the percent decrease.

	Force± SD (N)
Start of 10 s MVC	223.45 ±59.81
End of 10 s MVC	199.19 ±55.97
% Decrease ± SD	10.85 ±4.86

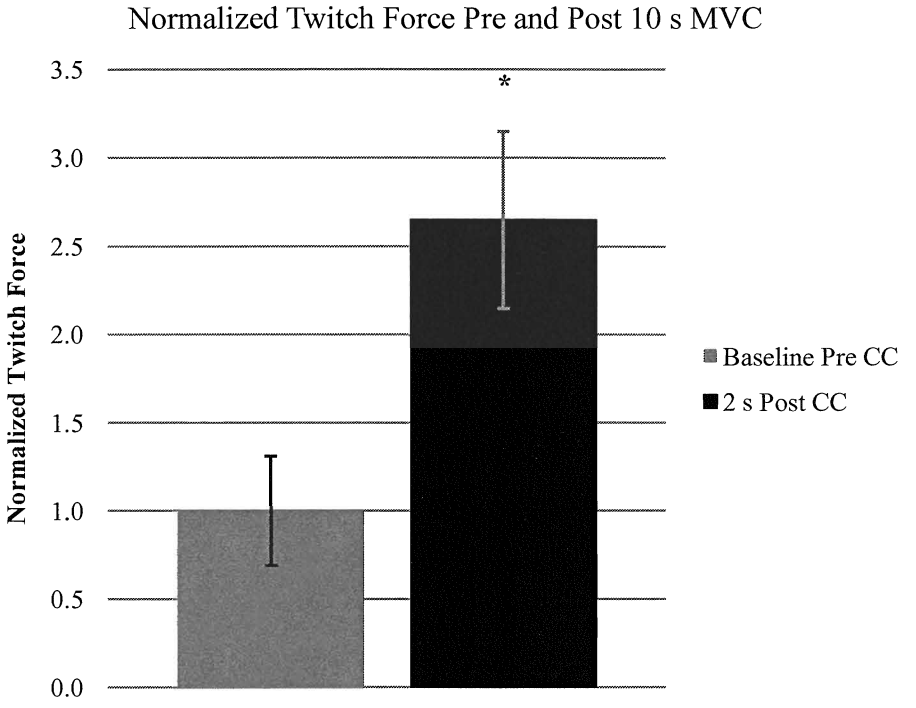


Figure 12. Normalized twitch force pre, 1.0 (±0.31) and post 10 s MVC. Bars represent means ± standard deviations. Potentiated twitch force 2 s post CC was on average 2.65 (±1.61) times that of the baseline twitch force. *denotes significance at the 0.05 level.

Motor Unit Firing Rate

Motor unit firing rate was recorded during a 5 second 50% MVC which was preceded by the conditioning contraction and 1 twitch used to verify the muscle was potentiated. A total of 33 motor units were identified during the control contraction and 27 during the potentiated contraction. Results revealed that mean firing rate decreased significantly from 20.15±2.90 pps in the unpotentiated muscle to 18.27±2.99 pps in the potentiated muscle ($P<0.05$) (Table 1, Figure 14). Motor unit firing rates decreased in 9 out of 10 subjects (Figure 13), as firing rate for one subject remained consistent pre to post conditioning contraction. Firing rates were analyzed during the most stable 3-4

seconds of the contraction. The number of motor units recruited ranged from 4 to 12 motor units depending on the subject.

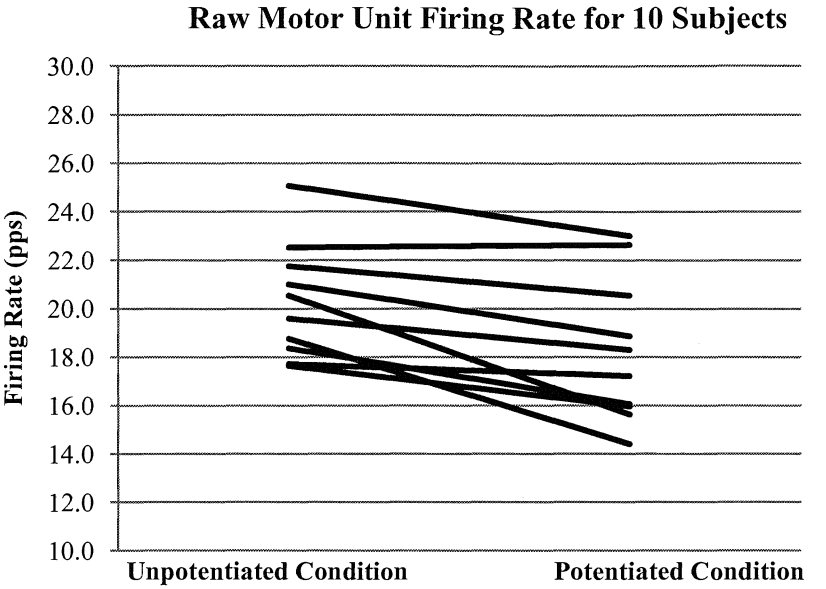


Figure 13. Raw motor unit firing rate (pps) for all 10 subjects. Motor unit firing rate decreased in 9 out of 10 subjects. Firing rate was not significantly different pre to post CC for one subject, changing from 22.5 pps to 22.6 pps.

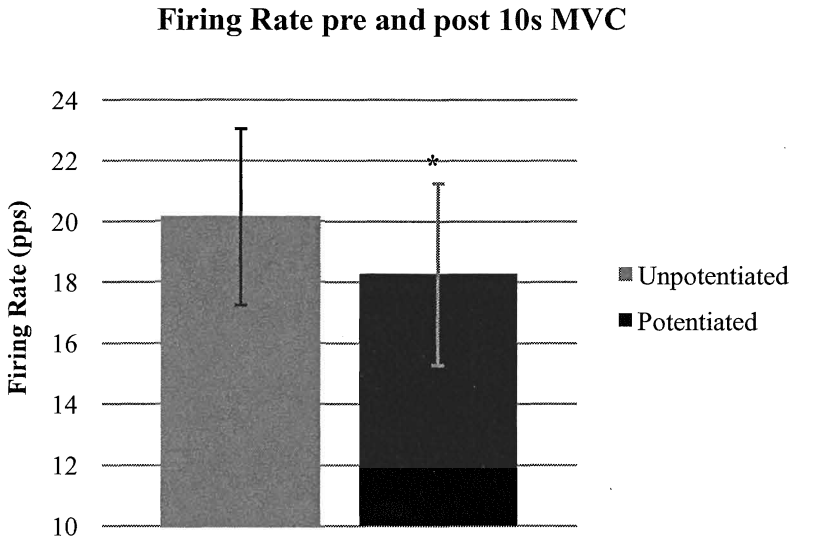


Figure 14. Motor unit firing rate in the tibialis anterior during a 5 second 50% MVC in the potentiated and unpotentiated condition. Bars represent means ± standard deviations. Firing rate decreased significantly from 20.15 (±2.90) pps to 18.27 (±2.99) pps. *denotes significance at the 0.05 level

Surface EMG Recordings

The 50% MVC in the potentiated and unpotentiated condition were analyzed for changes in mean power frequency (MPF), and root-mean-square (RMS). The potentiated and unpotentiated twitch was analyzed for changes in peak-peak amplitude of Mmax. Of the three sEMG parameters MPF was the only one to undergo significant changes pre to post conditioning contraction with RMS and peak-peak Mmax amplitude remaining unchanged (Table 1). Mean power frequency decreased significantly to 53.62±8.56Hz after the conditioning contraction (Figure 15) from a baseline of 60.13±7.75Hz (Table 1) during the control contraction ($P<0.05$) coinciding with the decrease in firing rate.

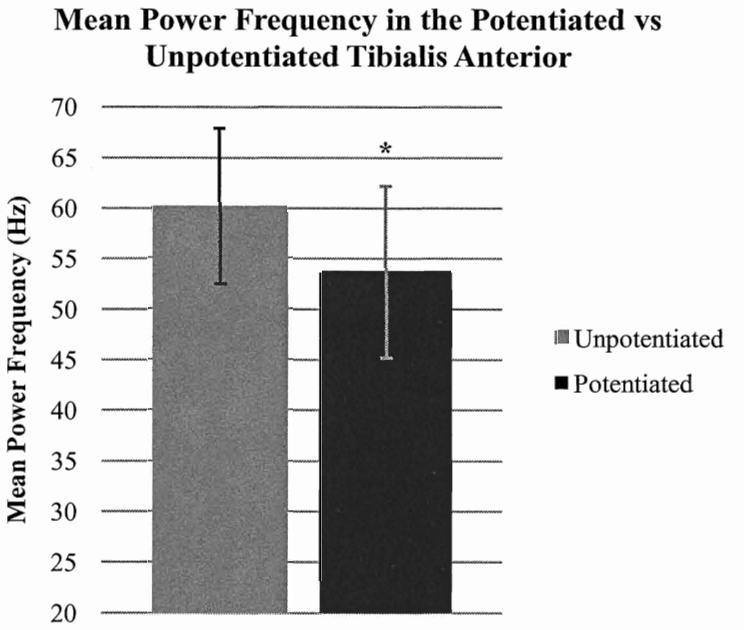


Figure 15. MPF in the tibialis anterior during a 5 second 50% MVC in the potentiated and unpotentiated condition. Bars represent means ± standard deviations. *denotes significance at the 0.05 level

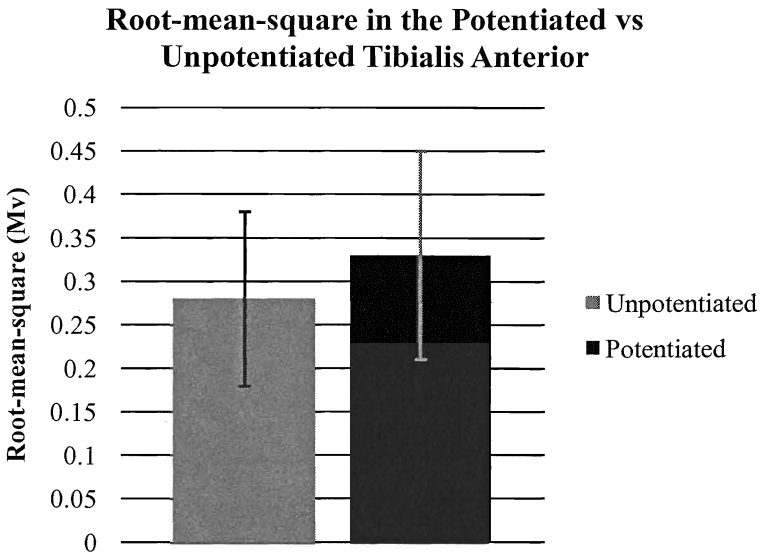


Figure 16. RMS in the tibialis anterior during a 5 second 50% MVC in the potentiated and unpotentiated condition. Bars represent means ± standard deviations. There was no significant change in RMS pre to post CC. 0.28±0.10 mV vs. 0.33±0.12 mV.

CHAPTER V

DISCUSSION

The purpose of the present study was to bring clarity to the research surrounding the effect of potentiation on motor unit firing rate in the tibialis anterior (TA). Motor unit firing rate was measured using a quadrifilar needle electrode while the TA was in a potentiated state. A 10 s conditioning contraction (CC) during which the subject maximally dorsiflexed was used to potentiate the TA; potentiation was verified by eliciting twitches immediately following the CC.

Regulation

Skeletal muscle activation is largely history dependent. The ability of muscle to generate force is a balance between two opposing phenomena, potentiation and fatigue. Skeletal muscle potentiation, in the human model, referred to as post activation potentiation (PAP), is characterized by the muscles ability to generate greater twitch force following a brief CC than before a contraction. Muscles made up of mostly type II fast twitch fibres, or a mix of slow and fast fibres are more susceptible to potentiation (Houston and Grange, 1990). The opposing effect, namely fatigue which is also more prevalent in fast twitch fibres, is defined as repeated muscle activation resulting in the decreased ability of the muscle to generate maximal tension and power (Vandenboom, 2004). The net effect of potentiation and fatigue results in the degree to which muscle can generate force.

The amount of force a muscle generates is also dependent on motor unit firing rate. The effect of muscle fatigue on motor unit firing rate has received considerable attention and more recently it has been proposed that firing rates throughout a fatigue

protocol display a tri-phasic pattern (Adam & De Luca, 2005). Subjects were required to maintain a series of contractions held at 20% MVC in the vastus lateralis for 50s with 6s rest in between contractions. Time to exhaustion averaged 8.2 contractions for 50s each. Firing rates decreased during the first 15-40s of the first contraction to perhaps adjust to a potentiated muscle. Adam & De Luca (2005) elicited twitches and analyzed RMS amplitude to verify that this decrease was not the result of fatigue. At this time point, there was no change in RMS amplitude and twitch force was potentiated by 111% confirming the absence of fatigue (Adam & De Luca, 2005). This decrease however is short lived as firing rates then increase to maintain force output, an increase which is accompanied by the recruitment of additional motor units with the inevitable decline in firing rate as force eventually dissipates at 100% endurance time (Adam & De Luca, 2005).

Although it has been hypothesized that the decrease in firing rates first observed during a fatigue protocol may be the muscle reacting to potentiation, conclusive evidence for this theory is scarce. Klein et al., (2001) observed a decrease in firing rate in the triceps brachii muscle following a conditioning contraction while Suzuki et al., (1988) reported a slight increase. We found that following at conditioning contraction (10 s MVC) the potentiated tibialis anterior displays decreased firing rates when compared the unpotentiated condition.

Characteristics of MUFR and sEMG recordings in the Potentiated Condition

Firing rate was measured via quadrifilar needle electrode in the potentiated as well as unpotentiated tibialis anterior. Firing rate was measured during a 5 second contraction at 50% MVC. Decomposing EMG signals obtained from indwelling electrodes can be quite time consuming as Adam and De Luca (2005), reported spending as much as 200 hours decomposing action potentials. As contraction intensity increases additional motor units are recruited and firing rates increase, both of which add to the complicated nature of the analysis. Contraction intensity however, does not impair the ability to successfully decompose the signal (Mambrito & De Luca, 1984). In an attempt to control the amount of time required for data analysis, firing rates in the present study were analyzed during contractions equal to 50% of MVC.

Results indicated that firing rates in the TA decreased significantly from 20.15±2.90 pps at baseline to 18.27±2.99 pps during the potentiated contraction. All subjects were able to maintain the 50% MVC for 5 s without significant deviation from that force level.

Surface measures of muscle activity were analyzed in conjunction with indwelling signals. Root-mean-square, which represents the signal power, (De Luca & Van Dyk, 1975) was used to assess changes in EMG amplitude while mean power frequency provided a means for spectral analysis. Mmax, an important measure of action potential characteristics was analyzed for changes in peak to peak amplitude. Of the three measures only mean power frequency changed to a significant degree decreasing from 60.13±7.75Hz to 53.62±8.56Hz. When the analogue signal recorded from the surface electrode is decomposed into the sinusoids that comprise that signal, the frequency of

each sinusoid as well as the weight of each sinusoid when plotted as a histogram result in the frequency spectrum. The mean power frequency represents the average frequency that contributes to the overall signal (Kamen & Caldwell, 1996). The observed decrease in mean power frequency suggests motor units with lower conduction velocities and firing rates contribute to a greater degree in the potentiated tibialis anterior.

There were no changes in RMS or peak to peak amplitude of the M-wave. Houston and Grange (1989) observed a 14% decline in force from the beginning to end of a 10 s MVC indicating the presence of some fatigue. The decline in force during the CC in the present study was only 10.85 ± 4.86%, slightly less than results from Houston & Grange (1989), indicating that fatigue was minimal. Surface measures of EMG support the fact that fatigue was most likely negligible. Root-mean-square amplitude increases with fatigue as a greater number of motor units are recruited and the rates they fire increases (Merletti et al. 1990), while M-wave amplitude decreases due to an elongated action potential caused by a membrane compromised by ionic imbalances (Nagata & Christianson, 1995). Neither RMS nor peak to peak amplitude of the M-wave changed significantly pre to post conditioning contraction in any of the subjects.

Maintaining Constant Force Output with Decreased Firing Rates

Muscle force can be graded neurologically by either a modulation in firing rate or the recruitment and derecruitment of motor units (Erim et al. 1996). While firing rate decreased subjects were able to maintain the 50% MVC contraction without the recruitment of additional motor units. Although the recruitment of new motor units was not followed in this study our MPF data suggest new motor units were not recruited. The

recruitment of higher threshold motor units to maintain force would result in an increase in MPF, whereas our results report a decrease in MPF. It is thus possible that this decrease in firing rate in the potentiated TA is necessary to prevent an overshoot in force. This is precisely the situation in the present study. Descending drive to the muscle is altered due to the peripheral change in the contractile unit. The conditioning contraction that precedes the contraction in which firing rate is measured results in the phosphorylation of the regulatory light chain on the myosin protein of the thick filament. When Ca^{2+} levels increase in the contracting muscle, Ca^{2+} binds to calmodulin activating myosin light chain kinase. MLCK is the kinase responsible for phosphorylating the thick filament (Stuart et al., 1987). When the light chain on the thick filament is phosphorylated the myosin head moves away from the backbone increasing the likelihood it will bind to actin to create a force generating cross-bridge (Sweeney et al. 1993).

The recruitment of additional motor units would be another mechanism to maintain force in the presence of decreasing firing rates (Erim et al., 1996); however, in the present study it is speculated that this was not the case.

As motor units are recruited to initiate and maintain force during muscle activation, the rate at which those motor units fire contribute to the frequency spectrum for that contraction (Kamen & Caldwell, 1996). Motor units are recruited as a rule from slow to fast, fatigue resistant to fatigue prone which translates into slow twitch fibres being recruited first while fast twitch fibres are recruited as contraction length or intensity increases (Mendell, 2005). Firing rates of motor units that fire earlier in a contraction fire faster than those recruited later, this organization of firing rates enables the optimization

of force and the time for which it can be maintained (Hatz & Buys, 1977). It would be unlikely that fast twitch fibres, which are more fatigable than slow twitch fibres, would be able to contribute to force production for a sustained period of time if their firing rates matched those of lower threshold motor units (Erim et al., 1996). This behavior is consistent up to 50% MVC after which firing rates across motor units begins to converge. Force is the priority at the expense of fatigue (Erim et al., 1996). This neurological design is quite intelligent as higher threshold motor units generate large amounts of force but are susceptible to fatigue. Therefore, lower firing rates at higher force levels should mitigate the onset of fatigue (Erim et al. 1996).

It could be postulated that potentiated muscles are more likely to recruit fast twitch muscle fibres earlier in a contraction which may somehow be beneficial. Post activation potentiation has been shown to affect fast twitch fibres (Houston et al., 1989) while phosphorylating the regulatory light chain in type I fibres has little effect on contractile function. Afferent input post conditioning contraction may alter descending drive to the motor unit, more selectively recruiting the now more efficient potentiated fast twitch fibres. Having a greater proportion of fast twitch fibres active at the onset of activity, following a conditioning contraction, firing at frequencies that are lower than the slow twitch fibres could account for the decrease in firing rate as well as well as the spectral compression illustrated by the change in MPF.

Mean power frequency and firing rate both decreased in the current study. It could be concluded that fatigue played a role in these decreases; however the presence of fatigue was not evident as subjects were able to easily maintain the 50% MVC contraction for the entire 5 seconds. Fatigue can be defined as the inability to voluntarily

maintain a given force level (Taylor & Gandevia, 2008). None of our subjects displayed this impairment. Additionally, as previously mentioned, RMS amplitude did not change pre to post conditioning contraction, while force decreased during the CC by only 10.85 ± 4.86%, suggesting fatigue was negligible. Therefore, the decrease in firing rate and MPF might be somehow related to the muscle being in a potentiated state. This has been hypothesized elsewhere (Adam & De Luca, 2005; Klein et al., 2001) and may be a mechanism to delay the onset of fatigue or attenuate energy consumption by the peripheral system. Action potentials are accompanied by an influx of Ca^{2+} into the muscle cell and sequestering that Ca^{2+} by the sarcoplasmic reticulum at the end of contraction comes at an energy cost as ATP is hydrolyzed during this process (Vandenboom, 2004). If firing rate to the muscle decreases due to the myosin light chain being phosphorylated fewer action potentials may lead to a conservation of energy and a delay of onset of fatigue.

Methodological Considerations

According to Vandervoort et al. (1983) a 10 s MVC is required to significantly potentiate the tibialis anterior and the results of this study support this finding. Houston & Grange (1989) compared the effect of using a 10 s MVC versus a 60 s MVC on twitch potentiation, phosphate incorporation and metabolite displacement. Twitch force 2 s following the 60 s MVC had decreased to 0.66 of baseline and was potentiated by only 21% at the 4 minute mark of recovery. Phosphate incorporation was significantly elevated from 0.42 mol phosphate/mol P-light chain pre 60 s MVC to 0.79 mol phosphate/mol P-light chain. ATP decreased 20%, lactate increased 15 fold, while

phosphocreatine (PCr) decreased 84%. According to Houston & Grange (1989) the 10 s MVC maximally potentiated the muscle by 1.53 times 2 s following the contraction with only modest metabolite displacement. ATP decreased by 10%, lactate increased 3 fold, and PCr decreased 35%. One min into recovery both PCr and ATP had returned to resting levels (Houston & Grange, 1989). Therefore, the CC chosen in this study was appropriate as it significantly potentiated the TA; and although metabolites were not measured, RMS amplitude data suggest fatigue was minimal.

Following the conditioning contraction, twitch torque was maximally potentiated at the 2 s time point by 2.4±0.58 fold. The decay of potentiation was tracked for 5 minutes (2 s, 5 s, 15 s, 30 s, 60 s, 120 s, 180 s, and 300 s) post conditioning contraction and although it had decreased to within 18% of the baseline twitch at 300 s, force was still significantly potentiated ($P<0.05$). This result is again consistent with Vandervoort et al., (1983) who reported a potentiated twitch that persisted for up to 8 to 10 minutes. Inserting the needle electrode on day 3 had no effect on potentiation as the 10 s MVC resulted in a twitch force that had increased by 2.65±1.61 fold.

Twitches were also elicited before and after the control conditions in which motor unit firing rate was recorded during a 5 s contraction at 50% MVC. No significant differences were found in twitch force thus signifying the need for contractions with intensities greater than 50% MVC to potentiate the tibialis anterior.

Mechanical Considerations

Stimulation to the tibialis anterior to generate dorsiflexion twitch force was applied over the common peroneal nerve. The peroneal nerve also innervates the peroneal muscles which act as plantar flexors in the lower leg. To minimize the opposing effect of the plantar flexors on dorsiflexion force the ankle was placed in 10-15° plantar flexion (Marsh et al., 1981, McNeil et al. 2005).

Shortening the plantar flexors significantly decreases their ability to generate force. Gandevia & McKenzie (1988), while studying the effect of shortened muscle length on voluntary activation discovered that when the tibialis anterior is shortened thus lengthening the plantar flexors, stimulation at the peroneal nerve spreads to the plantar flexors resulting in plantar flexion torque. Therefore putting the ankle in slight plantar flexion should shorten the muscle putting it on the descending portion of the length tension curve. This portion of the curve is characterized by impairment in the ability to generate maximal force due to decreased filament overlap (Josephson, R.K., 1999; Manal et al. 2006; Morgan et al. 1991).

However, in retrospect, if the purpose of this methodological control is to ensure maximal potentiated twitch force the merits must be revised taking into account muscles in a lengthened position potentiate less than those at a shortened length. These opposing effects may negate the need for this methodology (Vandervoort et al., 1983).

Alternate Interpretation

It should be acknowledged that a decrease in MPF is commonly observed during tasks that progress to fatigue as fast fatigable fibres are recruited and subsequently fatigue. Although the exact mechanism of fatigue at the contractile level is not clearly understood an accumulation of metabolites related to the hydrolysis of ATP is likely. Additionally, electrolyte imbalances found in a fatigued muscle compromise membrane integrity impairing the ability of the muscle cell to reach threshold to generate an action potential (Merletti et al. 1990). Mean power frequency is correlated to conduction velocity (CV) in such a way that if MPF is decreasing it is likely that CV is also decreasing (Merletti et al., 1990). A slowing of conduction velocity is another indication of fatigue. However, during fatiguing contractions action potentials are slower and thus are recorded as larger in amplitude exhibited by an increase in RMS, Adam & De Luca, 2005, an observation which is lacking in the present study.

One possible explanation for the ability of the CNS to detect the change in muscle activation due to potentiation may be group III and IV muscle afferents. The role of these sensory afferents is not well understood although it has been reported that they respond to mechanical and chemical changes associated with fatigue and the metabolic products of fatigue (Martin et al., 2008). The 10 s MVC used to potentiate the tibialis anterior with minimal fatigue, would have caused significant changes within the muscle cell, such as increases in lactate and inorganic phosphate as well as decreases in ATP and phosphocreatine concentrations. These changes may have been enough to trigger group III and IV afferents to relay signals to the spinal cord to adjust firing rate in order to maintain constant force output. Additionally, the force produced by the 10s MVC would

have been sensed by the golgi tendon organ (GTO). The GTO relays changes in tension in the muscle to the spinal cord allowing for alterations in descending drive to help coordinate smooth movement and locomotion (Leonard, 1998). It is possible that group Ib afferents, the afferents the GTO communicates through, signal Ib interneurons to alter descending drive down the α motoneuron, thus decreasing firing rate to the TA (Figure 17).

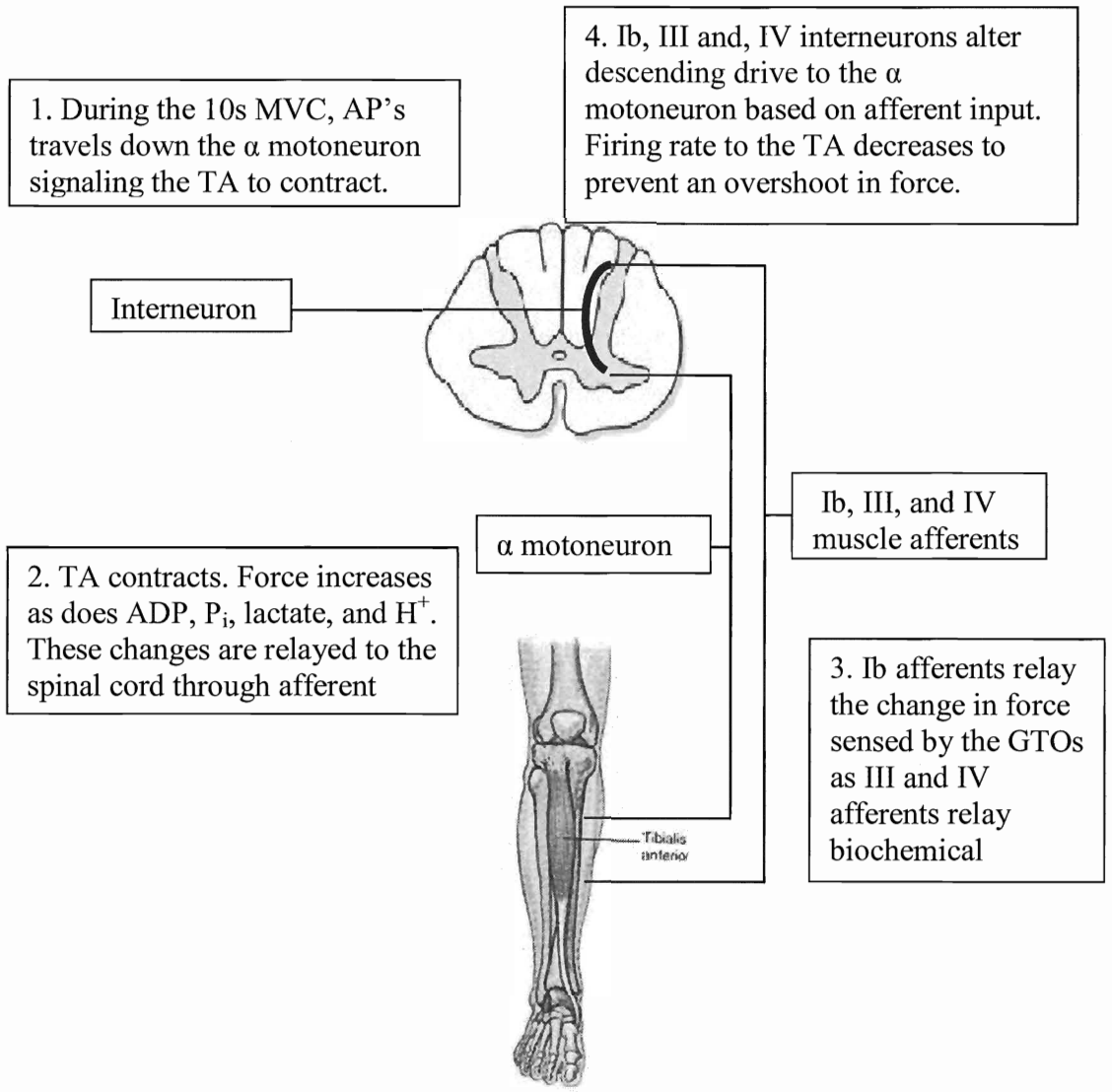


Figure 17. Schematic Sensory Diagram. Possible sensory mechanism responsible for decreasing motor unit firing rates in the potentiated tibialis anterior.

Future Directions

The present study was limited in regards to the population our results can be generalized to. We used healthy, college aged subjects free from neuropathies. Future studies should examine the effect of potentiation on firing rate in diverse populations such as those suffering from neurological impairments. Studying firing rate during potentiation at different muscle lengths and during contractions of varying intensities would also be beneficial. The sensory mechanism responsible for down regulating firing rate to the potentiated muscle is an area we touched on briefly, suggesting sensory afferents III and IV may be involved. Clarification of the sensory response to a potentiated muscle needs to be examined.

Conclusion

The purpose of this study was to measure motor unit firing rate in the potentiated tibialis anterior through the use of intramuscular needle electrodes to begin to understand the neurological role of potentiation.

The main finding was that motor unit firing rates decreased in the potentiated tibialis anterior when compared to the unpotentiated condition. Following a maximal CC of 10 s, firing rates decreased in the TA while the subject held a 5 s contraction at 50% MVC. We suggest that this decrease in firing rate is a means to counter balance the ability of the muscle to increase force per motor unit. The concurrent decrease in mean power frequency suggests that there may be a shift to higher threshold motor units innervating fast twitch muscle fibres. Fast twitch fibres, which are the most susceptible to potentiation, fire at lower frequencies during submaximal contractions and thus could

account for the observed spectral compression. Although, decreased firing rates and MPF are characteristics of fatigue, based on the analysis of RMS amplitude, PP M-wave amplitude and force data it is unlikely fatigue played a major role in firing rate and MPF. The sensory mechanism responsible for decreasing descending drive to the motoneuron is as of yet unknown and requires further investigation. Sensory afferents III and IV are sensitive to mechanical and metabolic changes within the muscle, and thus could contribute to the observed adaptation.

This study is significant in that is confirms and extends the findings by Klein et al. (2001) who reported decreasing motor unit firing rates in the triceps brachii following submaximal conditioning contractions. Additionally, the methodology employed here was carefully designed to maximize potentiation while minimizing fatigue. Therefore, the observed decrease in motor unit firing rate in the TA can be attributed to a mechanism related to potentiation with a significant degree of certainty. The mechanisms responsible for this adaptation, for example the sensory response to potentiation, should now be the focus of future research in this area.

Limitations

1- Metabolites and myosin regulatory light chain phosphate content were not measured pre or post conditioning contraction. Therefore some of the analysis of our results were made with the assumption these metabolic changes were present.
2- Needle electrodes measure firing rate in only a set portion of the muscle being investigated. Generalizations to the entire muscle based on signals recorded using this methodology must be approached with caution.
3- Isometric contractions were used to decrease the chance of motion artifact in the myoelectric signal and thus our results can only be extrapolated to similar contraction types.

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APENDICES

APENDIX A-INFORMED CONSENT DOCUMENT

Title of Project: Motor Unit Firing Rate in the Potentiated Tibialis Anterior

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This study has been reviewed and approved by the Brock Research Ethics Board (#02-283). The Brock Research Ethics Board requires written informed consent from participants prior to participation in a research study so that they can know the nature and risks of participation and can decide to participate or not to participate in a free and informed manner. You are asked to read the following material to ensure that you are informed of the nature of this research study and how you will participate in it if you consent to do so. Signing this form will indicate that you have been so informed and that you give you consent.

Introduction

You are being asked to participate in research being conducted by Jon Howard and supervised by David Gabriel Ph.D and Rene Vandenboom Ph.D. The electrical signal of skeletal muscle is measured from the skin surface, similar to electrocardiography (ECG) which measures the electrical activity of cardiac (heart) muscle. The skeletal muscle electrical signal is termed, electromyography (EMG) This electrical signal can also be measured from within the muscle by intramuscular needle EMG. The signal can then be analyzed in a number of different ways to evaluate muscle activation.

The testing will take place in the Electromyographic Kinesiology Laboratory (WH21). There will be three testing sessions, the first session will last less than an hour with subsequent sessions lasting approximately one and a half hours each and separated by at least 24 hours from the previous session.

Plan and Procedures

Jon Howard, Sean Lenhardt, Kyle McIntosh, and Greig Inglis will conduct all testing. The following procedures will take place. Prior to commencement you will be asked to complete the PAR-Q assessment of physical health status to ensure that you are not at health risk during the following experiment.

Next our lab technicians will take anthropometric measurements of each participants lower leg. These will include lengths, girths and the circumference around the peak of the calf muscle.

Upon completion of the assessment, the dominant lower leg will be prepared for testing. Small areas superficial of the tibialis anterior, patellar tendon, fibular head, and gastocnemius (calf) will be shaved, lightly abraded and cleansed with alcohol. These areas correspond to the location of the electrodes that will be taped to the skin surface. The electrodes will measure the electrical activity of tibialis anterior, similar to the more familiar electrocardiogram (ECG) that measures the electrical activity of the cardiac (heart) muscles.

We will locate the motor point (neuromuscular junction) with a light repeated superficial stimulation of the tibialis anterior. The motor point will be marked with indelible ink.

Next the participant will be placed in a chair and harnessed in to minimize synergistic activity from supporting muscles. The participants foot from their dominant leg will be placed in a foot harness where it will be secured by a clamp which is foam padded. Attached to the harness is a load-cell that will measure the amount of force upon dorsiflexion.

Day 1 Testing, MVC Familiarization

The dominant leg will be prepared for exercise testing. Small areas of the tibialis anterior, patellar tendon, calf, fibular head, and tibia head will be shaved, lightly abraded and cleansed with alcohol. These areas correspond to the location of the electrodes that will be taped to the skin surface. The electrodes will measure the electrical activity of leg muscles, similar to the more familiar electrocardiogram that measures the electrical activity of the cardiac (heart) muscles.

The subject will perform 3 maximal effort isometric (same length) contractions each separated by 2-3 minutes of rest with the purpose of familiarizing the subject with the protocol involved for determining maximal voluntary contractions (MVC).The contractions will be 5 seconds in duration. Strength measurements will be taken while seated with the leg secured in a chair designed to isolate the action of the dorsiflexors. Adjustable supports and straps on the chair will ensure stability and minimize extraneous movements. A padded foot clamp will be attached on the top of the foot. The leg not being tested will be resting beside the leg being tested. It is important not to hold your breath while strength testing.

Day 2 Testing: Baseline and Fatigue Measurements

The subject will perform 3 maximal effort isometric (same length) contractions each separated by 2-3 minutes of rest with the purpose of determining your maximal voluntary contraction which will then be used for the remainder of the study. The contractions will be 5 seconds in duration. Strength measurements will be taken while seated with the leg secured in a chair designed to isolate the action of the dorsiflexors.

Adjustable supports and straps on the chair will ensure stability and minimize extraneous movements. A padded foot clamp will be attached on the top of the foot. The leg not being tested will be resting beside the leg being tested. It is important not to hold your breath while strength testing.

Mild electrical stimulation will be applied to the peroneal nerve located laterally to your knee to induce dorsiflexion. You will then be asked to perform a maximal voluntary contraction (MVC) for 10 seconds. Dorsiflexion will again be electrically induced following the 10 second MVC.

The subject will maximally contract the TA, during this contraction electrical stimulation will be applied. A twitch will also be electrically induced immediately following the MVC. After a brief rest the subject will maximally contract for 10 seconds (10 s MVC). This will be followed quickly by another brief MVC with electrical stimulation being applied during as well as immediately after the MVC.

The subject will maximally contract the TA, during this contraction electrical stimulation will be applied. A twitch will also be electrically induced immediately following the MVC. After a brief rest the subject will contract at 50% of MVC for 5 seconds. This will be followed quickly by another brief MVC with electrical stimulation being applied during as well as immediately after the MVC.

Day 3 Testing: Motor Unit Firing Rate Measurement

The dominant leg will again be prepared for exercise testing in the same way it was for Day 1 Testing. Tibialis anterior will be mildly stimulated, the subject will then contract at 50% MVC for 5 seconds, this will be followed by another sequence of mild stimulation.

Tibialis anterior will be mildly stimulated, subject will the contract maximally for 10 seconds, mild stimulation will follow, subject will perform a 50% MVC for 5 seconds, mild stimulation will follow.

Tibialis anterior will be mildly stimulated, the subject will then contract at 50% MVC for 5 seconds, this will be followed by another sequence of mild stimulation.

Recording Voluntary Muscle Activity

Before electrode placement, the skin surface will be shaved, lightly abraded, and cleansed with alcohol to limit the at the skin-electrode impedance. Monopolar surface electrodes will record voluntary muscle activity at the skin surface of the tibialis anterior. The positions of the electrodes will be marked with indelible ink to ensure the consistency of the placement. In addition, a four-wire electrode will be inserted into the volume of tissue just below the surface electrode. We will use a 25-guage cannulua that contains four 50-μm diameter platinum-iridium wires epoxied in a side port. The same recording needle will be used for each subject but will be sterilized by autoclave prior to its reuse.

Risks and Discomforts

It is not possible to predict all possible risks or discomforts that volunteer participants may experience in any research study. Based upon previous experience, the present investigator anticipates no major risks or discomforts will occur in the present project.

1. Participants sometimes experience mild discomfort when the skin is gently cleaned and rubbed with a mild abrasive in preparation for electrode placement. On occasion, some subjects may experience skin irritation associated with the placement of the electrodes. This is usually very mild and goes away in a few hours, or a day.

2. There may be discomfort related to the delayed onset of muscle soreness associated with isometric contractions of the arm muscles. If muscle soreness does occur, it is usually very mild and should dissipate within 72 hours.

3. Infection. Placement of a needle electrode into the muscle can be somewhat uncomfortable. If pain is experienced, the needle will be moved to another area. Like any foreign body, the needle electrode poses a risk of infection. The needle electrodes to be used in this investigation will be thoroughly sterilized using the following procedure. First, the electrode cannula will be mechanically sterilized using alcohol upon removal from the participants muscle, then autoclaved prior to its reuse. Again, this is a common procedure used without incident in Biomechanics Laboratories.

4. Maximal effort isometric contractions are associated with an increase in blood pressure. You must make sure that you do NOT hold your breath during maximal

exertions. If you have received medical clearance and/or are already physically active, the risks are minimal.

Voluntary Participation

Participation in this study is voluntary. Refusal to participate will NOT result in loss of access to any services or programs at Brock University to which you are entitled. You will inform the investigator, Jon Howard of your intention to withdrawal prior to removing yourself from this study.

Discontinuation of Participation

Participation in this research study may be discontinued under the following circumstances. The investigator, Jon Howard or supervising faculty David A. Gabriel, Ph.D., or Rene Vandenboom, Ph.D, may discontinue your involvement in the study at any time if it is felt to be in your best interest, if you not comply with study requirements, or if the study is stopped. You will be informed of any changes in the nature of the study or in the procedures described if they occur. It is important to remember that you are free to terminate your participation at any time, for any reason.

Potential Benefits

Participants will receive no direct benefits from participating in this study. However, participants should know that their willingness to serve as a subject for this experiment will help a Brock University researcher and other scientists develop new theories of exercise that will benefit individuals in the future.

Costs and Compensation

The cost of the test and procedures are free. You will not receive any form of compensation for your participation in this study.

Confidentiality

Although data from this study will be published, confidentiality of information concerning all participants will be maintained. All data will be coded without personal reference to you. Any personal information related to you will be kept in a locked office, to which only the investigator has access. Four investigators will have access to the data, however, names of participants or material identifying participants will not be released without written permission except as such release is required by law.

Persons to Contact with Questions

The investigator will be available to answer any questions concerning this research, now or in the future. You may contact the investigator, Jon Howard by telephone 905-227-4007 or by e-mail at jonathan.howard@brocku.ca. You may also contact the supervisor David A. Gabriel by telephone 905 688-5550 ext. 4362 or Rene Vandenboom at 905-688-5550 ext. 4726. Also, if questions arise about your rights as a research subject, you may contact the Office of Research Services at (905) 688-5550 ext. 3035. If you wish to speak with someone not involved in the study, please call the Chair of the Department of Physical Education and Kinesiology at (905) 688-5550 ext. 4538.

Consent to Participate

Certify that you have read all the above, asked questions and received answers concerning areas you did not understand, and have received satisfactory answers to these questions. Furthermore, you have completed the PAR-Q questionnaire indicating that you are physically able to participate. You willingly give consent for participation in this study. (A copy of the consent form will be given to you).

Name of Participant (Please Print): _____

_____ _____

Signature of Participant Date (day/month/year)

In this document, the investigator fully intends to conduct all procedures with the subject's best interest uppermost in mind, to insure the subject's safety and comfort.

I have fully explained the procedures of this study to the above volunteer. I believe that the person signing this form understands what is involved in this study and voluntarily agrees to participate.

_____ _____

Date (day/month/year) Jon Howard, MSc Canadidate Department of Physical Education and Kinesiology

APENDIX B- EMG DATA COLLECTION SHEET

Subject Name:_____ Number_____
Age_____

Gender of Subject_____

ANTHROPOMETRIC

Type	Measurement (cm)
Low Leg Length	
Low Leg Circumference	
Foot Length	

EXPERIMENTAL SET-UP

Load Cell Distance	

Date:_____

Day _____

EQUIPMENT SETTINGS

AMPLIFICATION:CH1_____ CH2_____CH3_____

FILTER SETTINGS:CH1_____CH2_____CH3_____

SKIN IMPEDENCE PRE:_____ SKIN IMPEDENCE POST:_____

SKIN TEMPERATURE PRE:_____ SKIN TEMPERATURE POST:_____

TIBIALIS ANTERIOR 100% MVC

	Trail 1	Trial 2	Trial 3
Voltage			
Amplification			
Filter Low			
Filter High			
Volts/Div			
Divisions			
Time			

Max M-Wave

Volts/Division				
Time Window				
Peak to Peak				
Amplification				
Stimulation Unit	Rate	Delay	Duration	Volts
Stimulation Box				

APPENDIX C: RAW DATA AND SUBJECT CHARACTERISTICS

Table 3- Raw Force Tracking Potentiation. Raw force values in Newtons (N) tracking the decay in potentiation for 5 minutes following a 10 s MVC conditioning contraction (CC). Means ± SD are included.

Subject	Baseline	2s	5s	15s	30s	1m	2m	3m	5m
1	10.47	31.94	27.13	20.57	18.03	14.18	13.59	12.67	10.26
2	17.93	36.14	33.38	29.81	26.00	25.32	22.92	23.29	21.20
3	22.31	51.55	47.83	40.78	35.74	31.38	28.11	26.83	21.97
4	13.83	27.18	24.64	20.11	16.22	16.22	14.53	13.56	13.86
5	23.08	39.20	36.92	32.20	31.04	31.13	28.93	29.33	25.82
6	13.34	48.90	43.27	36.50	30.66	27.99	23.33	21.87	15.75
7	17.93	36.14	33.38	29.81	26.00	25.32	22.92	23.29	21.20
8	21.23	48.59	42.40	39.12	37.54	34.73	28.59	27.39	26.92
9	15.10	34.99	32.77	31.67	28.19	25.69	22.76	22.71	20.28
10	28.37	74.53	70.78	66.46	58.35	51.16	50.88	50.90	44.67
Mean	18.36	42.92*	39.25*	34.70*	30.78*	28.31*	25.66*	25.18*	22.19*
±SD	±5.43	±13.6	±13.2	±13.1	±11.8	±10.2	±10.3	±10.5	±9.4

Table 4- Relative Decay in Potentiation. Decay in potentiation 5 minutes post CC relative to the baseline measurement. Means ± SD are included. Immediately following the CC twitch force was elevated by 2.40 (±0.58) times.

Subject	Baseline	2s	5s	15s	30s	1m	2m	3m	5m
1	1.0	3.05	2.59	1.96	1.72	1.35	1.30	1.21	0.98
2	1.0	2.02	1.86	1.66	1.45	1.41	1.28	1.30	1.18
3	1.0	2.31	2.14	1.83	1.60	1.41	1.26	1.20	0.98
4	1.0	1.97	1.78	1.45	1.17	1.17	1.05	0.98	1.00
5	1.0	1.70	1.60	1.40	1.34	1.35	1.25	1.27	1.12
6	1.0	3.66	3.24	2.73	2.30	2.10	1.75	1.64	1.18
7	1.0	2.02	1.86	1.66	1.45	1.41	1.28	1.30	1.18
8	1.0	2.29	2.00	1.84	1.77	1.64	1.35	1.29	1.27
9	1.0	2.32	2.17	2.10	1.87	1.70	1.51	1.50	1.34
10	1.0	2.63	2.49	2.34	2.06	1.80	1.79	1.79	1.57
Mean	1.0	2.40*	2.17*	1.90*	1.67*	1.53*	1.38*	1.35*	1.18*
±SD	±0.30	±0.58	±0.49	±0.41	±0.34	±0.27	±0.23	±0.24	±0.18

* denotes significance set at the 0.05 level

Table 5- Subject Characteristics- Values are raw measurements for height (m), weight (kg), BMI (kg/m^2), lower leg length and circumference (cm), and foot length (cm). Group means ± standard deviations are included.

Subject (n=10)	Sex	Age	Height (m)	Weight (kg)	BMI (kg/m^2)	Lower Leg length (cm)	Lower leg circumference (cm)	Foot length (cm)
1	m	21	1.73	75.00	25.14	53	34	25
2	m	24	1.88	104.55	29.59	54	45	26
3	m	21	1.88	81.82	23.16	58	39	31
4	f	24	1.63	65.91	24.94	52	41	25
5	m	25	1.80	86.36	26.56	56	41	26
6	m	21	1.73	72.73	24.38	53	36	26
7	m	21	1.80	86.36	26.56	55	42	24
8	f	23	1.63	56.82	21.50	41	34	21
9	m	23	1.88	81.82	23.16	59	41.5	28
10	m	26	1.88	81.82	23.16	60	38	29
Mean		22.9	1.78	79.32	24.81	54.10	39.15	26.10
SD		±1.85	±0.10	±12.92	±2.32	±5.34	±3.64	±2.77

Table 6- Raw Force Data for 10 s MVC. Raw force data for all 10 subjects during the 10s MVC conditioning contraction. Data presented as force in Newtons (N).

	Force (N) at start of CC	Force (N) at end of CC
Subject 1	164.92	145.43
Subject 2	264.66	236.54
Subject 3	250.80	232.29
Subject 4	192.06	174.27
Subject 5	273.10	232.29
Subject 6	222.15	190.36
Subject 7	245.63	201.42
Subject 8	242.16	238.63
Subject 9	90.17	76.01
Subject 10	288.80	264.66

APPENDIX D- RAW ACTION POTENTIALS AND FORCE TRACE

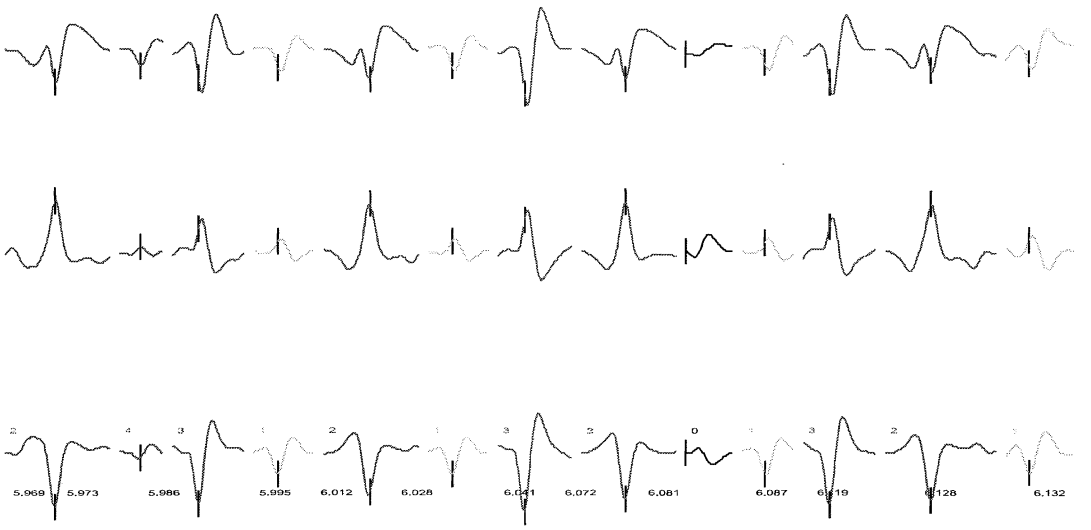

Figure 18- Three Channels of Myoelectric Activity in One Subject. A quadrifilar needle electrode inserted into the TA of one subject measured motor unit firing rate during a 5 second 50% MVC. This figure displays different views of action potentials from the three recording channels. This information was then used to calculate motor unit firing rate.

Figure 19- Raw Force Trace and Three Elicited Twitches. Force trace of a 50% MVC and 3 twitches in one subject. Vertical repeating bars represent the firing rate of 3 motor units. X axis if time in seconds, Y axis is % MVC.