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Force and Fatigue Development Following Electrical Stimulation: The Effect of Frequency and Fiber Type

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Force and Fatigue Development Following Electrical Stimulation: The Effect of Frequency and Fiber Type

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ABSTRACT

Background: Functional electrical stimulation (FES) has been used broadly during rehabilitation and sport injuries to expedite tissue and muscle recovery. More recently, attention has been given to the use of electrical stimulation at the cellular level for muscle regeneration. One of the challenges, however, is understanding the optimal FES characteristics to stimulate a muscle without causing fatigue or adverse injury to the muscle. Furthermore, since the proportion of fiber types differ in each muscle, FES application must consider fiber composition with the ideal FES application frequency.

Purpose: The purpose of this research is to evaluate the effect of different FES frequencies (10, 35, and 50Hz) on the onset of skeletal muscle fatigue, defined by a drop in muscle force. Further, to evaluate the electrical activity generated during FES application using surface electromyography (sEMG) and its relation to time to fatigue (TTF) at each frequency.

Methods:

Design: Repeated measures design. Subjects acted as their own control and each was tested randomly at the three stimulation frequencies for each muscle.

Participants: Ten healthy individuals between the ages of 18-30 years were consented according to the IRB approved protocol.

Procedure: Two different muscles with different fiber type composition were evaluated; the abductor pollicis brevis (APB) with high composition of type I fibers and vastus lateralis (VL) with high composition of type II fibers. Muscle and frequency evaluations were randomized using a random-ordered procedure to prevent carryover or learning effect.

Results: Results indicated significant differences between muscle types. The large VL required a greater stimulation amplitude to generate the FES-induced initial force of 25%MVC than the

small APB. Additionally, the required amplitude at 10Hz was greater than the other frequencies for both muscles. TTF was faster in the VL than the APB. Moreover, within the APB, TTF did not significantly differ by frequency, while for the VL, FES at 10Hz caused significantly longer TTF than at 35 or 50Hz. Further, FES-induced force decline presented earliest in both muscles with stimulation at 35Hz. An association with electrical activity behavior and the moment of initial significant force decline with FES was identified.

Conclusion: The results of our study support our main hypothesis that the frequency of FES may play a role in force generation and fatigue development. Findings support recommendations that stimulation at 50Hz may be most optimal for small muscles of predominantly type I fiber composition while stimulation at 10Hz may be most optimal for large muscles of predominantly type II fiber composition to prolong TTF. Future research should also consider examining a greater variety of muscle types at larger sample sizes. Additionally, improving filtering techniques to better extract pure muscle activity with FES should be investigated.

INTRODUCTION

Typically, muscle contraction is ordered such that the body intends to only activate the amount of fibers or proportion of a muscle necessary to perform a task. Specifically, the nervous system will transmit action potentials to neurons innervating particular muscle fibers (i.e. a motor unit) to initiate contraction (Bol, Weikert, & Weichert, 2011). Tasks requiring lower effort can be achieved with the activation of fewer motor units, but as the required effort increases the body will activate additional motor units and increase the rate of their firing activation. This pattern of ordered recruitment is known as *Henneman's size principle* (Figure 1) (Kraemer & Looney, 2012). The size principle provokes the slow-twitch, type I motor units formed of small diameter fibers to be activated first and as the required effort increases, fast-twitch, type II motor units formed of larger diameter fibers will then be activated. With this process, muscle tension is modulated based on the amount of motor units recruited and the rate of motor unit activation (Andrzejewska, Jaskolski, Jaskolska, Gobbo, & Orizio, 2014).

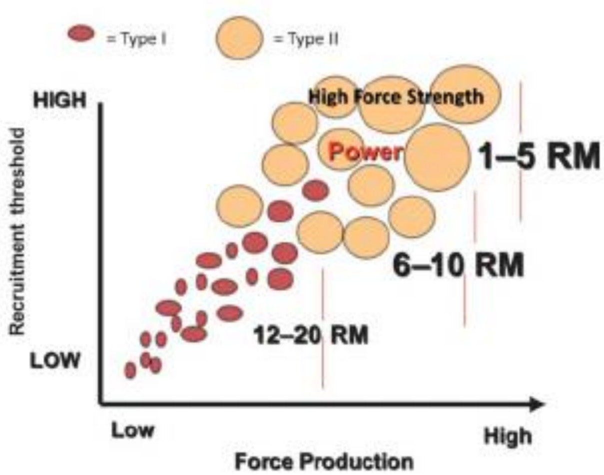


Figure 1: Motor unit recruitment according to the size principle (Kraemer & Looney, 2012).
(RM = repetition maximum)

Muscle fatigue develops after repeated or constant contraction of a muscle or muscle group during task performance leads to the muscle's inability to maintain the level of force required to accomplish the task (Dreibati, Lavet, Pinti, & Poumarat, 2010; Enoka, 1995). Since voluntary contraction follows the size principle, by activating the slow-fatiguing type I fibers first, the development of fatigue is minimized. Type I fibers contain more mitochondria and higher intramuscular fat stores so they are more fatigue resistant than type II fibers. Type II fibers, contain reduced mitochondria and greater myofibrillar ATPase activity (an enzyme that breaks down ATP in the myosin head) for enhanced force production (Fleck, S.J. & Kraemer, W.J., 2004). On the other hand, functional electrical stimulation (FES)-induced activation of muscles does not follow the size principle. FES recruits fibers based on the location of the stimulating electrodes, electrode geometry, and muscle characteristics such as fiber composition (Ibitoye, Estigoni, Hamzaid, Wahab, & Davis, 2014). As such, FES causes *synchronous recruitment* of fibers or recruits fibers in *reverse order* of the size principle (Estigoni et al., 2014; Ibitoye et al., 2014). With synchronous recruitment, all motor units under the stimulating electrode are activated together regardless of the force required by the task (Ibitoye et al., 2014). With the reverse recruitment of fibers, type II motor units that are more superficially located are activated and utilized to induce low levels of force and as the demanded force increases type I motor units will then be recruited (Estigoni et al., 2014; Morf, Wellauer, Casartelli, & Maffiuletti, 2015). Therefore, whether the fibers are synchronously recruited or reversely recruited, FES leads to faster fatigue development because more type II fibers are utilized earlier than with voluntary activation (Dreibati et al., 2010). For this reason, continued research should examine which stimulation frequencies best address promoting muscle activation based on fiber composition while reducing the development of fatigue.

Research inclusive of predominately type I muscles mostly assessed the tibialis anterior (TA), first dorsal interosseous (FDI), and abductor pollicis brevis (APB) while research inclusive of predominately type II muscles mostly assessed the biceps brachii (BB) and knee extensors (vastus lateralis (VL), vastus medialis, rectus femoris). In particular, TA fiber composition consists of predominately 60-85% type I fibers, FDI of predominantly 55% type I fibers, and the APB of predominantly 63% type I fibers (Polgar, Johnson, Weightman, & Appleton, 1973), while BB fiber composition consists of predominantly 50-70% type II fibers and VL fiber composition consists of predominantly 60-70% type II fibers (Edgerton, Smith, & Simpson, 1975; Gerdle, Karlsson, Crenshaw, Elert, & Friden, 2000; Polgar et al., 1973). Moreover, in larger muscle groups where superficial and deep tissue biopsy distinction is feasible, it has been noted that type II fibers are located superficially and type I fibers are located deeper in the tissue (Polgar et al., 1973); though, a similar proportion superficially and deep in the VL tissue has also been reported (Edgerton et al., 1975). These fiber arrangements are important to consider when applying FES since the signal is transferred superficially through the skin as opposed to internally from the CNS.

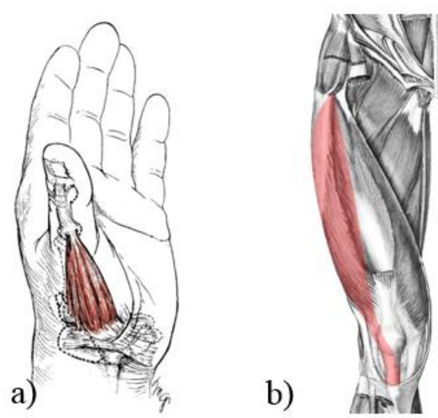


Figure 2: Anatomy of (a) abductor pollicis brevis (APB) (Troy, 2017) and (b) vastus lateralis (VL) (Bernacikova, Kalichova, & Berankova, 2010).

Additionally, size of the muscle also plays a role in fiber recruitment. For example, smaller muscles, like the APB of the hand (Figure 2a), typically rely on increases in motor unit discharge rates to achieve greater force production. Specifically, as force demands near 30% of the maximal voluntary contractile force (MVC), small muscles will have already recruited all of their available motor units, therefore the body must rely on increasing the rate of motor unit activation to achieve increased force outputs (Andrzejewska et al., 2014). In contrast, larger muscles, like the VL of the quadriceps (Figure 2b), are able to recruit additional motor units until force demands approach 60-90%MVC; it is only after this level of effort that the larger muscles then solely rely on increases in motor unit discharge rates to enhance force development (Andrzejewska et al., 2014).

With this understanding, consideration of fiber composition and muscle size should be had when implementing FES as opposed to a 'one size fits all' approach. Clinically, lower frequencies are preferred for FES as they are less fatiguing on the muscle than higher frequencies (Alexandre, Derosiere, Papaiordanidou, Billot, & Varray, 2015). It was observed in previous research that frequencies below 40-50Hz recruited more slow-twitch, type I fibers, that are more fatigue-resistant, but the higher frequencies recruited more fast-twitch, type IIa and IIb fibers, that fatigue more easily (Dreibati et al., 2010). Stimulation with frequencies ranging from 20-100Hz caused the percentage of MVC force to drop by 40-50% after five minutes and by nearly 60% after ten minutes. For this reason, it was suggested that FES training protocols not extend beyond 20 minutes of active FES and, with sports or clinical rehabilitation, a protocol of five minutes of FES spaced by ten minutes of rest, repeated four times maximally, may be most advantageous to prevent the development of fatigue (Dreibati et al., 2010).

Experimentally, fatigue development was most often defined by changes in force measures related to baseline MVC force when FES treatment was selected for a given duration or particular number of contractions (Bickel, Slade, Warren, & Dudley, 2003; Chesler & Durfee, 1997; Chou & Binder-Macleod, 2007; Gregory, Dixon, & Bickel, 2007). However, others have used gradual declines in force during FES as markers of fatigue, such as a 50% drop in force from the initial force induced by FES (Behringer et al., 2015). Additionally, electromyography (EMG) has been used to evaluate fatigue by monitoring changes in the electrical activity of the muscle.

By utilizing both force changes and EMG during FES, the understanding of fatigue progression is enhanced. Surface EMG (sEMG) signal assessment for fatiguing protocols has involved m-wave peak differences, generally decreasing with fatigue as the motor unit stops firing and m-wave duration and latency changes, corresponding to a decrease in conduction velocity (Estigoni et al., 2014). Moreover, analysis of motor unit action potential (MUAP) patterns has evaluated temporal features of the EMG signal: peak-to-peak amplitude (Kotan, Kojima, Miyaguchi, Sugawara, & Onishi, 2015), mean average amplitude, area under the signal (iEMG) (Garland & McComas, 1990), root mean square average (RMS) (Chesler & Durfee, 1997; Gonzalez-Izal, Lusa Cadore, & Izquierdo, 2014; Kawamoto, Aboodarda, & Behm, 2014; Tepavac & Schwirtlich, 1997), rise time to peak amplitude and spectral features of the EMG signal: median frequency (MDF) (Chesler & Durfee, 1997; Gonzalez-Izal et al., 2014; Kang, Jeon, & Lee, 2015; Tepavac & Schwirtlich, 1997) and mean frequency (Gonzalez-Izal et al., 2014).

Ultimately, understanding the response of muscles that differ in fiber composition to alternate FES stimulation frequencies may help develop better muscle-specific FES protocols

aimed to prevent fatigue and improve muscle recovery. Since smaller muscles are generally more reliant on motor unit discharge rates to produce force and are often type I predominant while larger muscles are more reliant on the recruitment of additional motor units and often type II predominant, the variability of muscle features should be more closely examined. In an attempt to address these considerations, the APB and VL were selected for the following investigation.

Purpose: The primary purpose of this investigation was to evaluate the effect of different FES frequencies on muscle fatigue in the APB and VL, which differ by size and fiber characteristics.

Specific Aims and Hypotheses

Aim 1: <u>To evaluate and compare changes in the force and electrical activity of each muscle pre- and post-fatigue.</u>

Hypothesis: It is hypothesized that the force, RMS, iEMG, and MDF measures obtained during MVC will decline. Furthermore, sEMG will detect greater electrical activity and firing rate in the APB for all frequencies due to its small size and concentration of motor units in the electrode region.

Aim 2: <u>To evaluate the FES amplitude to generate 25% MVC during each frequency (10, 35, and 50Hz) and compare between the two muscles.</u>

Hypothesis: It is hypothesized that lower FES frequency will require higher FES amplitude and between muscles the VL will require higher FES amplitude at all frequencies due to its larger size.

Aim 3: <u>To evaluate time to fatigue at different stimulation frequencies (10, 35, and 50Hz) for each muscle.</u>

Hypothesis: It is hypothesized that the larger muscle (VL) will fatigue more quickly than the smaller muscle (APB) at all stimulation frequencies since the VL contains a greater proportion of fast-fatiguing fibers. Further, within each muscle 10Hz will produce longer time to fatigue compared to the higher frequencies since higher frequencies recruit more type II fibers.

Aim 4: <u>To evaluate and compare the progression towards fatigue for each muscle during the FES-induced fatigue protocols.</u>

Hypothesis: It is hypothesized that the changes in RMS, iEMG, and MDF parameters over time will relate with the changes in force over time to function as predictors of fatigue.

LITERATURE REVIEW

Skeletal Muscle Composition and the Relation to Recruitment

Voluntary contraction, unique to skeletal muscle, is regulated through the brain, spinal cord, and peripheral nervous system, which transmit electric signals via motor neurons to the muscle (Bol et al., 2011). These electric signals, or action potentials, are communicated to the muscle at neuromuscular junctions (NMJs). Together, the motor neuron axon terminals and the innervated muscle fibers that form a NMJ compose a motor unit (Bol et al., 2011). Within a muscle fiber are bundles of myofibrils, composed of adjoining sarcomeres, the basic contractile units of muscle. Sarcomeres are arranged of actin and myosin protein filaments that functionalize the mechanism of contraction (Figure 3) (Bol et al., 2011).

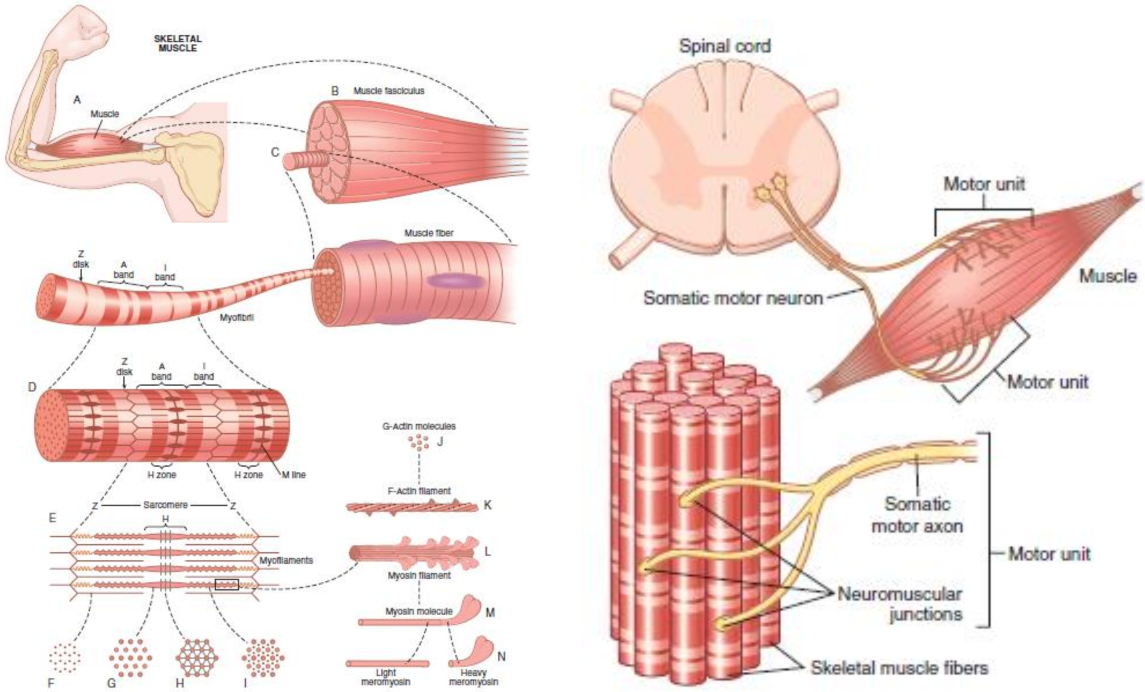


Figure 3: Structural hierarchy of muscle (left) and motor unit (right) (Hall, 2016).

The release of acetylcholine (ACh), a chemical neurotransmitter stored in vesicles at the motor neuron axon terminals, triggers the influx of extracellular sodium into muscle cells, increasing the muscle cell resting potential leading to muscle fiber depolarization (Bol et al., 2011; Hall, 2016). Depolarization above a certain threshold, particular to that motor unit, will initiate action potential propagation along the muscle fibers (Bol et al., 2011; Hall, 2016). Propagation increases the permeability of voltage-gated calcium channels, freeing more calcium from storage in the sarcoplasmic reticulum (an intracellular network surrounding myofibrils) to enter the sarcoplasm (Bol et al., 2011). In the sarcoplasm, calcium ions bind to troponin, a protein entwined with actin, changing its conformation to allow myosin binding. Adenosine triphosphate (ATP) provides energy to shorten the sarcomere length, producing muscle contraction (Bol et al., 2011).

Fatigue

In the most general sense, fatigue manifests as a reduction in force, but prior to force decline, the onset of fatigue is delayed by potentiation, a phenomenon that increases the mechanical response of muscle despite the repeated motion of the action causing a decline in the contractile properties of muscle (Papaiordanidou, Guiraud, & Varray, 2010). Potentiation was observed to be more prevalent with greater type I fiber composition (De Luca, LeFever, McCue, & Xenakis, 1982). Thereafter, mechanical force decline becomes more evident. Internally, it is the reduction in ACh release pre-synapse, desensitization of ACh receptors post-synapse, impairment in cross-bridge formation, reduction in myofibrillar calcium sensitivity resulting from increased myoplasmic inorganic phosphate, limited ATP availability, increased magnesium concentrations, and increased intracellular hydrogen that contribute to contractile impairment and, ultimately, fatigue (Papaiordanidou et al., 2010).

It is these intrinsic mechanisms that help distinguish fatigue into two main components:

Central Fatigue – is described as involving the neural processes before the signal reaches the NMJ (Papaiordanidou et al., 2010). Any impairment in the CNS's ability to provide sufficient control and transmission for task execution may limit one's ability to sustain an action, thus leading to fatigue caused by central nervous commands (Enoka, 1995). Moreover, central fatigue is associated with a lack of motivation, in which the body may prematurely fail to sustain the action due to sensations of pain and discomfort (Enoka, 1995). Thus, low tolerance individuals are more likely to fatigue sooner than others who perceive pain less severely.

Peripheral Fatigue – is described as involving the processes that follow signal transfer at the NMJ (Papaiordanidou et al., 2010). Particularly, the inability of the muscle or muscle

group to maintain adequate force production, related to the availability of muscle glycogen (reduced with high-intensity exercise) and excitation-contraction coupling processes (impaired with low-to-moderate intensity exercising) (Enoka, 1995).

For this investigation, with the transcutaneous application of FES to muscle, the progression of peripheral fatigue is the primary component of interest. The following sections will present research on muscle response with the development of peripheral fatigue through volitional activation and FES.

Voluntary Activation and Fatigue

Experiments involving voluntary muscle activation in relation to the components of fatigue include research by Kawamoto et al. (2014) who tested unilateral dynamic fatigue (i.e. one leg contracting while the other leg remains at rest) through resistive exercise at 40%MVC and 70%MVC. Results identified reduced strength and endurance capacity in the contralateral homologous muscle group (i.e. the leg that remained at rest) at both exercise levels. The study concluded that the manifestation of peripheral fatigue in the active limb can lead to the onset of crossover fatigue, the fatiguing of a different/non-exercised muscle due to fatigue in the exercised muscle (Kawamoto et al., 2014).

Komi & Tesch (1979) investigated muscle fatigue on the vastus lateralis in young, healthy individuals who differed in muscle fiber distribution. Fatigue, defined as a decline in force, was induced through 100 repeated maximum knee extension contractions at a constant angular velocity using isokinetic equipment. EMG signal changes were examined through iEMG and mean frequency. Findings indicated that individuals with more predominant type II fiber

composition generated higher torque and were more susceptible to fatigue than individuals with more predominant type I composition. Further, iEMG and mean frequency values declined regardless of fiber type, but only significantly in the type II predominant muscles. This suggested that contraction failure may be associated with motor unit recruitment pattern changes, of which, the changes occur more rapidly in type II predominant muscles (Komi & Tesch, 1979).

Tesch, Dudley, Duvoisin, Hather, & Harris (1990) examined the effect of maximal concentric and eccentric quadriceps contractions on fatigue. Fourteen healthy male participants performed three series of 32 unilateral MVCs while sEMG was recorded on the vastus lateralis and rectus femoris. iEMG, mean frequency, and MDF were measured in addition to torque. At baseline, eccentric MVC generated greater torque than concentric MVC. However, at exercise completion concentric-induced torque declined significantly while eccentric-induced torque remained similar to baseline. The sEMG measures at baseline identified that concentric MVC induced greater iEMG, but for both concentric and eccentric contractions in the VL, the iEMG increased slightly with exercise progression. In contrast, the mean frequency decreased with exercise during concentric MVC but did not change from baseline during eccentric MVC. These outcomes suggested that force maintenance with repeated MVC is more efficient during eccentric contraction than concentric contraction; therefore, the factors contributing to fatigue may differ between eccentric and concentric motions (Tesch et al., 1990).

Pasquet, Carpentier, Duchateau, & Hainaut (2000) induced fatigue in the ankle dorsiflexor muscles to examine peripheral mechanisms of fatigue. Participants performed concentric and eccentric contractions in a protocol that consisted of five sets of 30 MVCs. Contraction rate was constant at 50º/s for 30º range of motion. Torque was measured and sEMG of the tibilais anterior was recorded throughout the protocol. M-wave amplitude, post-activation

potentiation, and interpolated-twitch responses were extracted from the sEMG data. During concentric contraction, results indicated more significant force decline and a greater decrease in electrical activity detected by the sEMG compared to eccentric contraction. Additionally, the twitch response and potentiation measures were greater in the concentric contractions suggesting that intracellular calcium-controlled excitation-contraction coupling processes are more affected than with eccentric contractions (Pasquet et al., 2000).

Overall, research on voluntary contraction as it related to the mechanisms of peripheral fatigue found that eccentric and concentric contractile movements differ in their expression of fatigue. In addition, mean frequency measures extracted from sEMG indicated declines with fatigue progression, while changes in iEMG have reportedly increased and decreased with fatigue. Moreover, the fatiguing action of an isolated muscle group can also initiate fatigue in the contralateral muscle group.

FES and Fatigue

FES in relation to fatigue at the cellular level was examined by Clausen (2015). It was hypothesized that the passive and active changes in sodium and potassium levels impacted muscle fatigue and force recovery. FES was applied continuously for 5-300 seconds at 10-60Hz in rat extensor digitorum longus muscles. Measures examined the time-course of excitation-induced changes in the two ions. After 60 seconds of FES at 60Hz there was a rapid increase in sodium influx, but the rate was six-fold in the first 15 seconds compared to the last 45 seconds. This decline in the rate of FES-induced sodium uptake after the first 15 seconds of stimulation was conjectured to be a cause of increased extracellular potassium, which reduced the rate of force production. Overall, it was suggested that the influx of sodium and efflux of potassium are

self-limiting, leading to weakened action potentials as the levels plateau; the decrease of extracellular sodium and increase in extracellular potassium correlates with fatigue (Clausen, 2015).

Furthermore, Martin, Stein, Hoeppner, & Reid (1992) investigated the effect of stimulation on muscle morphology and metabolic properties in paralyzed tibialis anterior muscles. Stimulation was delivered over 24 weeks in 6 week blocks of 8hrs/day while the contralateral muscle remained untreated. With stimulation, the muscle significantly increased the proportion of type I fibers. Additionally, stimulation enhanced the succinate dehydrogenase activity of both fibers in the paralyzed tibialis anterior tissue. Generally, this reflected enhanced oxidative capacity and endurance properties of the tissue, but overall, stimulation did not impact fiber size or strength (Martin et al., 1992).

Moreover, stimulation of healthy tissue to examine the effects at the cellular level was investigated by Hultman & Sjöholm (1983). Stimulation was delivered using intramuscular electrodes in quadriceps at 20Hz to achieve forces between 50-75%MVC. Force decline was rapid, dropping by 78% of the initial force value after 50 seconds. During stimulation, up to four muscle biopsies were extracted from each leg. Hultman & Sjöholm (1983) noted that the ATP turnover rate decreased in the later contractions and the rate of decline was similar to the rate of force decline. Further, the phosphocreatine (PCr) stores decreased exponentially during contraction and were depleted by the completion of stimulation. It was also observed that glycolysis began five seconds after contraction was first initiated and the rate (measured by lactate accumulation) increased throughout the contraction duration.

In regards to recovery, Kang et al. (2015) examined the effectiveness of FES as a healing technique when muscle was fatigued. Kang and associates (2015) applied low frequency

stimulation (0.3Hz) to the erector spinae after the muscles were fatigued through repeated lifting and lowering movements. Results showed increases in ATP and proteins in the stimulated region, restoring cells and facilitating healing. It was also effective in improving cumulative fatigue recovery by reducing the amount of creatine kinase in the blood of individuals with delayed onset muscle soreness (Kang et al., 2015).

At the applied level, research by Grospretre, Gueugneau, Martin, & Lepers (2017) analyzed FES through different protocols designed based on a similar total torque-time integral. The investigation targeted the relationship between muscle activation pathways and fatigue. FES was delivered to ten young, healthy participants at 20, 60, or 100Hz (randomized) on the triceps surae. Muscle response was observed through the mean twitch number, the soleus H-reflex, and the m-wave, while fatigue was measured through changes in MVC and evoked forces, in addition to EMG signal changes. Outcomes revealed that delivering FES at a high stimulation frequency (60 or 100Hz) with a low pulse amplitude contributed to the greatest fatigue. Moreover, stimulation at 20Hz mainly caused changes at the muscle level (peripheral components), not at the activation pathways, but the higher frequencies also influenced both spinal and supraspinal processes (Grospretre et al., 2017).

Further, Papaiordandidou et al. (2010) investigated the occurrence of fatigue in the APB. FES was applied as a protocol of three series of 17 trains at 30Hz. MVC and electrically evoked measures (level of activation, RMS/m-wave, and H reflex) before and after each series of trains were compared. Results indicated significant decreases in force generation through both changes in MVC and the electrical responses. Additionally, muscle excitability was reduced, suggesting impairment in muscle contractile properties. Papaiordanidou et al. concluded that stimulation at

lower frequencies, like 30Hz applied in their study, only generates peripheral fatigue (Papaiordanidou et al., 2010).

Chesler & Durfee (1997) investigated the effectiveness of sEMG to identify fatigue during FES-induced isometric contractions of the quadriceps. For their experiment, a hardware device that delivered the FES and recorded the EMG was constructed. Stimulation was delivered to 20 able-bodied adults and three spinal cord injured adults. RMS and MDF measures were extracted from the sEMG to quantify fatigue. Three stimulation amplitude levels were determined for each participant by first stimulating to the maximum tolerated, then to the lowest level that induced contraction, and lastly the average of these two values was calculated as the moderate level. For the spinal cord injured participants, maximum stimulation amplitude was defined by the machine maximum of 150mA. To induce fatigue, FES was delivered for four-60s stimulation trials with 5-15 minutes of rest between trials. After determination of the RMS and MDF, slopes of the trends were compared to the changes in torque over time. Outcomes showed that over time the RMS declined and the waveform broadened. Similarly, the MDF also decreased with time. Overall, however, Chesler & Durfee did not confirm complete reliability of RMS and MDF as fatigue indicators due to the large variability between subjects.

Similarly, Tepavac & Schwirtlich (1997) utilized a personally developed EMG amplifier and FES artifact filtering unit to examine the correlation between changes in sEMG and force with fatigue development. Isometric contractions were induced through FES on the wrist flexors in both able-bodied and spinal cord injured participants. FES was applied at 25Hz to a sub-maximal amplitude between 25 and 40mA for five minutes. Seven sEMG parameters were extracted: RMS, mean average, peak-to-peak amplitude, iEMG, power spectrum density, MDF, and mean frequency. Torque versus EMG evoked potential plots were generated in an attempt to

determine an association between force decline and muscle electrical response. For this investigation, force and MDF were most strongly correlated, both decreasing with fatigue progression (Tepavac & Schwirtlich, 1997).

Overall, research has shown that FES-induced fatigue presents due to impairment at the metabolic level and under a range of frequencies and amplitudes. Further, several sEMG parameters have been investigated in relation to fatigue development, where MDF was most consistently associated with a decline in force.

Altogether, it is evident that muscle mechanics and fatigue progression are important considerations, especially when performing tasks for rehabilitation and recovery. However, limited investigation of fiber composition in relation to FES-induced fatigue makes it necessary to expand the understanding of fatigue development as it relates to the characteristics of muscle during FES.

Electrical Stimulation and Skeletal Muscle Response

The following section addresses research on different features of FES: stimulation frequency, stimulation amplitude, and delivery method and electrode placement as it relates to the development of fatigue. Additional discussion on hybrid activation, the combination of FES and voluntary activation, as it relates to muscle response will also be included.

Stimulation Frequency

Stimulation frequency is defined as the rate at which an electrical pulse is delivered in one second. The higher the frequency the more pulses delivered to the muscle in the one-second period. Sequentially, the muscle is stimulated more rapidly. Bickel et al. (2003) investigated

changes in frequency as it related to muscle fatigue. Frequency manipulation was achieved through FES delivery following variable-frequency trains (VFTs, first inter-pulse interval was 5ms, then four pulses were delivered at 70ms pulse-width) or constant-frequency trains (CFTs, delivering six pulses at 70ms pulse-width) applied to ten recreationally-active men. Stimulation was directed to the quadriceps femoris and tibalis anterior muscles. Results showed that VFTs achieved greater force production in fatigued muscle, regardless of fiber type (Bickel et al., 2003). This determination suggested that VFTs may be the more optimal FES protocol design to guide functional movement and lessen fatigue.

Another study by Dreibati et al. (2010) examined the impact of different FES frequencies on force and fatigue development with consideration of sports and clinical rehabilitation training protocols. FES was applied to the quadriceps of 26 healthy adults at 20, 50, and 100Hz. The stimulation session for each frequency lasted 20 minutes, consisting of 60 - five second contractions with 15 seconds of rest between each contraction. MVCs were collected before, during, and after FES to compare changes in force production. Dreibati and colleagues found significant differences in the initial FES-induced forces between 20 and 100Hz and 50 and 100Hz, where 100Hz achieved a greater force at ~71% MVC compared to the ~62%MVC at 50Hz and ~55%MVC at 20Hz (differences were non-significant between 20 and 50Hz). Further, in the 50 and 100Hz protocols, contractile effort significantly declined after the 15th contraction, which coincided with five minutes of applied FES. Moreover, at 100Hz intense fatigue was induced by protocol completion at which point a force of only 27%MVC was achievable. Therefore, it was concluded that the higher the FES frequency the greater the loss of muscle force. At a stimulation frequency of 20Hz fatigue development was slowest, thus it was

encouraged that lower frequencies are more suitable for rehabilitation while higher frequencies that help achieve maximal force could be utilized when training athletes (Dreibati et al., 2010).

Furthermore, other studies combined the manipulation of a few FES parameters, inclusive of frequency, to determine which parameter most significantly contributed to fatigue. Behringer et al. (2015) implemented a crossover design that compared frequency (80Hz versus 20Hz), intensity/amplitude (80%max tolerable versus 20%max tolerable), and pulse-width (400µs versus 150µs) variation on fatigue. These parameters were arranged into six unique protocols: HI (high intensity)-HF (high frequency)-LW (low pulse width), HI-HF-HW, HI-LF-HW, LI-HF-LW, LI-HF-HW, LI-LF-HW. FES was applied to the right and left VL of 13 athletic males at 4sON/4sOFF. The participant would receive two different protocols per visit, one protocol to each leg. Visits occurred every other day, totaling three days. After a three-week washout period, the protocol assignment given previously to each leg was switched so that both legs received all six protocol variations. Fatigue was quantified based on the slope of the linear regression trend over all tetani (tetani is the FES-induced peak force range over four seconds of activation) and the number of tetani whose force was above 50% of the initial tetanus. Behringer and associates found that these measures were significantly lower during high frequency FES but had no effect from amplitude or pulse-width. Additionally, it was determined that the fatigue index based on number of tetani is a better quantifier in low frequency (~20Hz) FES protocols while the fatigue index based on slope analysis is preferred in high frequency (~80Hz) protocols. Ultimately, it was shown that frequency was the only stimulation parameter affecting fatigue (Behringer et al., 2015).

Similar conclusions regarding frequency were noted by Gorgey, Black, Elder, & Dudley (2009) who examined FES on the quadriceps with variable frequency (100Hz and 25Hz), pulse

rate (450μs and 150μs), and force demand (75%MVC and 45%MVC). Seven healthy adults received four different FES protocol combinations: 100Hz/450μs/75%MVC, 100Hz/150μs/75%MVC, 25Hz/450μs/75%MVC, 100Hz/450μs/45%MVC. Peak torque was measured at the beginning and end of each protocol. As determined by Behringer et al., Gorgey et al. (2009) noted that lower stimulation frequency reduced the development of fatigue as defined by the percent drop in force, but changes in the level of force demand and pulse rate did not affect fatigue. Therefore, it was concluded that the reduced torque coinciding with the reduced stimulation frequency between protocols may be the cause of reduced fatigue (Gorgey et al., 2009).

Stimulation Amplitude

Stimulation amplitude, sometimes referred to as intensity, describes the amplitude or size of the pulse that is delivered to the muscle. Binder-Macleod, Halden, & Jungles (1995) examined the effects of stimulation amplitude on muscle response in the quadriceps femoris. Fifty healthy adults received stimulation trains delivered for 180 contractions with a one-second period between trains. Amplitudes required to achieve 20%MVC, 50%MVC, and 80%MVC were compared through normalized force-frequency relationships. Outcomes showed that between 20%MVC and 50%MVC the normalized force-frequency relationship was no different, and at 80%MVC there was only a slight leftward shift in the relationship, meaning that at slightly lower stimulation frequencies a higher contractile force was achievable due to the increased amplitude level. Further, the association between stimulation frequency at 20, 40, and 60Hz with fatigue using the amplitudes to reach 20%MVC and 50%MVC, showed that an increase in frequency increased fatigue. However, for a given frequency, the amount of fatigue caused from an amplitude level required to reach 20%MVC or 50%MVC did not differ. Thus, it was suggested

that the fatigue characteristics of the recruited motor units were similar at all stimulation amplitudes, displaying a less ordered recruitment than with voluntary activation (Binder-Macleod et al., 1995). With FES, the recruitment of fast-fatiguing type II motor units at much lower force demands was postulated by Binder-Macleod et al. (1995) to partially explain the increased rate of fatigue development.

In other research, Chou & Binder-Macleod (2007) examined stimulation amplitude as it related to the level of force output with fatigue development. FES was applied to the quadriceps femoris of ten healthy adults at variable amplitudes and frequencies using the burst superimposition technique, the application of stimulation as subjects performed MVCs. The pre-fatigue protocol consisted of 300ms testing trains at 10, 12.5, 20, 30, 40, and 60Hz and 100, 200, 300, 400, and 600µs pulse durations. This provided information on the muscles force responses to different frequencies and intensities prior to fatigue. For the fatiguing protocol, FES was applied in 300ms trains at 40Hz with a 600µs pulse duration for a total of 180 trains. Lastly, post-fatigue stimulation was delivered using the same sequence of trains as the pre-fatigue protocol, but in this instance, each train was separated by two trains used in the fatiguing protocol to help maintain a steady state of fatigue. Results compared pre-fatigue and post-fatigue force-amplitude relationships. During the fatiguing protocol, a rapid decline in peak force was observed during the first 60 contractions, with a more gradual decline between contractions 60 and 150, and a stabilization in the last 30 contractions. On average, the peak decline in force was estimated at ~50%. Chou and Binder-Macleod (2007) determined an exponential relationship between muscle force and stimulation amplitude and the normalized force-amplitude relationship did not change with stimulation frequency or fatigue. This posed a potential standard quantifier for all FES protocols (Chou & Binder-Macleod, 2007).

Delivery Methods and Electrode Placement

Aside from stimulation frequency and amplitude, other critical features to be considered with FES are the amount of delivery sites, type of waveform, and particular electrode size and placement. Advancing technology has contributed to the development of FES electrode arrays that involve multiple sites for delivery in contrast to the conventional method that uses two sites: active and indifferent (Morf et al., 2015). Morf et al. (2015) performed an investigation comparing applications of conventional FES to multipath FES (a garment integrated with FES that offers multiple current pathways and large electrodes) on twenty post-total knee arthroplasty patients. Primary measures included FES-induced torque during knee extension with self-reported discomfort via a visual analog scale as a fatiguing protocol was performed. Outcomes indicated that the multipath FES system evoked significantly greater torque, but the total decrease in FES-evoked torque was not significantly different between conventional and multipath FES. However, comparison of the pre-post MVC torque values did show that multipath FES generated a significantly lower MVC torque reduction, meaning it was less fatiguing (Morf et al., 2015). Further, it was noted that the deep tissue region below the surface electrodes showed increased muscle fiber activation with increased levels of FES-induced torque. In addition, more superficially located axons, nearer the stimulation electrodes, can depolarize more easily, thus depending on the fiber composition of a particular muscle, more type I or type II fibers may be stimulated first (Morf et al., 2015).

Research comparing electrode size and positioning on the muscle through a simulation model generated in CMISS software was investigated by Kim, Trew, Pullan, & Rohrle (2012). Kim and colleagues' main objective was to develop a computational framework that estimated how differing FES trains and electrode configuration impacted the excitability and fatigability of

muscle. Simulated FES was applied to the tibialis anterior for four different test cases: 1) single electrode effect on transmembrane potential, 2) optimal distance between cathodal electrodes, 3) changes in potassium ion concentration and calcium release, and 4) muscle fatigue development when using electrodes placed at a fixed distance. For test case 1, surface stimulation electrode sizes of 2.6mm^2 and 4.5mm^2 were compared with stimulation delivered at 22.5mA through monophasic pulses. For test case 2, the same stimulation conditions were set and inter-electrode distance was varied between 0mm and 10mm. For test case 3, biphasic and monophasic waveforms were compared through stimulation at 75 and 125Hz. For test case 4, muscle fatigue was examined with FES simulation using one 9mm^2 electrode versus two 4.5mm^2 electrodes at an inter-electrode distance of 4mm. Stimulation frequency was set at 75Hz, amplitude at 45mA, and waveform to monophasic. Fatigue was defined by changes in the potassium ion concentration of the t-tubule and calcium release from the sarcoplasmic reticulum. Further, comparison of electrode size of was performed. Stimulation at 75Hz showed less influx of sodium and less calcium release but higher potassium in T-tubules at lower frequencies and biphasic waveform than with higher frequencies and monophasic waveform suggesting the delay of fatigue with lower frequency/biphasic waveforms. Outcomes of the four test cases demonstrated that lower frequencies and biphasic waveforms are less fatiguing than monophasic waveforms at higher frequencies. Additionally, electrode size had no effect on muscle response, rather, finding that muscle fiber activation was sensitive to the thickness of the skin and adipose tissue (Kim et al., 2012).

Hybrid Activation

Another aspect of FES is the integration of FES with voluntary contraction. When circumstances allow, supplementing FES with volitional movements can enhance muscle recovery. In terms of muscle healing, Reinold et al. (2008) examined FES impact on infraspinatus for external rotation after rotator cuff surgery. Participants performed three isometric external rotation contractions (no FES and FES). Peak force production was significantly greater with FES than without across all ages and injury severities, thus it was suggested that FES and exercise together could enhance force production and minimize rotator cuff inhibition after surgery (Reinold et al., 2008).

In terms of muscle response to FES, one study investigated the recruitment of type II motor units during FES when achieving a force level of 40%MVC in the knee extensors (Hamada, Kimura, & Moritani, 2004). MVCs were performed at five-minute intervals starting with a pre-FES application then during a repeated activation protocol that involved either FES application set to induced 10%MVC effort for 20 minutes or voluntary contraction to induce 10%MVC for 20 minutes. Outcomes identified that the mean motor unit spike amplitude, particularly the motor units with larger amplitude spikes, declined significantly while the smaller spike amplitudes increased their firing rate during 40%MVC contraction after FES. With this, the RMS also increased after FES but not with voluntary activation. This suggested evidence of a reverse fiber recruitment pattern.

Similarly, research comparing the effects of hybrid activation on a predominately type I (APB) muscle and predominately type II (VL) muscle revealed that a higher stimulation frequency generated a higher electrical output, described by an increased recruitment of fibers (Stratton & Faghri, 2016). Hybrid activation measured less electrical output than FES alone. This

was supposed as a result of FES activating more type II fibers while voluntary follows more coordinated recruitment where more type I fibers activated in the deep tissue may be missed by the sEMG electrodes (Stratton & Faghri, 2016).

Overall, stimulation at the cellular level was shown to promote type I fiber expression and greater oxidative capacity in the tissue, though the induction of fatigue with stimulation was a result of impairment in sodium-potassium levels and ATP depletion. At the applied level, assessment of FES features found that stimulation frequency was the one factor that most affected fatigue progression. Further, research suggested that alternating the delivery of FES within a protocol or combining it with voluntary activation may efficiently enhance recovery.

Monitoring Muscle Response

Electromyography

A technique widely used to monitor muscle response is electromyography (EMG) (Estigoni et al., 2014; Ibitoye et al., 2014; Ryait, Arora, & Agarwal, 2009). EMG signals, or m-waves, are the sum of all action potentials generated by the given motor unit(s) activated; a combination of the generated electrical nerve and muscle tissue responses (Estigoni et al., 2014; Farfan, Politti, & Felice, 2010; Ibitoye et al., 2014; Sezgin, 2012). The production of an EMG signal begins with the central nervous system (CNS) commanding muscle response through motor neuron impulses sent to the NMJ innervation zone in the muscle belly (Kauppi, Hahne, Muller, & Hyvarinen, 2015). Depolarization of muscle fibers associated with a particular motor unit creates a motor unit action potential (MUAP). MUAPs travel to the muscle termini causing contraction. A slightly delayed version of the propagated MUAP is detected by the EMG sensor,

which is connected to a hardware device recording the signal (Kauppi et al., 2015). Dispersion of the innervation zone, number of active motor units, conduction velocity of the motor unit distribution, the intramuscular motor unit locations, subcutaneous tissue thickness, electrode orientation, electrode type, muscle cross-sectional area, muscle temperature, muscle length (Ibitoye et al., 2014), and environmental noise (Zhang & Huang, 2015) are all factors that impact EMG recording.

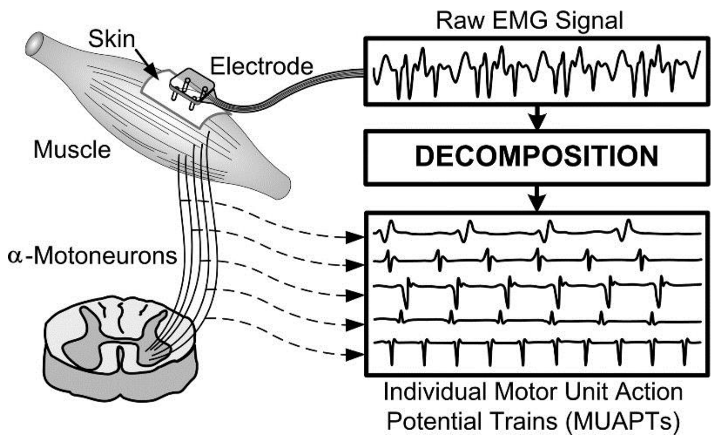


Figure 4: Acquiring a surface electromyogram (sEMG) signal (De Luca, Adam, Wotiz, Gilmore, & Nawab, 2006).

Surface electrodes are an effective means for obtaining EMG signals with the purpose of assessing the degree and timing of muscle activation (Botter, Vieira, Loram, Merletti, & Hodson-Tole, 2013). Surface EMG (sEMG) signals are preferred for understanding general muscle activity within the whole muscle as the sensor region contains a greater number of motor units and are easier to use for dynamic movements (Botter et al., 2013; Ibitoye et al., 2014; Nawab, Chang, & De Luca, 2010). Moreover, sEMG recordings follow easier preparation protocols and avoid the risks of disease transmission and muscle tissue damage as it is noninvasive (Nawab et al., 2010). However, sEMG signals are more easily affected by the amount of biological tissue (i.e. adipose, skin) between the muscle and electrode, directly impacting signal frequency and

amplitude (Ibitoye et al., 2014). Such tissue impedance reduces the bandwidth to often less than 400Hz (Ibitoye et al., 2014).

Signal Processing

For all EMG collection, it is recommended that the signal be transferred through a differential amplifier, which measures the voltage difference between two electrodes (Konrad, 2005; Rose, 2014). During this transfer, the amplifier system applies an analog bandpass filter to the raw signal before it is converted to a digital signal displayed on the computer (Rose, 2014). Bandpass filters simultaneously remove both high and low frequency components of the data outside the bandwidth of the EMG signal activity range. Specifically, the low frequency cutoff helps remove any baseline drift that arises from movement artifacts and sweat during data collection and helps remove the DC offset (i.e. eliminates the mean amplitude displacement from zero) (Clinical Biomechanics Research Group, 2006; Robbins, 2014; Rose, 2014). The high frequency cutoff helps remove high frequency noise and prevents aliasing (i.e. the loss of signal during analog to digital conversion) (Rose, 2014). Outcomes from the Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles (SENIAM) project determined that the low frequency cutoff should be around 10-20Hz and the high frequency at ~500Hz (Hermens & Freriks, 2006). Similarly, the International Society of Electrophysiology and Kinesiology (ISEK) advises a bandpass range of 5-500Hz for sEMG (Podium Conference Specialists, 2015).

After conversion from analog to digital, EMG data typically requires additional manipulation to extract parameter measures unless the amplifier-associated software includes such measurement calculations in the program. For the former, EMG data is imported into user-

programming software, like MATLAB, for preprocessing, filtering and measurement extraction, and spectral analysis (The MathWorks, 2016). Preprocessing involves wave rectification to fit the signal into one polarity, usually positive, for measurement quantification. Full-wave rectification essentially takes the absolute value of the signal so that the negative components swing to the positive polarity (Rose, 2014).

Filtering and measurement extraction typically involves the application of a low pass filter ranging from a 5-100Hz cutoff then determining the linear envelope to provide signal shape (Rose, 2014). Determination of mean value/moving average estimates as a window of specified size slides along the signal, is also common (Rose, 2014). Window size specifications are dependent on speed of movement and length of signal; the smaller the window the more detectable rapid changes in EMG, but also the less smooth the signal becomes, whereas larger window sizes increase smoothing while sacrificing some detection of signal trends (Robbins, 2014). Overlapping the window during root mean square (RMS) averaging helps obtain the benefits of both smaller and larger window sizes (Robbins, 2014). Both the Finite Impulse Response (FIR) filter types and Infinite Impulse Response (IIR, e.g. Butterworth or Chebyschev) filter types, as well as RMS (Konrad, 2005; Robbins, 2014; Rose, 2014), are possible approaches for averaging the data (Rose, 2014). FIR and IIR filters are non-adaptive, thus the filtering algorithm estimates do not update based on signal changes with time, as they would with adaptive filtering algorithms (Ortolan et al., 2003). Both FIR and IIR types estimate noise in the signal and subtract it from the raw data to output a smoother filtered signal. They differ in that FIR filters resembles a moving average that only considers the past inputs (the algorithm samples a section of the signal (i.e. the input) and processes it to create an output; these outputs are summed at the end of the filtering process to regenerate the overall smoothed signal) of the

impulse while IIR filters consider both past inputs and past outputs before summing the components into the overall smoothed signal (Iowegian International Corporation, 2015).

Linear envelope and/or averaging allows for several temporal characteristics of the EMG signal (i.e. time-to-peak events, area under the waveform, signal on/off times, amplitude features) to be extracted (Konrad, 2005; Robbins, 2014; Rose, 2014). With fatigue, it was suggested that a high pass filter below 20Hz be applied for m-wave assessment (Dimitrov, Arabadzhiev, Hogrel, & Dimitrova, 2008). For spectral analysis, signal data must be converted to the frequency domain, commonly achieved through the application of a Fourier transformation (i.e. Fast Fourier Transform (FFT) or Short-time Fourier Transform (STFT)) (Robbins, 2014; Rose, 2014).

EMG Acquisition with Electrical Stimulation

Evoked EMG signals, those generated through FES, help monitor changes in motor unit electrical activity as the muscle contracts and help monitor general electrical patterns induced by FES (Ibitoye et al., 2014). However, evoked EMG signals also record electrical activity from the FES device itself, known as stimulation artifacts, due to the close proximity between electrodes and the EMG detection region (Ibitoye et al., 2014). This adds noise to the m-waves, expanding the sEMG amplitude to ranges of ~10-20mV, impairing clear distinction of the muscle electrical activity which typically has an sEMG amplitude of ~500μV maximally (Estigoni et al., 2014; Ibitoye et al., 2014). Further, cross-talk between adjoining agonist and antagonist muscles during contraction can impact detection of where the signal originated (Ibitoye et al., 2014).

Artifact Removal Techniques

Stimulation artifacts can be eliminated by hardware devices via switch circuits that shut down amplifier control, or software algorithms that utilize blanking windows and filters: IIR with exponential forgetting, adaptive linear prediction filter, Gram-Schmidt canceller, comb, zero phase forward, and reverse digital (Zhang & Ang, 2007). IIR with exponential forgetting involves an unbiased averaging and smoothing of the signal. Adaptive linear prediction filters involve algorithms that adjust during the filtering process to predict the values of the next portion of the signal based on the past and current signal data. Gram-Schmidt cancellation involves orthonormalizing (i.e. making the signal orthogonal and normalized) the signal via linear algebra. Comb filtering adds a delayed version of the signal to itself for destructive/constructive interference. The forward and reverse filtering help to avoid phase distortion (Zhang & Ang, 2007).

Each of these filtering approaches can be effective under certain conditions, but the extensive stimulus artifacts that arise in FES-induced EMG signals makes application of these techniques less efficient. Alternatively, a wavelet transformation can be applied. Wavelet transformation manipulates the signal in time-series without changing signal shape, thus, no artificial information is introduced to the signal (Andrade, Nasuto, Kyberd, Sweeney-Reed, & Van Kanijn, 2006). However, the process uses a predefined mother wavelet as a filtering reference during the transformation, which makes an assumption about the signal’s time-series input (Andrade et al., 2006).

A newer method is empirical mode decomposition (EMD), which, unlike wavelet transformation, makes no time-series input assumptions, rather using a sifting process (Andrade et al., 2006). EMD was designed as a mathematical algorithm to be applied to any nonlinear,

non-stationary data, decomposing such complex data into a small, finite number of intrinsic mode functions (IMFs) that admit well-behaved Hilbert transforms, a linear operator that takes the signal and produces another signal as an analytical representation (Huang et al., 1998). Pilker et al. (2012) evaluated the efficacy of the EMD to remove stimulus artifact from evoked EMG signals during FES. The EMD algorithm separated the raw EMG signal into several IMFs based on the energy associated with various intrinsic time scales. The separation was achieved through a sifting process that identified local maxima and minima, which were then connected forming an envelope that was spline filtered. The maxima and minima values were entered into an equation that extracts some of the wave as an IMF and the process was repeated to generate additional IMFs until the stopping criterion was reached (Figure 5) (Pilkar, Yarossi, & Forrest, 2012; Stratton & Faghri, 2016).

The primary limitations of EMD as a method of stimulus artifact removal for EMG are the loss of some high frequency m-wave activity and mode-mixing. The first few intrinsic mode functions (IMFs) are dominated by stimulus artifacts, but also contain some high frequency muscle data. As such, the large presence of stimulus artifacts requires the omission of those IMFs. Mode-mixing is a result of the algorithm's simultaneous consideration for the time domain and frequency domain. Different IMF components may have variable intermittency; a component comes into existence or disappears from the signal entirely, depending on the time scale. During reconstruction of the IMFs, these components may not correctly sum, particularly in the frequency domain. Proposed solutions for cases in which the components are not well separated in frequency, or are embedded in high frequency artifact, include creating a scale limit to the envelope extrema, or adding then subtracting a masking signal (Deering, R. & Kaiser, J.F., 2005) or white noise (Wu, Wei, Cai, Ding, & Law, 2015).

Modification of the algorithm through the addition of white Gaussian noise to the raw EMG signal and each residual of the EMG signal during the sifting process achieved more detailed decomposition of the static sEMG signal during fatigue assessment (Wu et al., 2015). White Gaussian noise was used to fill the time-frequency space through which EMD applies a dyadic filtering process. In determination of the final mean of the IMFs, the white noise was canceled out, providing more detailed decomposition of the static sEMG signal. The Hilbert Transform was applied to each IMF to calculate the instantaneous frequency, from which the mean instantaneous frequency (MIF) was determined. Results showed a declining trend in MIF values as the fatiguing process continued.

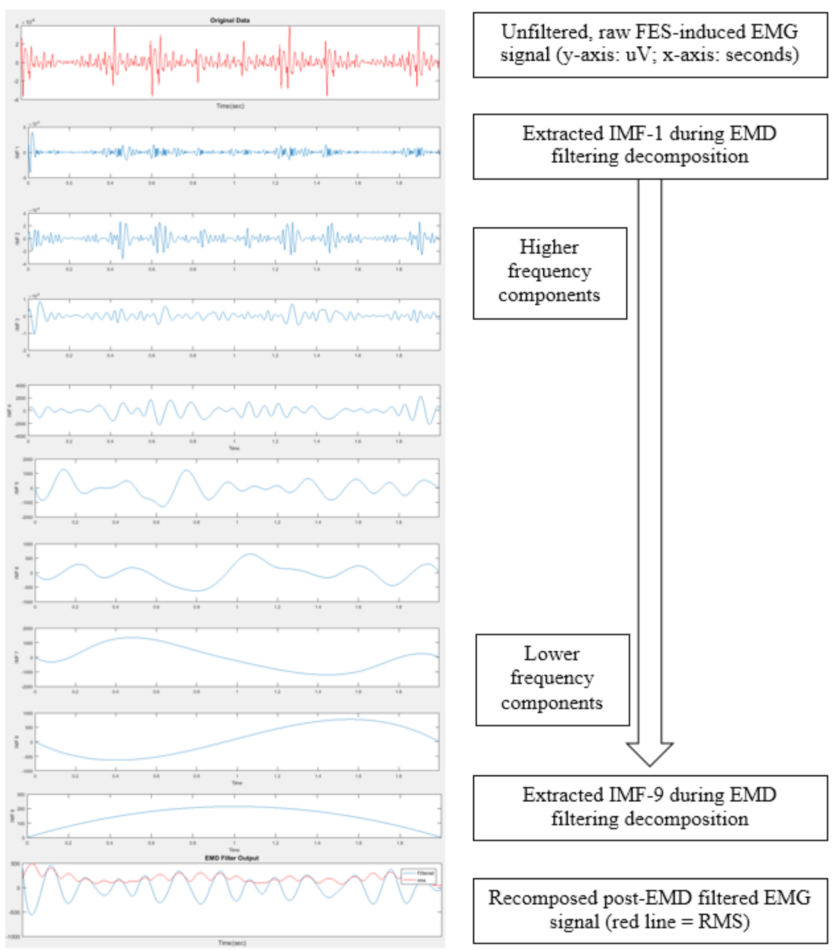


Figure 5: Filtering process during empirical mode decomposition (EMD).

The benefits of EMG as a useful tool for monitoring muscle activity are clear, but the challenges with extracting pure muscle signals from FES-induced protocols are also evident. For the following investigation, basic EMD, that without the addition of white noise or a masking signal, was the most effective filtering option available. As such, modifications to EMD like those utilized by Wu and colleagues (2015) were not attempted. Future investigations should continue to evaluate more efficient ways to filter FES-induced EMG signals and to quantify signal changes, particularly as it relates to fatigue development.

SIGNIFICANCE

Functional electrical stimulation (FES) as a tissue regenerative technique at the cellular level and rehabilitative technique at the applied level is well-documented. More recently, interest has directed FES application towards tissue regeneration. At the cellular level, studies have shown that FES aids cell proliferation in connective tissue and improves the rate of new collagen formation in injured tendons, suggesting that more collagen-producing cells are directed to the injury site with FES (Araújo, Franciulli, Assis, Souza, & Mochizuki, 2007; Maffulli & Furia, 2012). Additionally, greater blood flow due to FES was also suggested to release nitric oxide-like humoral agents that are integral for blood vessel development (Faghri, Van Meerdervort, Glaser, & Figoni, 1997; Faghri, Glaser, & Figoni, 1992). Further, FES was observed to promote vascular endothelial growth factor (VEGF) expression, enhancing blood vessel growth, in rat skeletal muscle (Kanno et al., 1999).

At the applied level, accelerated rat and dog tendon healing was achieved with FES by promoting greater blood flow and fibroblast number to the injured region (Ekaputra, Prestwich, Cool, & Hutmacher, 2011; Jabbarzadeh et al., 2008). Moreover, FES training was found to

reverse long-term denervation muscle atrophy and dystrophy by increasing myofiber diameter size by more than ~50% and regenerating new myofibers (Kern et al., 2004). In addition, the integration of FES with a traditional volitional isometric training program for post-anterior cruciate ligament repair was shown to be more effective at improving muscle function after five weeks compared to isometric training alone and to prevent atrophy by limiting the reduction in oxidative enzyme activity (Eriksson & Häggmark, 1979). Muscle fiber type transformation has also been reported with application of 'slow pattern' FES, that which resembled the firing rate of the predominantly type I soleus (Windisch, Gundersen, Szabolcs, Gruber, & Lømo, 1998). Windisch et al. (1998) applied FES to both denervated and intact nerve rat extensor digitorum longus muscles for four months and found that after just three weeks most of the type IIb and type IIx fibers were converted to type IIa or type I fibers and after two months of stimulation the type IIa fibers began converting to type I fibers.

These findings highlight the potential for FES as a key component for effective tissue regeneration, but for complete effectiveness, understanding the influence of different muscle characteristics is critical. Human musculature consists of tissue that varies in size, fiber composition, and fiber arrangement so consideration of these characteristics should be had when implementing FES. This variability contributes to tissues receiving activation at different motor unit thresholds; therefore, the optimal FES protocol should be aligned with the motor unit response rate as to avoid overstimulation or understimulation. More so, as FES is reported to increase the rate of fatigue development, individual muscle type responses to FES should be understood to maximize efficiency of an FES treatment. The following investigation attempts to elucidate some of these considerations by examining how varied muscle type is affected by FES under alternate stimulation frequencies. Identification of measures such as time to fatigue and

EMG can help advise clinicians after which durations in different muscles manipulation of certain FES parameters (stimulation amplitude, frequency) should be executed to sustain muscle activity and offer a force-alternative quantifier of fatigue that could be utilized for real-time feedback, respectively.

METHODOLOGY

Participants

Ten healthy male and female participants (23.2 ± 3.0 years; age ± SD) were recruited for this study. Inclusion criterion was individuals between the ages of 18 and 30 years. Exclusion criteria were having a known history of orthopedic diagnosis such as arthritis, ligament injuries, meniscus tears, joint instability due to a previous ligament injury, a known history of musculoskeletal problems such as Achilles tendinitis or patellar tendinitis, a known history of nerve pain/damage such as carpal tunnel syndrome, a known history of cardiovascular problems, having a pacemaker, and a known allergy to silver/silver chloride surface electrodes or adhesive on tape. Full-board IRB approval and written consent was obtained prior to participation.

Design

This study followed a quasi-experimental, repeated measures within participants design.

Instruments

1- FES: The Respond Select® (Empi, Inc., St. Paul, Minnesota) neuromuscular electrical stimulation system was used to evoke contractions. The device can deliver stimulation

intensities between 0-100mA at rates between 2-110Hz. Pulse-width was set at 300μs for this device. Duty cycle was set at 4sON/4sOFF.

2- EMG: The Nexus-10 EMG device (MindMedia B.V., Netherlands) was used to continuously record muscle activity during FES-induced activation and voluntary contractions. Sampling frequency was set at 2048Hz.

3- Force: Isometric force measures in the hand (APB) were collected using the PASCO PASPort (PS-2104) high resolution force sensor (range: ± 50N, resolution: 0.002N; PASCO Scientific Inc., Roseville, CA). Isometric force measures in the quadriceps (VL) were collected using the Shimpo Instruments (ELECTROMATIC Equipment Co., Cedarhurst, NY) Javelin FGV-HXY Force Gauge (maximal capacity: 500lb/250kg; accuracy: +/-0.2% F.S.).

Experimental Setup

In preparation for EMG Ag/AgCl electrode placement, excess hair was shaved with caution to avoid skin irritation and bleeding, then the area was scrubbed with an abrasive gel and cleaned with alcohol.

FES electrodes: Self-adhering, reusable, latex-free bipolar stimulating surface electrodes (90x50mm square for VL; 3.5cm round for APB) were placed according to EMPI, Inc. guidelines (Figure 6) and connected to the FES device for muscle activation.

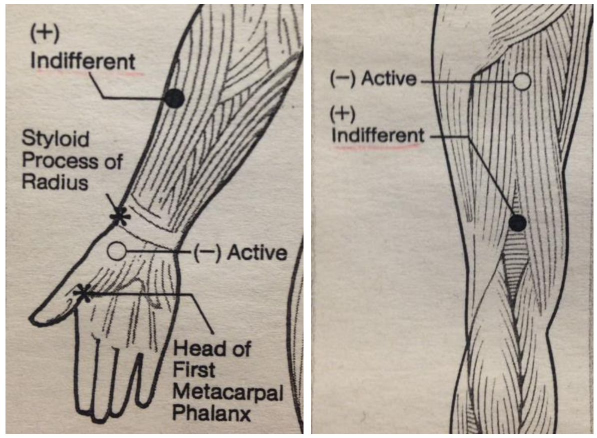


Figure 6: FES electrode placement for the APB (left) and VL (right).

<u>EMG electrodes:</u> Pre-gelled, self-adhesive Ag/AgCl EMG surface electrodes were placed across the muscle belly in parallel with the muscle fibers according to Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles (SENIAM) guidelines (Figure 7).

APB – along the lateral surface of the metacarpal of the thumb, slightly medial of the distal 1/4 of the 1st ossa metacarpalia

VL – at 2/3 down the line from the anterior spina illaca superior to the lateral patella

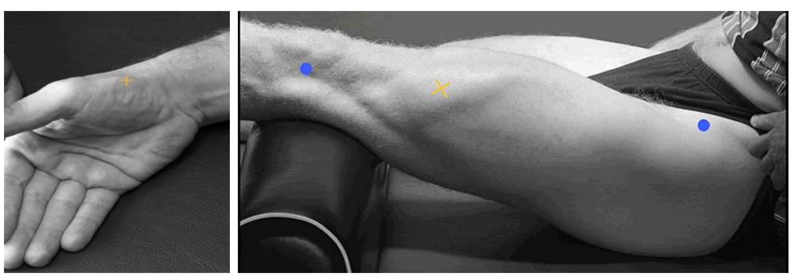


Figure 7: EMG electrode placement for the APB (left) and VL (right).

EMG electrodes and stimulation electrodes were then placed over the muscle belly and muscle motor points, respectively. The EMG electrodes were placed directly between the active and indifferent FES electrodes to record muscle activity between them. A reference electrode was placed on a bony site at the distal end of the ulna. All electrodes and wires were fixed to the skin

with adhesive tape to avoid possible motion artifacts, disruptions in the EMG signal due to the shifting of electrode position atop the skin if poorly adhered.

For all MVC collection, one rater was used throughout the experiment and intra-rater reliability of r = 0.782 was established. To determine the intra-rater reliability for both force measurement systems, all subjects performed two MVC trials for each muscle.

Procedure

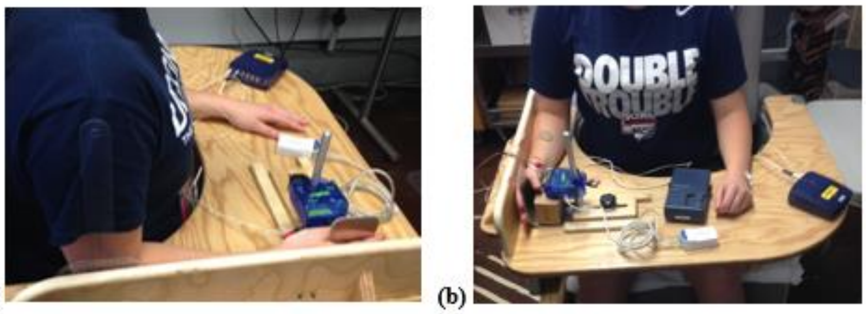
A random number generator was used to assign participant numbers and randomize the order of the muscle group and frequency to be tested.

Acclimation and consent procedure: Each participant was first invited to the lab for familiarization and consent procedure. After explaining the entire protocol, participants were given the chance to ask questions, after which an IRB approved consent form was signed. A familiarization session that introduced appropriate posturing (Figures 8 and 9) and execution of MVC procedure (described below) and acclimation to FES sensation was provided to all participants prior to the fatiguing protocol. By having the participant come to the lab before the testing day, it allowed the participant to feel more comfortable with the environment where the testing would occur and the tasks they would be required to perform so that nervousness and anxiety would not impact the ability for the patient to relax when receiving FES.

FES-Induced Fatigue in the APB

APB protocol: Participants first performed a 5-minute warm-up consisting of low intensity walking. Subjects were seated (Figure 8) in an upright chair with the right arm in 90° elbow

flexion. Hand and forearm muscles were isolated via resting on a board that pins the fingers, only to let the thumb contract (Stratton & Faghri, 2016). The thumb force sensor was affixed to a rod projecting from the table surface so as to be positioned facing the palm of the hand at thumb height (Figure 8).



(a) (b)

Figure 8: (a) Side view of APB setup displaying elbow and shoulder at 90°. (b) Top view of APB setup displaying dynamometer arrangement and metacarpal isolation.

1- Pre-fatigue MVC Protocol: Each subject performed a total of six pre-fatigue 100%MVCs and three 25%MVCs while EMG was recorded. Before FES was applied to the APB, the subject performed two trials of 100%MVC. Each contraction was held for 5 seconds and 30 seconds of rest was given between trials. These two trials were averaged to obtain the preMVC measure. From the preMVC measure, the 25%MVC measure was calculated. The subject then performed a 25%MVC contraction, holding for 5 seconds. Another 30 seconds of rest was given. The subject then began the fatiguing protocol for a given stimulation frequency as described below (see 2- Fatigue Protocol). The process described above was repeated two additional times to precede the two additional fatiguing protocols delivered at the remaining two stimulation frequencies. Table 1 displays the pre-fatigue MVC protocol.

Table 1: Pre-fatigue MVC protocol for the APB.

<table>
<tr><th>Stimulation Frequency</th><th colspan="2">Pre-10Hz*</th><th colspan="2">Pre-35Hz*</th><th colspan="2">Pre-50Hz*</th></tr>
<tr><td>100%MVC</td><td>Trial 1 (T1)</td><td>Trial 2 (T2)</td><td>Trial 1 (T1)</td><td>Trial 2 (T2)</td><td>Trial 1 (T1)</td><td>Trial 2 (T2)</td></tr>
<tr><td>PreMVC</td><td colspan="2">Mean of T1 and T2</td><td colspan="2">Mean of T1 and T2</td><td colspan="2">Mean of T1 and T2</td></tr>
<tr><td>25%MVC</td><td colspan="2">Trial 1</td><td colspan="2">Trial 1</td><td colspan="2">Trial 1</td></tr>
</table>

*Note: Stimulation frequency was randomized so the order displayed is not consistent for all protocols.

2- Fatigue Protocol: Electrical stimulation was delivered to the APB utilizing stimulation frequencies at 10, 35, and 50Hz in a random order as EMG was recorded. FES was applied, incrementally increasing stimulation amplitude until the force level corresponding to 25%MVC (see above: 1- Pre-fatigue MVC Protocol) was achieved. The stimulation intensity necessary to achieve the initial force level (25%MVC) was recorded. The FES was delivered at 4sON/4sOFF (Behringer et al., 2015) until the initial force dropped by 50% to a force level corresponding to 12.5%MVC for three consecutive contractions, or if 30 minutes of FES was completed after which the FES was stopped and time to fatigue or 30 minutes was recorded for future analysis. The participant then performed two post-fatigue 100%MVC trials, holding each contraction for 5 seconds. Five seconds of rest was given between trials. The two trials were averaged to determine the postMVC value. At least 30 minutes of rest was given between fatiguing protocols. The above procedure was repeated two more times for the remaining two stimulation frequencies. Table 2 displays the fatigue protocol.

Table 2: FES-induced fatigue protocol for the APB.

<table>
<tr><th>Stimulation Frequency</th><th colspan="2">10Hz*</th><th colspan="2">35Hz*</th><th colspan="2">50Hz*</th></tr>
<tr><td>FES to 25%MVC</td><td colspan="2">Value from Table 1</td><td colspan="2">Value from Table 1</td><td colspan="2">Value from Table 1</td></tr>
<tr><td>FES drop to 12.5%MVC</td><td colspan="2">0.5*Value above</td><td colspan="2">0.5*Value above</td><td colspan="2">0.5*Value above</td></tr>
<tr><td>100%MVC</td><td>Trial 1 (T1)</td><td>Trial 2 (T2)</td><td>Trial 1 (T1)</td><td>Trial 2 (T2)</td><td>Trial 1 (T1)</td><td>Trial 2 (T2)</td></tr>
<tr><td>PostMVC</td><td colspan="2">Mean of T1 and T2</td><td colspan="2">Mean of T1 and T2</td><td colspan="2">Mean of T1 and T2</td></tr>
</table>

*Note: Stimulation frequency was randomized so the order displayed is not consistent for all protocols.

FES-Induced Fatigue in the VL

<u>VL protocol:</u> Participants first performed a 5-minute warm-up consisting of low intensity walking. Subjects were seated (Figure 9) in an upright chair (hips at 90˚ flexion) as such that feet did not make contact with the ground and the back of the knee joint contacts the edge of the chair seat. Arms were relaxed on the chair arms. A strap was placed firmly around the waist to isolate the quadriceps (Bohannon, Kindig, Sabo, Duni, & Cram, 2012; Stratton & Faghri, 2016). A manually constructed unit, designed to hold the force sensor so that it is positioned facing the lower anterior leg, was affixed from the side of the chair.

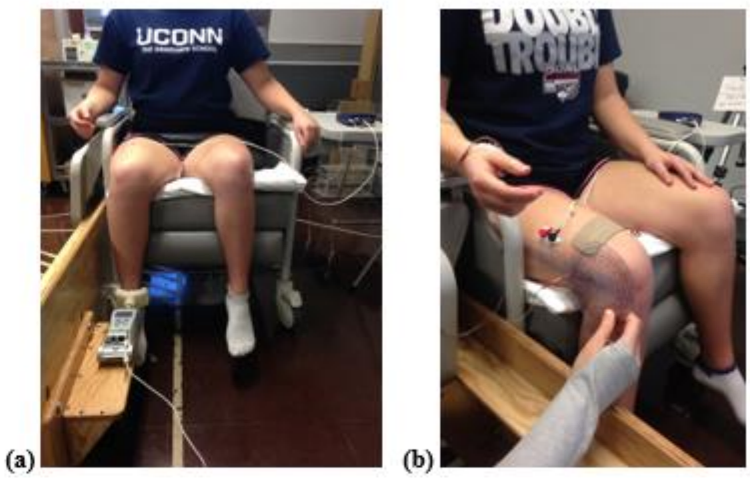


Figure 9: (a) Front view of VL setup displaying dynamometer against lower tibia. (b) Side view of VL setup displaying knee at 90°.

3- Pre-fatigue MVC Protocol: Each subject performed a total of six pre-fatigue 100%MVCs and three 25%MVCs while EMG was recorded. Before FES was applied to the VL, the subject performed two trials of 100%MVC. Each contraction was held for 5 seconds and 30 seconds of rest was given between trials. These two trials were averaged to obtain the preMVC measure. From the preMVC measure, the 25%MVC measure was calculated. The subject then performed a 25%MVC contraction, holding for 5 seconds. Another 30 seconds of rest was given. The subject then began the

fatiguing protocol for a given stimulation frequency as described below (see 4- Fatigue Protocol). The process described above was repeated two additional times to precede the two additional fatiguing protocols delivered at the remaining two stimulation frequencies. Table 3 displays the pre-fatigue MVC protocol.

Table 3: Pre-fatigue MVC protocol for the VL.

Stimulation Frequency	Pre-35Hz*		Pre-50Hz*		Pre-10Hz*	
100%MVC	Trial 1 (T1)	Trial 2 (T2)	Trial 1 (T1)	Trial 2 (T2)	Trial 1 (T1)	Trial 2 (T2)
PreMVC	Mean of T1 and T2		Mean of T1 and T2		Mean of T1 and T2	
25%MVC	Trial 1		Trial 1		Trial 1	

*Note: Stimulation frequency was randomized so the order displayed is not consistent for all protocols.

4- Fatigue Protocol: Stimulation was delivered to the VL three separate times, once for each stimulation frequency (10, 35, and 50Hz), ordered randomly as EMG was recorded. FES was applied, incrementally increasing stimulation amplitude until the force level corresponding to 25%MVC (see above: 1- Pre-fatigue MVC Protocol) was achieved. The stimulation amplitude necessary to achieve the initial force level (25%MVC) was recorded. At this moment time began recording and the FES remained on, delivered at 4sON/4sOFF(Behringer et al., 2015) until the initial force dropped by 50% to a force level corresponding to 12.5%MVC for three consecutive contractions, defining fatigue, or 30 minutes of FES was completed. The FES was immediately shut off and the recording time was stopped. The participant then performed two post-fatigue 100%MVC trials, holding each contraction for 5 seconds. Five seconds of rest was given between trials. The two trials were averaged to determine the postMVC value. At least 30 minutes of rest was given between

fatiguing protocols. The above procedure was repeated two more times for the remaining two stimulation frequencies. Table 4 displays the fatigue protocol.

Table 4: FES-induced fatigue protocol for the VL.

Stimulation Frequency	**35Hz***		**50Hz***		**10Hz***	
FES to 25%MVC	Value from Table 1		Value from Table 1		Value from Table 1	
FES drop to 12.5%MVC	0.5*Value above		0.5*Value above		0.5*Value above	
100%MVC	Trial 1 (T1)	Trial 2 (T2)	Trial 1 (T1)	Trial 2 (T2)	Trial 1 (T1)	Trial 2 (T2)
PostMVC	Mean of T1 and T2		Mean of T1 and T2		Mean of T1 and T2	

*Note: Stimulation frequency was randomized so the order displayed is not consistent for all protocols.

Data Analysis

Analysis involved signal processing to remove stimulus artifacts prior to parameter extraction for statistical testing.

EMG Signal Processing

All EMG signals were initially filtered using a Butterworth filter (20-500Hz cutoff, 5^{th}-order) inherent in the amplifier's BioTrace+ software (MindMedia B.V., Netherlands). Data was then transferred to MATLAB (The MathWorks, Inc., Natick, Massachusetts) for further processing.

<u>MVC:</u> The three preMVC and the three postMVC trials were each cut down to one second. RMS averaging was then performed on all trials. The one-second signals were then full-wave rectified and noise minimized with a 4^{th}-order, zero-lag, low-pass filter at 5Hz cutoff frequency to form the linear envelope. From the linear envelope, iEMG was calculated. The Fast Fourier Transform (FFT) was applied to the full-wave rectified signal to form the power density spectrum in the frequency domain. From this, the MDF was calculated.

<u>FES:</u> EMG signals for each frequency obtained during FES were cut down to two seconds at eleven different time points (initial, 10% of time to fatigue, 20%, 30%, 40%, 50%,

60%, 70%, 80%, 90%, 100% fatigued) and filtered through EMD (Pilkar et al., 2012; Wu et al., 2015). The time points were determined by dividing the total time to fatigue (seconds) by 10 and then adding the quotient to the start time to obtain the time marking 10% of time to fatigue (TTF) and then adding the quotient to the time marking 10%TTF to obtain time at 20%TTF, etc. Since FES was applied at 4sON/4sOFF, the middle two seconds (1-3 seconds) of each contraction corresponding with the time points was isolated. These two second segments were then filtered through EMD. The post-EMD filtered signal was then averaged with RMS. The signals were then full-wave rectified and smoothed with a 4th-order, zero-lag, low-pass filter at 5Hz cutoff frequency for iEMG calculation. FFT was applied to the EMD filtered signals to form the power density spectrum, from which MDF was calculated.

Statistical Analysis

Statistical testing was performed in SAS V9.4. Significance was set at $p < 0.05$.

MVC: EMG activities generated for each muscle for each FES frequency pre-post fatigue were evaluated using RMS, iEMG, and MDF. Furthermore, the pre-post MVC forces were also evaluated. The distributions of each parameter pre-fatigue and post-fatigue were determined to confirm normality. Pre-post parameter comparisons were completed using paired student's t-tests. Differences between muscle groups and frequency levels were evaluated with ANOVAs and Tukey post-hoc tests to assess moderators of fatigue. Correlation and linear regression analyses were used to evaluate the effectiveness of iEMG, RMS, and MDF as moderators of fatigue when compared with changes in MVC.

AMPLITUDE: The required stimulation amplitude to achieve the initial force level of 25%MVC was recorded for every FES application. The distribution was determined to confirm

normality. Differences within a muscle group between stimulation frequencies were evaluated using ANOVA and Tukey post-hoc tests. Differences between muscle group and stimulation frequency was evaluated using MANOVA and Tukey post-hoc tests.

TIME: Time to fatigue (TTF) was measured for every FES application. The distribution was determined to confirm normality. Differences in time to fatigue within and between muscle groups for each stimulation frequency were evaluated using ANOVA and Tukey post-hoc tests.

FES: After signal processing, values for each of the 11 time points included force, RMS, iEMG, and MDF. The distributions of each parameter at each time point were determined to confirm normality. Differences within and between muscle groups for each stimulation frequency were evaluated using ANOVA and Tukey post-hoc tests. Paired t-tests were used to compare the difference in parameter values at initial TTF and the percent TTF.

RESULTS

The results are presented based on the specific aims proposed by the investigation:

Aim 1: To evaluate and compare changes in the force and electrical activity of each muscle pre- and post-fatigue.

Changes in MVC force were evaluated pre-post fatigue after each frequency. There was a significant drop in MVC force at 35Hz and 50Hz for the APB ($p<0.05$). For the VL, there was significant drop in force at 10Hz and 35Hz ($p<0.05$).

Furthermore, we examined changes in electrical activity acquired through pre-post MVC for each frequency. Generally, for all frequencies combined, both the APB and VL showed reduced electrical activity as depicted in RMS and iEMG following the fatigue protocol. When

evaluating each muscle separately, the APB showed a significant decline in electrical activity following stimulation at 35Hz and 50Hz ($p<0.05$). For the VL, significant declines were noted at 35Hz ($p<0.05$). Table 5 displays the pre-post MVC force and electrical activity changes by protocol. Additionally, when comparing the pre-fatigue electrical activities generated by the APB and VL during 100%MVCs, it was noted that the APB generated higher electrical activity (RMS, iEMG, and MDF) than the VL ($p<0.05$).

Table 5: Changes in pre-post-fatigue MVC force and electrical activity in the APB and VL.

	Force (N)		RMS (µV)		iEMG (mV.s)		MDF (Hz)	
	Pre-Post	**p-value**	**Pre-Post**	**p-value**	**Pre-Post**	**p-value**	**Pre-Post**	**p-value**
APB10	0.339±1.57	0.8342	491.42±624.25	0.4514	1182.73±896.73	0.2198	-18.37±21.67	0.4186
VL10	16.15±5.85	0.0221*	280.30±274.90	0.3376	454.03±444.18	0.3366	5.34±4.80	0.2981
APB35	2.43±0.90	0.0273*	-12.07±138.29	0.9324	168.21±35.89	0.0011*	0.5±17.43	0.9777
VL35	19.50±8.79	0.0537*	16.10±7.16	0.0511*	26.24±11.91	0.0550*	9.26±3.01	0.0132*
APB50	3.07±1.09	0.0200*	42.85±16.14	0.0263*	73.91±27.05	0.0231*	0.002±5.17	0.9997
VL50	8.10±5.06	0.1438	5.16±2.68	0.0863	8.44±4.05	0.0672	1.45±3.53	0.6911

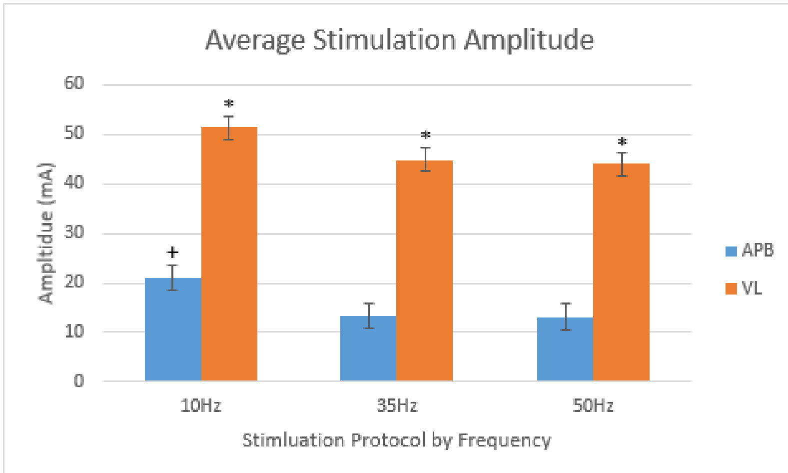
(*) denotes significance ($p<0.05$). Values reported as mean ± standard error.

Aim 2: To evaluate the FES amplitude to generate 25%MVC during each frequency (10, 35, and 50Hz) and compare between the two muscles.

In general, for all frequencies combined, the APB required a significantly lower stimulation amplitude than the VL to reach the initial required force level of 25%MVC (mean APB: 15.77mA, mean VL: 46.77mA, $p<0.0001$) (Figure 10). There was a significant interaction between muscle type and frequency as it related to the amplitude to achieve a force at 25%MVC, suggesting the potential moderating effect of frequency on amplitude required for each muscle.

When muscles were evaluated individually, the amplitude to achieve 25%MVC for the APB was significantly greater at 10Hz ($p<0.0001$) compared to the other frequencies (mean at 10Hz: 20.9±0.97mA, mean at 35Hz: 13.3± 1.14mA, mean at 50Hz: 13.1± 0.96mA). There was

no significant difference between these frequencies for the VL to achieve 25%MVC (mean at 10Hz: 51.4±2.42mA, mean at 35Hz: 44.9±2.85mA, mean at 50Hz: 44.0±2.60mA).



(*) denotes significant differences between muscles ($p<0.05$). (+) denotes significant differences within muscle ($p<0.05$).

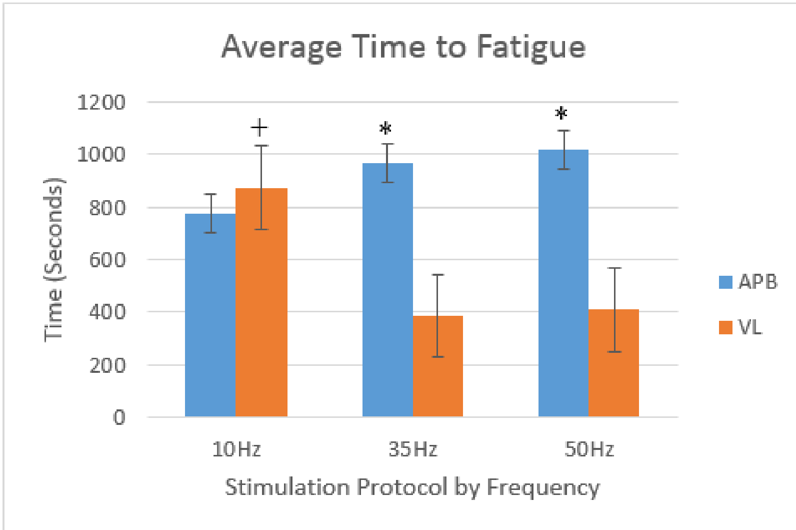
Figure 10: Required stimulation amplitude to achieve initial contractile force (25%MVC).

Aim 3: To evaluate time to fatigue at different stimulation frequencies (10, 35, and 50Hz) for each muscle.

Fatigue was indicated by a drop in force of 50% of the initial FES-induced level of 25%MVC. The time to reach the 50% drop in force was recorded as the time to fatigue (TTF) and was compared between muscles and frequencies. In general, for all frequencies combined, the APB had a higher TTF than the VL ($p<0.05$, mean APB: 918.7 seconds, mean VL: 556.14 seconds). There was a significant difference in TTF between muscles at 35Hz and 50Hz ($p<0.05$), with VL having a shorter TTF (Figure 11).

Evaluating each muscle individually, the APB appeared to fatigue faster at 10Hz, although not significantly. However, the trend for the VL suggested that fatigue was faster at 50Hz and slower at 10Hz. For the VL, there was significant difference in TTF between 10Hz and

the other frequencies (35 and 50Hz, $p<0.05$). Table 6 depicts the TTF for each muscle at each stimulation frequency.



(*) denotes significant differences between muscles ($p<0.05$). (+) denotes significant differences within muscle ($p<0.05$).

Figure 11: Time to fatigue at different stimulation frequencies between muscle types.

Table 6: Time to fatigue between muscles at each frequency.

	10Hz		35Hz		50Hz	
	TTF (seconds)	**p-value**	**TTF (seconds)**	**p-value**	**TTF (seconds)**	**p-value**
APB	773.9±212.04	0.7186	966.7±174.74	0.0056*	1015.5±258.11	0.0418*
VL	872.2±164.41		386.5±59.61		409.8±98.73	

(*) denotes significance ($p<0.05$). Values reported as mean ± standard error.

Aim 4: To evaluate and compare the progression towards fatigue for each muscle during the FES-induced fatigue protocols.

The recorded force and EMG activity during FES-induced fatigue protocols were divided into time points, defined by the percentage of time to fatigue (50% drop from initial force of 25%MVC), in order to evaluate the progression towards fatigue. Overall, when combining all frequencies for both muscles, force significantly declined with progression of TTF ($p<0.0001$). Figure 12 shows the average trend in force decline for each muscle.

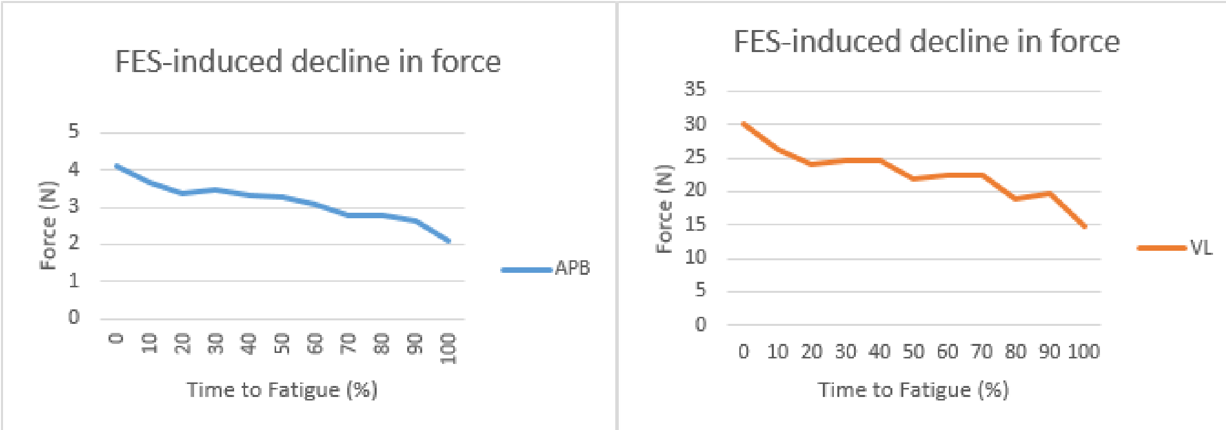


Figure 12: FES-induced decline in force from initial (25%MVC) in the APB (left) and VL (right).

Comparison between frequencies with progression of TTF for each muscle indicated that stimulation of the APB at 10Hz lead to significant force decline after 40%TTF (Figure 13a). This time point identified that fatigue first presented in the APB during stimulation at 10Hz after ~5.2 minutes of FES application. However, stimulation of the APB at 35Hz generated more immediate significant FES-induced force decline beginning at 10%TTF and consistently declining until fatigue (Figure 13b). Under these stimulation conditions, fatigue presented after ~1.6 minutes. For stimulation of the APB at 50Hz, significant FES-induced force decline became prominent at 70%TTF and continued to decline until fatigue (Figure 13c). This time point denoted that fatigue presented after ~11.8 minutes of FES application.

Likewise, comparison between frequencies within in the VL revealed similar force trends to the APB. For the VL, stimulation at 10Hz indicated a significant decline in force after 60%TTF (Figure 13a). This time point identified that early indications of fatigue for the VL at 10Hz began after ~8.7 minutes of FES application. Further, stimulation of the VL at 35Hz induced a significant and consistently increasing decline in force from 10%TTF to fatigue (Figure 13b). This suggested that first signs of fatigue were evident after ~39 seconds. For the

VL at 50Hz, a significant decline in force became prominent at 80%TTF and continued to decline until fatigue (Figure 13c). Under these stimulation conditions, fatigue presented after ~5.5 minutes.

When comparing force trends with electrical activity behavior a consistent pattern was noted for each muscle. For the APB, stimulation at 10Hz induced a spike in electrical activity (Figure 14) at the time point in which significant force decline began (40%TTF). Furthermore, spikes in the electrical activity behavior corresponding to the time points of initial significant force decline were observed at 10%TTF with stimulation at 35Hz (Figure 15) and at 70%TTF with stimulation at 50Hz (Figure 16) in the APB. In contrast, the electrical activity behavior of the VL indicated drops in electrical activity with each presentation of initial significant force decline: at 60%TTF with stimulation at 10Hz (Figure 14), at 10%TTF with stimulation at 35Hz (Figure 15), and at 80%TTF with stimulation at 50Hz (Figure 16).

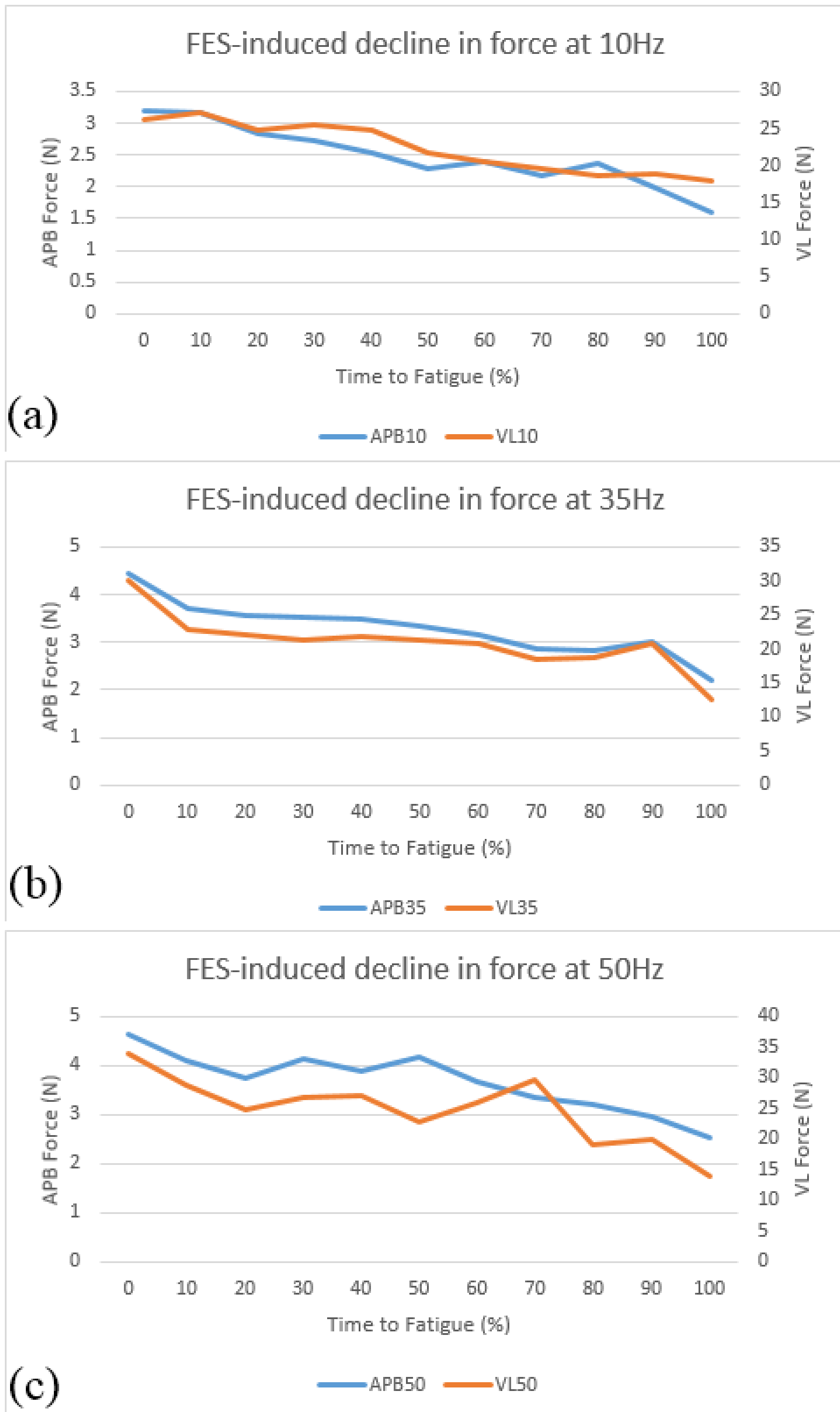


Figure 13: FES-induced decline in force at (a) 10Hz, (b) 35Hz, and (c) 50Hz.

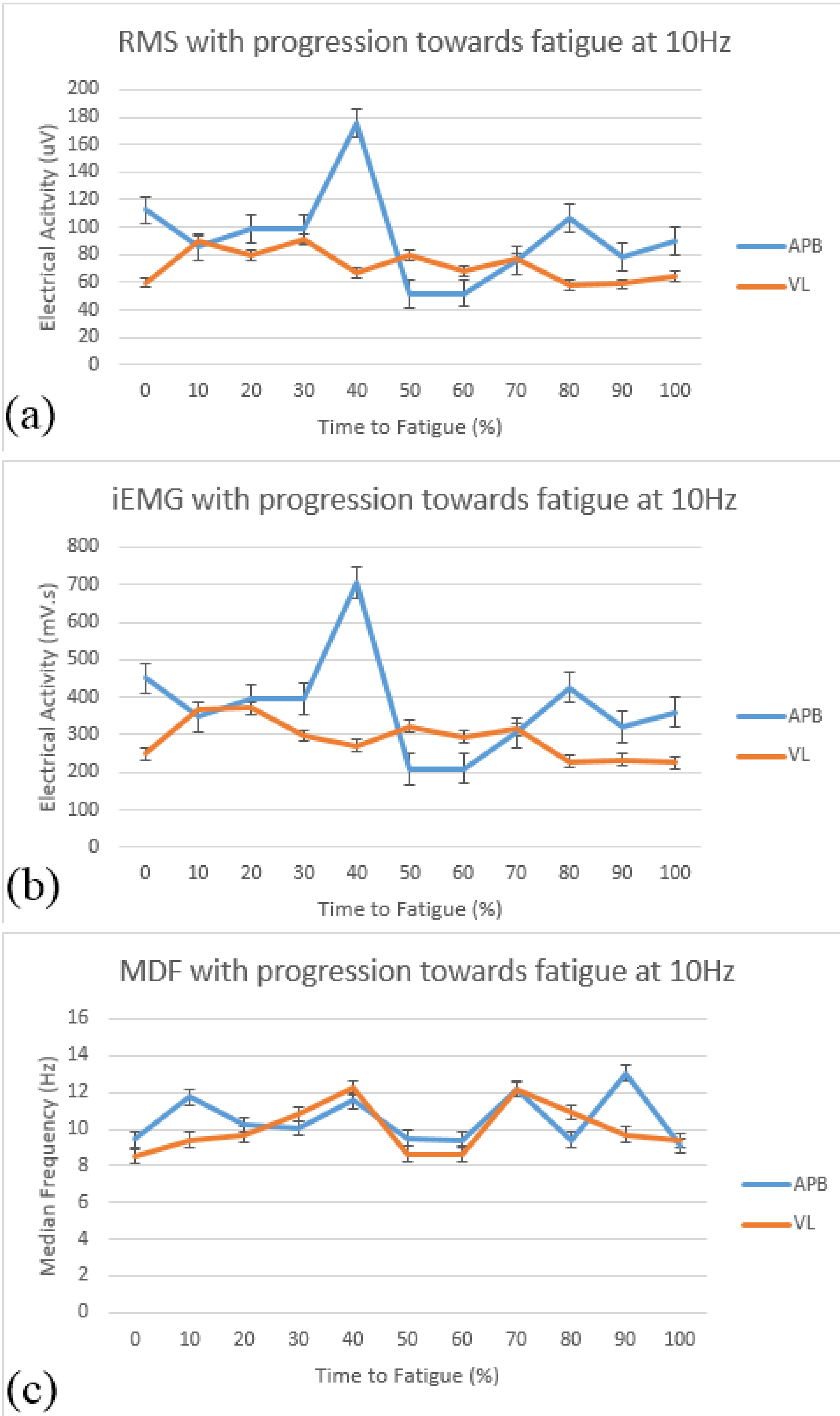


Figure 14: FES-induced (a) RMS, (b) iEMG, and (c) MDF changes with progression towards fatigue at 10Hz.

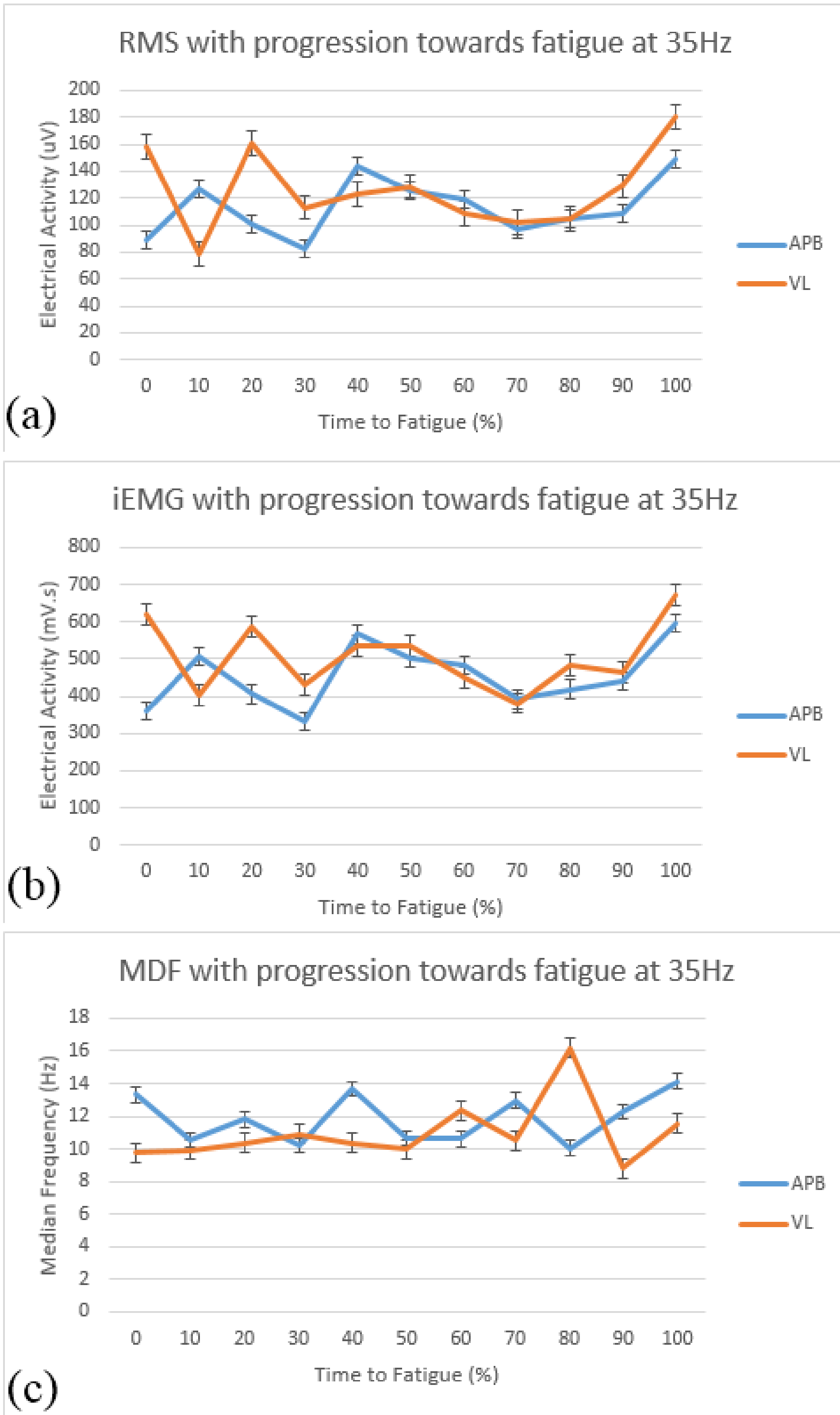


Figure 15: FES-induced (a) RMS, (b) iEMG, and (c) MDF changes with progression towards fatigue at 35Hz.

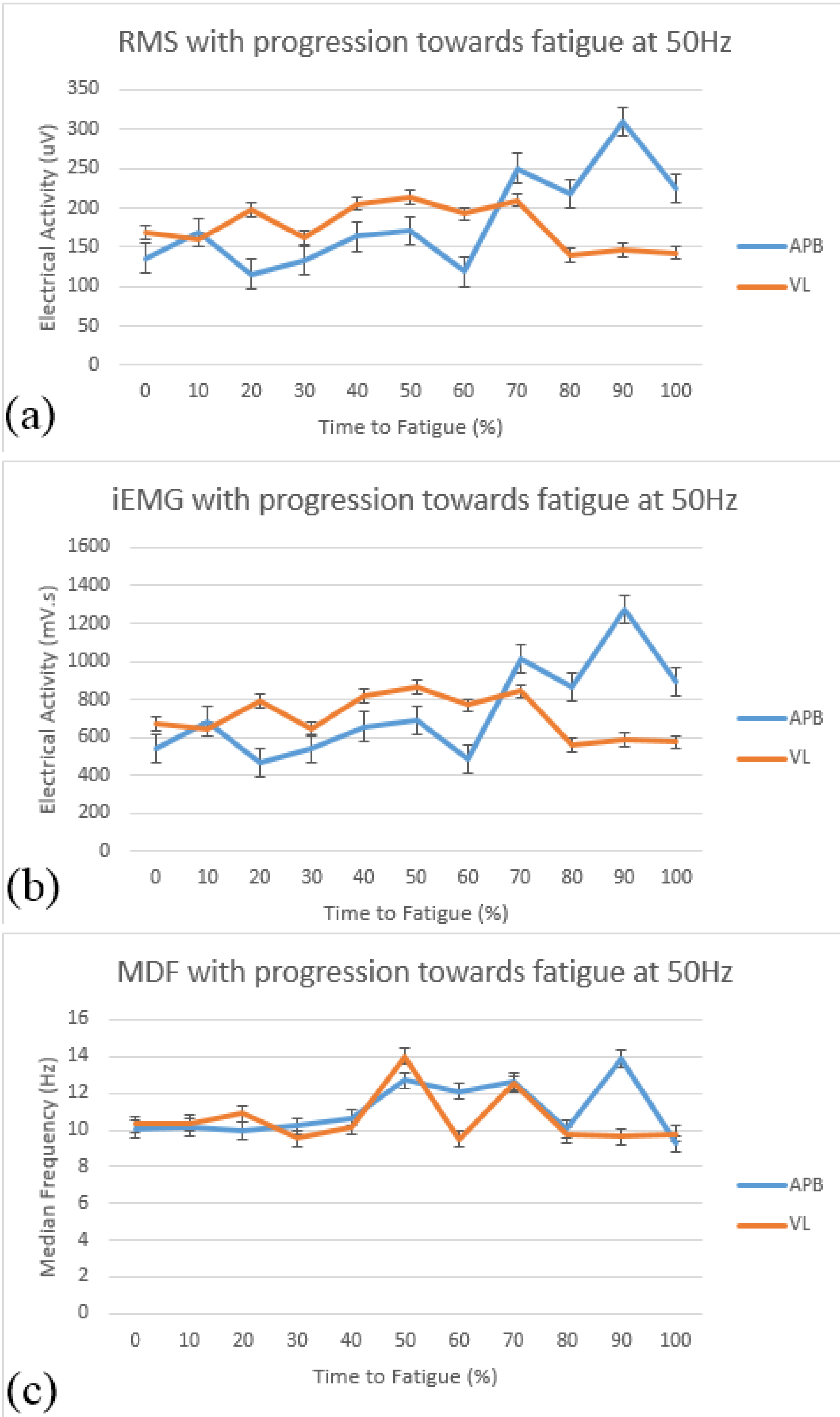


Figure 16: FES-induced (a) RMS, (b) iEMG, and (c) MDF changes with progression towards fatigue at 50Hz.

DISCUSSION

FES is applied during rehabilitation to promote functional gains through increased force generation and improved muscle integrity. In addition to the reported effects of increased cell proliferation (Maffulli & Furia, 2012) and angiogenesis (Kanno et al., 1999), enhanced muscle integrity and force generation is further achieved by the FES-induced exercise-like movement that leads to fiber type transformation (Andersen, Mohr, Biering-Sørensen, Galbo, & Kjaer, 1996; Crameri, Weston, Climstein, Davis, & Sutton, 2002; Windisch et al., 1998) and increased muscle cross-sectional area (Kern et al., 2004). With this knowledge, the aim of this investigation was to better understand how different muscle fiber compositions can impact optimal FES performance, as research comparing the application of FES on muscles of varying fiber proportion was limited. Further, fatigue-related research often manipulated the stimulation amplitude and used a fixed stimulation frequency to allow for force-amplitude comparisons (Chou & Binder-Macleod, 2007). This has established the recommendation to increase amplitude as fatigue develops, irrespective of stimulation frequency. In the present investigation, a fixed stimulation amplitude was chosen to focus on the effects of differing stimulation frequencies on the development of fatigue. Particularly, frequency manipulation during FES-induced fatigue as it pertained to time to fatigue and the changes in force and EMG over time was evaluated. The following will present a discussion of the outcomes by hypotheses.

First hypothesis: It is hypothesized that the force, RMS, iEMG, and MDF measures obtained during MVC will decline. Furthermore, sEMG will detect greater electrical activity and firing rate in the APB for all frequencies due to its small size and concentration of motor units in the electrode region.

In regards to the changes in MVC force and EMG measures, the results of our study were consistent with our hypothesis for the APB at 35 and 50Hz and the VL at 10 and 35Hz. Under these conditions, significant decline in force occurred. Additionally, electrical activity also declined. This indicated that a decline in electrical activity is reflective of a significant decline in force. Similar findings were noted in other research by Dreibati et al. (2010) who observed decline in MVC force with stimulation at 20, 50 and 100Hz in the quadriceps. Further, Griffin, Jun, Covington, & Doucet (2008) examined force output during fatigue with increasing stimulation frequency from 20 to 40Hz for three minutes, decreasing stimulation from 40 to 20Hz for three minutes, and three minutes of constant stimulation at 20Hz in the thenar muscle of the thumb. Griffin et al. (2008) found that force and EMG m-wave amplitude significantly declined with all fatiguing tasks. Thus, for our study, when force decline was not significant, it would be expected that electrical activity would also change non-significantly.

For the comparison between muscles, in our study the APB generated greater electrical activity than the VL with maximal voluntary contraction, supporting our hypothesis. Likewise, Stratton & Faghri (2016) made the same observation that electrical activity was greater in the APB than the VL when comparing maximal contractions. They concluded that the small muscle size provided for a greater fiber density under the EMG electrodes, increasing the detectable electrical activity. Additionally, research by Trevino et al. (2016) and Watanabe et al. (2016) found that the mean frequency value was related to the proportion of type I fibers where more type I generated a greater mean frequency. Also, De Luca & Hostage (2010) identified a reverse relationship between recruitment threshold and firing rates. This supports our findings that the more type I predominant APB would generate a greater MDF because type I fibers have lower recruitment thresholds.

Second hypothesis: It is hypothesized that lower FES frequency will require higher FES amplitude and between muscles the VL will require higher FES amplitude at all frequencies due to its larger size.

The results of our study indicated that both muscles required a higher stimulation amplitude at 10Hz to reach the initial force of 25%MVC. However, within each muscle type, the necessary amplitude at 10Hz was only significantly greater in the APB. This may be explained by the fact that lower frequencies have been reported to recruit fewer fibers (Stratton & Faghri, 2016; Dreibati et al., 2010), therefore a larger amplitude would be needed to attract additional fibers of lower threshold (type I fibers) located deeper in the tissue.

Between muscles, the higher required amplitude in the VL at all frequencies can be explained by the greater proportion of type II fibers, which have higher firing thresholds and larger diameters. Kuriki et al. (2012) reported that muscle control differs between sizes. Small muscles, like the APB, assist in fine motor movements by innervating fewer fibers per motor unit; therefore, they are less force producing in exchange for greater precision. In contrast, large muscles, like the VL, assist in gross motor function, generating significantly greater forces than smaller muscles by innervating upwards of 100 to 1000 fibers per motor unit (Kuriki et al., 2012). Therefore, greater amplitude is necessary to activate the larger motor units of the large muscle.

Third hypothesis: It is hypothesized that the larger muscle (VL) will fatigue more quickly than the smaller muscle (APB) at all stimulation frequencies since the VL contains a greater proportion of fast-fatiguing fibers. Further, within each muscle 10Hz will produce

longer time to fatigue compared to the higher frequencies since higher frequencies recruit more type II fibers.

For the VL, the results supported our hypothesis. The quicker TTF in the VL can be explained by the greater proportion of type II fibers as reported by Polgar et al. (1973). Also, it was previously reported by Dreibati et al. (2010) that higher frequencies of 50 and 100Hz fatigued the quadriceps more quickly than stimulation at 20Hz because they recruit more fast-twitch fibers (Dreibati et al., 2010). Further, it is possible that the VL is overstimulated at higher frequencies, meaning that the activated motor units are firing more often than necessary, leading to faster fatigue. This is supposed as it is one of four knee extensors that typically functions in conjunction with the other muscles of the quadriceps, but was preferentially stimulated in this investigation. Additionally, since motor unit recruitment thresholds for the VL range from ~5.5 to 22.7Hz (Trevino et al., 2016), it could be inferred that the higher stimulation frequencies (35 and 50Hz) would maximize motor unit recruitment and therefore lead to fatigue more quickly in larger muscles of predominant type II fiber composition.

For the APB, the hypothesis was not confirmed. TTF was non-significantly different between frequencies and it was slightly faster at 10Hz and slower at 50Hz. The non-significant difference in time may be explained by the greater proportion of the type I fibers (Polgar et al., 1973) allowing the same motor units to be activated for all frequencies, thus leading to the similar rate of fatigue development. Further, the faster time to fatigue at 10Hz may be explained by the greater discomfort experienced by the participants at the low frequency. Previously, it was reported that lower frequencies are less comfortable because they generate a tapping sensation in which individual pulses are sensed, while higher frequencies are perceived as smoother signals that elicit a more comfortable tingling effect (Sluka & Walsh, 2003). Additionally, higher

stimulation amplitudes also reportedly contributed to greater discomfort (Baker, Wederich, McNeal, Newsam, & Waters, 2000). Therefore, it is possible that the increased sensation of discomfort may have led to reduced muscle performance as a resistance to further pain or discomfort.

Fourth Hypothesis: It was hypothesized that changes in the EMG measures over time during FES would relate to the changes in force over time to function as predictors of fatigue.

Results of our investigation support our hypothesis that an association exists between FES-induced force decline and EMG changes. The corresponding peak in electrical activity of the APB and drop in electrical activity of the VL when FES-induced force declined may be due to the better glycogen storage maintenance and utilization ability of type I fibers. Gregory, Williams, Vandenborne, & Dudley (2005) researched the influence of metabolic characteristics and phenotypic expression of individual fibers in predicting glycogen utilization during FES. Biopsies of the VL were extracted at baseline and after 30 minutes of non-fatiguing stimulation. Outcomes demonstrated that both an enzyme ratio of succinate dehydrogenase and quantitative-actomyosin ATPase (SDH:qATPase) and fiber phenotype significantly predicted glycogen utilization, where type I fibers are the most proficient at utilizing and maintaining glycogen stores. Therefore, it is possible that the fibers of the APB are capable of producing an electrical spike at the moment of significant force decline as a final mechanism attempting to sustain force before fatigue while the fast-fatiguing type II fibers do not sustain the same energy capacity and thus, the electrical activity drops at the moment of significant force decline.

LIMITATIONS

EMD filtering was able to successful extract an EMG signal that resembled the activity range observed with voluntary recruitment, however, there is a chance that some higher frequency muscle data was sacrificed in applying this technique, which researchers should recognize when quantifying the signal with frequency measures like MDF. Further, a larger sample size could strengthen confirmation of the outcomes, but a sample of ten individuals is within the common range for this type of research. Additionally, we selected a sample population between the ages of 18 and 30 years to limit the confounding issues that arise from changing muscle morphology with age. Though this provided us with a more homogenous population, findings cannot be inferred beyond 30 years or below 18 years. Lastly, research could be enhanced by evaluating how increasing amplitude during FES, based on the observed times to fatigue in this research, may help prolong progression to fatigue.

CONCLUSIONS AND CLINICAL IMPLICATIONS

Overall, research indicated that frequency influenced force and fatigue development. It was evident that the type I fibers are more endurance-related and that type II fibers are better force generators. Therefore, clinicians should consider the function of the muscle and the fiber type when developing FES treatment protocols. Increases in muscle strength through FES reportedly require the maintenance of 60%MVC (Dreibati et al., 2010). Since force production is less of a concern in APB function, rather the sustained repetition of fine motor movements is preferred, lower-moderate frequency FES ranges may be most suitable. However, as force generation is the primary function of the VL, if improved muscle strength is desired then higher frequencies should be applied to try to achieve force production near 60%MVC.

Further, additional research is needed to evaluate the combination and interactions between amplitude, frequency, and muscle type. However, the findings from this investigation may provide some guidelines for health professionals to adjust the amplitude based on fatigue development. For example, it was previously suggested that FES training protocols not extend past 20 minutes (Dreibati et al., 2010), yet with our participants, fatigue was reached after ~6 to 15 minutes when only stimulating to 25%MVC. With consideration of time to fatigue at the different frequencies, the outcomes of this investigation suggest that low frequency stimulation (~10Hz) may be most optimal for large muscles of greater type II fiber composition while moderate-high frequency (~50Hz) stimulation may be most optimal for small muscles of greater type I fiber composition. Therefore, it could be postulated that one can maximize FES treatment by keeping the stimulation amplitude constant for the first ~8 minutes in large muscles and ~11 minutes in small muscles and then increase amplitude proportionally as needed to encourage the recruitment of additional motor units to sustain force development and prevent early fatigue.

Lastly, continued research can benefit from enhanced filtering techniques to achieve completely purified muscle activity with EMG. Currently, our research group is working on completing the construction of new hardware technology that amplifies the EMG signal and removes the FES stimulus artifacts. Preliminary testing has produced promising results, in which clear EMG signals were obtained from FES-induced contractions. With formal validation, this technology may evolve as an industry standard for EMG signal acquisition with FES. Particularly, as FES application is increasingly utilized for tissue regeneration and healing, the availability of a real-time feedback filtering device will greatly advance the efficiency of treatment.

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