EFFECT OF STIMULATION FREQUENCY AND INTENSITY ON SKELETAL MUSCLE FATIGUE DURING REPETITIVE ELECTRICAL STIMULATION

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Biomechanics and Movement Science

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ABSTRACT

The goal of this project was to study strategies to improve muscle performance during functional electrical stimulation (FES). Specific Aim 1 compared isometric muscle performance during repetitive stimulation, with 3 different combinations of frequency and pulse duration to generate the same initial peak force: Protocol #1used a long-pulse duration (600-μs) and low-frequency; Protocol #2 used a medium-frequency (30-Hz) and medium-pulse duration; and Protocol #3 used high-frequency (60-Hz) and short-pulse duration. The results showed that if the frequency and intensity are kept constant during FES, repetitive stimulation using a low-frequency, long-pulse-duration (Protocol #1) maximized muscle performance. Specific Aim 2 compared isometric muscle performance during repetitive electrical stimulation using 3 different strategies: constant pulse-duration and stepwise increases in frequency (frequency-modulation), constant frequency and stepwise increases in pulse-duration (pulse-duration-modulation), and constant frequency and pulse-duration (no-modulation). The results showed that frequency-modulation can help improve muscle performance during FES.

Chapter 1

INTRODUCTION

The central nervous system (CNS) achieves a precise control over a wide range of task-specific muscle forces by varying the number of activated motor units (recruitment) and the firing frequency of the active motor units (firing rate) [44, 43]. In individuals with paralysis due to upper motor neuron dysfunction, functional electrical stimulation (FES) of peripheral nerves can be used to substitute for loss of voluntary motor control [50, 48]. FES can help individuals with spinal cord injury (SCI) to regain the ability to stand [14, 3, 4, 2, 77], walk [39, 48, 53, 47, 69, 84, 1, 41], grasp objects [51], and regain bladder and bowel control [63]. FES is also used for the management of foot drop in individuals with hemiplegia following stroke [73, 40, 64, 79]. Other innovative applications of FES include restoration of a functional cough in ventilator-dependant tetraplegics [74] and the restoration of swallowing in patients with dysphagia [27]. Analogous to the two CNS mechanisms for skeletal muscle force control, stimulation intensity and frequency can be modulated to control muscle force output during FES.

FES-tasks such as walking and grasping require the repetitive generation of a targeted muscle force. However, muscle fatigue causes a decline in the force-generating ability of the muscle and impedes FES task-performance [14, 2, 53, 60, 62]. Muscle fatigue is defined as a decline in the force generating ability of muscle due to previous activity [7]. Muscle fatigue can limit the number of steps an FES-user can perform or the duration for which an FES-user can stand or grasp an object [14, 2, 53, 60, 62]. Muscle fatigue is one of the major limitations preventing the widespread application of FES [14, 2, 53, 60, 62].

In addition to controlling force, stimulation frequency [7, 52, 29, 10, 54] and intensity [10, 33] can also affect the amount of muscle fatigue produced during FES. Although both the stimulation frequency and intensity can be increased to compensate for the decline in force generating ability due to muscle fatigue, most current FES systems deliver a constant frequency and only vary the intensity to control muscle force [57, 61, 73, 24]. An understanding of the effects of stimulation frequency and intensity on muscle fatigue during repetitive electrical stimulation can help to identify strategies that maximize muscle performance during FES. The overall goal of this study, therefore, was to systematically study the effects of stimulation frequency, intensity, and the modulation of frequency and intensity on muscle performance during repetitive electrical stimulation.

1.1 Definitions and Terminology

During FES, single pulses are grouped together to form stimulation trains. Either the duration or the amplitude of each pulse within a train can be varied to control the stimulation intensity. For our study, we fixed the stimulation amplitude and only varied the pulse duration to control the stimulation intensity. The interval between successive pulses within a train (inter-pulse interval) determines the stimulation frequency of a train. Stimulation pulse-duration and frequency can either be modulated within or across trains. In the present study, the pulse-duration and frequency were modulated across trains.

1.2 Muscle Fatigue During Repetitive Stimulation

Various stimulation parameters such as stimulation frequency [7, 52, 29, 10, 54], intensity [10, 33], and duty cycle [56, 23] affect the amount of muscle fatigue produced during repetitive stimulation. In addition, the peak force or force time integral generated in response to each train have been shown to influence the amount of muscle fatigue during repetitive activation [16, 6, 36, 66]. A previous study from our laboratory showed that peak force was a more important determinant of fatigue than the force-time integral or the number of pulses within the stimulation train, when the rest time was held constant across protocols during repetitive stimulation with short-duration trains [66]. However, during FES, the duty cycle and targeted peak force level will be determined by the requirements of the task. We therefore maintained a constant duty cycle and similar initial target peak forces for all the fatigue protocols compared in our study. For all fatigue protocols tested in our study, a 30% duty cycle (300-ms train duration and 700-ms rest time) was maintained. In addition, all fatigue protocols tested in our study generated similar initial peak forces, i.e., 20% of the subject's maximal voluntary isometric contraction (MVIC) force. The fatigue protocols tested in this study have been shown to produce fatigue in the human quadriceps muscle in previous studies [10, 23, 33, 66].

1.3 Effects of Stimulation Intensity and Frequency on Muscle Fatigue

During surface electrical stimulation, stimulation intensity affects the number of motor units recruited by a train. Only a few studies have investigated the relationship between stimulation intensity and muscle fatigue [10, 33]. Binder-Macleod and colleagues tested the rate and amount of fatigue during repetitive stimulation of the human quadriceps muscle with trains at stimulation amplitudes that produced 20%, 50%, and 80% MVIC forces and found less fatigue during repetitive stimulation at higher compared to lower stimulation amplitudes [10]. In contrast, Godfrey and colleagues [33] recently showed greater declines in force due to fatigue at high (supra-maximal) compared to low (sub-maximal) stimulation intensities. Thus, previous literature does not provide conclusive evidence about the stimulation intensity levels that can help to minimize fatigue when used for repetitive stimulation.

The stimulation frequency controls the extent of temporal summation produced in response to the individual pulses within a stimulation train. Most current FES systems deliver the lowest frequency that can generate a fused tetanic contraction. The rationale for using low frequencies in FES is based on the premise that higher frequencies cause greater fatigue [52, 10]. Although several previous studies have shown that fatigue is a function of the stimulation frequency or the number of pulses [52, 10], these studies have often ignored the effect of either the differences in initial peak force or stimulation intensities on muscle fatigue. Interestingly, Russ and colleagues [66] recently showed that increasing the frequency or number of pulses did not affect the amount of muscle fatigue produced during repetitive isometric contractions if the initial force produced by the stimulation trains was controlled. Another study showed that intermittent high-frequency stimulation produced less fatigue than low-frequency repetitive stimulation in able-bodied and spinal cord injured subjects [54]. Thus, previous literature does not provide conclusive evidence about the isolated effect of stimulation frequency on muscle fatigue.

1.4 Combinations of stimulation intensity and frequency

During FES, because prescribed forces are determined by the requirements of the task, stimulation intensity and frequency are inter-dependant parameters. In addition, because the frequency [7, 52, 10, 54], intensity [10, 33], and the force generated in response to electrical stimulation [16, 6, 36, 66, 66] have been shown to affect muscle fatigue, it is difficult to design a study that can isolate the effect of stimulation frequency versus intensity on muscle fatigue while controlling for the initial peak force levels. However, for FES applications, it may not be important to study the isolated effects of frequency versus intensity on muscle fatigue, but to determine the combinations of frequency and intensity that can minimize muscle fatigue while repetitively generating the force level required for an FES-task. It has been previously suggested that using the combination of the lowest frequency and the highest stimulation intensity during repetitive electrical stimulation may minimize fatigue [12]. However, no previous studies have systematically investigated the effect of different combinations of frequency and intensity on muscle fatigue and performance during repetitive electrical stimulation. It is important to determine the combination of stimulation frequency and intensity that minimizes fatigue while generating the desired force level to enable a better choice of stimulation parameters for use in clinical FES systems.

The lack of evidence regarding the combination of frequency and intensity that can generate a targeted muscle force while minimizing muscle fatigue during repetitive electrical stimulation formed the motivation for Specific Aim 1 of our present study. We hypothesized that for any two no-modulation fatigue protocols that generate the same initial peak force, a greater amount of fatigue and poorer muscle performance will be produced during the fatigue protocol consisting of a lower stimulation intensity compared to the one using a greater intensity of stimulation [12, 10]. The underlying premise for this hypothesis was that the metabolic effects of fatigue would be shared among a relatively greater number of motor units during stimulation at higher compared to lower intensities. In addition, repetitive stimulation with the combination of low-frequency and high-intensity would deliver relatively fewer pulses, and thereby produce lesser fatigue compared to repetitive stimulation using a combination of high-frequency and low-intensity.

1.5 Modulation of Stimulation Frequency and Intensity

Stimulation trains of different combinations of frequency and intensity can initially generate the muscle force required to perform a functional task during FES. However, with repetitive activation, the muscles will fatigue and an increase in either the frequency or the intensity of stimulation will be required to allow the maintenance of the targeted muscle force. Sigmoid relationships between the firing frequency and isometric muscle force and between the stimulation intensity

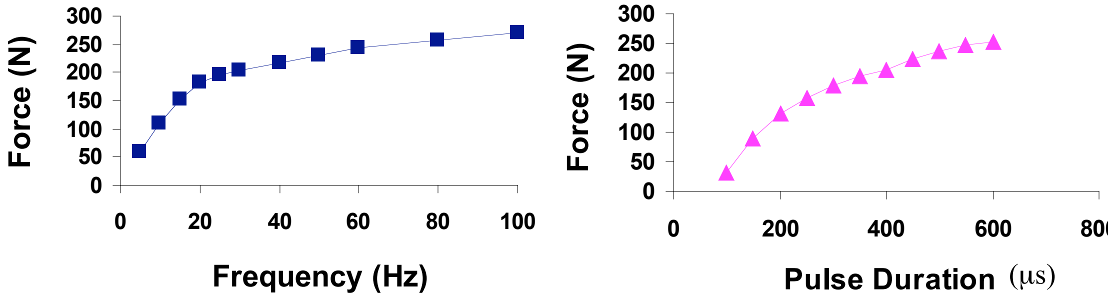


Figure 1.1: Graphs showing the relationship between stimulation frequency (Hz) and skeletal muscle peak force (left), and between stimulus pulse duration (μs) and peak force (right). Both curves were plotted using 300-ms long trains, with a 10-second rest time between the trains. For the force-frequency curve, trains with 600-μs pulse duration were delivered. For the force pulse duration curve, 60-Hz frequency trains were delivered.

and isometric muscle force have been reported [8, 10, 11]. Increasing the frequency or intensity of electrical stimulation will, therefore, produce an increase in skeletal muscle force output, especially for frequencies and intensities on the steep portion of the force-frequency and force-intensity curves (Figure 1.1).

Surprisingly, although both the stimulation intensity and frequency can be used to increase muscle force, most current FES systems deliver a constant frequency and only increase the stimulation intensity to increase force output as the muscle fatigues [57, 61, 73, 24]. The results of previous studies on animal muscles showed that using a simultaneous modulation of stimulation intensity and frequency produced better control of muscle force and an improved ability to compensate for recruitment nonlinearities and changes in external load during electrical stimulation [19, 49]. A stochastic modulation of the inter-pulse intervals within stimulation trains was shown to decrease muscle fatigue compared to stimulation at a constant frequency [34]. In addition, a recent study from our laboratory involving repetitive nonisometric contractions found that repetitive stimulation with trains starting at a low-frequency and then switching to a high-frequency showed better performance than stimulation starting at high- and switching to low-frequencies [45, 46]. In contrast, a recent study showed that random modulation of frequency (mean: 40-Hz), amplitude (mean: 75%maximal tetanic force), and pulse duration (mean: 250-s) by 15%of their mean values every 100-ms did not effect the rate of fatigue during isometric contractions of the tibialis anterior and quadriceps femoris muscles of 7 spinal cord injured subjects [76]. However, in spite of the lack of evidence in the literature supporting the use of intensity for modulation of muscle force, only the intensity is varied in most clinical FES systems [57, 61, 73, 24]. No previous studies on human muscles systematically compared the effects of increasing the stimulation frequency versus intensity on muscle fatigue and performance during repetitive electrical stimulation. In addition, during volitional activation, both the firing rate and the number of motor units recruited are modulated throughout the contraction [44, 43]. The use of frequency and intensity-modulation in FES would therefore make the control mechanisms of FES similar to those utilized during physiological contractions.

The lack of studies on human skeletal muscles investigating the effects of increasing frequency versus intensity on muscle performance and fatigue during FES formed the motivation for Specific Aim 2 of our study. We hypothesized that with each stepwise increase of frequency and intensity, muscle force output would increase due to temporal and spatial summation of forces respectively. Repetitive stimulation using strategies involving the modulation of frequency or intensity would, therefore, result in better performance compared to repetitive stimulation with a constant frequency and intensity. In this thesis, we studied intensity-modulation and frequency-modulation separately as we believed that it was a necessary first step towards understanding the basic mechanisms underlying these strategies before attempts could be made to test complex stimulation strategies involving the simultaneous modulation of both intensity and frequency during FES. In future studies, we would like to investigate the effects of combined modulation of frequency and intensity in different muscle groups (e.g. ankle dorsiflexors) of patient populations (e.g. stroke, spinal cord injured).

1.6 Modulation of Intensity Using Pulse Duration versus Stimulus Amplitude

Both the amplitude and the duration of the stimulation pulses can be used to modulate the stimulation intensity during electrical stimulation. For our study, we used stimulus pulse duration to vary the intensity because it was easier to control and provided a more consistent muscle force response compared to stimulation amplitude [55, 35]. Previous studies using direct nerve stimulation of animal muscles showed that the same force could be elicited with a smaller charge per stimulus using pulse-duration-modulation compared to amplitude modulation for the control of intensity [21, 55]. In addition, during electrical stimulation via nerve cuff electrodes, fixing the stimulus amplitude at a high level allowed greater force generation at relatively shorter pulse durations, and increased the difference between the recruitment thresholds of nerve fibers [35, 72].

1.7 Summary

Muscle fatigue is an important factor limiting the widespread application of FES [14, 2, 53, 60, 62]. Although both the frequency [7, 52, 29, 11, 54] and intensity [10, 33] used for repetitive stimulation affect muscle fatigue, no previous studies have determined the combination of frequency and intensity that maximizes muscle performance during FES. In addition, although both the frequency and intensity can be used to increase muscle force output during FES, most current FES systems use a constant frequency and only increase the intensity to increase muscle force as the muscle fatigues. There is a need to understand the effects of stimulation frequency and intensity, the two primary parameters for the control of muscle force during electrical stimulation, on muscle performance during FES. This thesis represents the first step of a larger project that aims to develop electrical stimulation strategies to improve FES-task performance.

1.8 OVERALL GOALS

The goal of this study was to identify the effect of frequency and pulse duration, and of the modulation of thes etwo 2 parameters on muscle fatigue and performance during repetitive electrical stimulation.

1.9 SPECIFIC AIMS AND HYPOTHESES

Specific Aim 1: To compare the muscle fatigue and isometric performance in response to repetitive surface electrical stimulation with three different combinations of frequencies and pulse-durations that were each selected to produce the same initial peak force: #1 low frequency, long-pulse duration (600-μs); #2 medium frequency (30-Hz), medium pulse duration; and #3 high frequency (60-Hz), short-pulse duration. The quadriceps femoris muscles of healthy individuals were tested.

Hypotheses:

1.1: Protocol #1 will produce the best and Protocol #3 the poorest isometric muscle performance in response to the fatiguing trains.

<u>Rationale:</u> We postulated that muscle performance in response to the fatiguing trains would be a function of the amount of muscle fatigue and the degree of low-frequency fatigue produced by the fatiguing trains. We hypothesized that Protocol #1 would produce the least muscle fatigue (see Hypothesis 1.2) and the least degree of low-frequency fatigue (see Hypothesis 1.3). Protocol #1 would, therefore, demonstrate the best performance in response to the fatiguing trains among the 3 fatigue protocols tested.

1.2: Protocol #1 will produce the least and Protocol #3 the most muscle fatigue in response to the testing trains.

Rationale: Among the 3 fatigue protocols, the fatiguing trains delivered during Protocol #1 would recruit the largest motor unit population to generate the same initial peak force. Due to this, Protocol #1 would result in the least ATP utilization by the actin-myosin ATPase per muscle fiber [13, 59, 67]. In addition, Protocol #1 would deliver the lowest frequency, thereby generating the fewest action potentials and the resulting in the least ATP utilization by $Ca^{2+} - ATPase$ and $Na^{+} - K^{+} - ATPase$ [52, 82, 17, 18, 26]. Because metabolic demand is related to muscle fatigue [20, 67, 81], Protocol #1 would produce the least muscle fatigue among the 3 protocols.

1.3: Protocol #1 will produce the least and Protocol #3 the most low-frequency fatigue.

Rationale: Low-frequency fatigue is the result of impairment in excitation-contraction coupling that is thought to result from increased levels of intracellular Ca^{2+} during muscle activation [82, 17, 18]. Intracellular Ca^{2+} concentrations have been shown to be directly related to the stimulation frequency [17]. Because the lowest stimulation frequency was used during Protocol #1, Protocol #1 would, therefore, result in the lowest levels of intracellular Ca^{2+} and therefore the least amount of low-frequency fatigue among the 3 fatigue protocols [82, 17, 18].

Specific Aim 2: To compare the muscle fatigue and isometric performance of the healthy human quadriceps femoris muscles during repetitive surface electrical stimulation with a frequency-modulation protocol, a pulse-duration-modulation protocol, and a no-modulation protocol. Specifically, the three fatigue protocols that were compared produced the same initial peak forces and consisted of: constant pulse duration (600-μs) and stepwise increases in frequency (frequency modulation);

constant frequency (60-Hz) and stepwise increases in pulse duration (pulse-duration-modulation); and the no-modulation protocol showing best muscle performance from 1.1.

Hypotheses:

2.1: The no-modulation protocol will show poorer performance in response to the fatiguing trains compared to the frequency- or pulse-duration-modulation protocols.

Rationale: During no-modulation, the same motor unit population would be recruited and a constant level of temporal summation would be produced throughout the fatigue protocol. In contrast, the modulation protocols involved either a stepwise increase in the extent of temporal summation (frequency-modulation) or a stepwise recruitment of previously inactivated, unfatigued motor units (pulse-duration-modulation). The no-modulation protocol would therefore show poorer performance than either the frequency- or pulse-duration-modulation protocols.

2.2: The frequency-modulation protocol will show better performance than the pulse-duration-modulation protocol.

Rationale: During the frequency-modulation protocol, the frequency was increased stepwise from low- to high during the fatigue protocol. In contrast, a high-frequency (60-Hz) was maintained throughout the pulse-duration-modulation protocol, contributing to greater muscle fatigue [52, 10]and perhaps high-frequency fatigue [42]. In addition, our preliminary data supported the hypothesis that frequency-modulation would demonstrate better performance than pulse-duration-modulation.

2.2: There will be differences in the amount of muscle fatigue and low-frequency fatigue produced among the 3 protocols.

Rationale: During the frequency-modulation protocol, a greater level of motor unit recruitment was maintained throughout the protocol; and low-frequency trains were used initially and high-frequency trains delivered only during the latter portion of the protocol; and both of these factors may contribute to minimizing muscle fatigue. During the pulse-duration-modulation protocol, the recruitment of previously inactive, unfatigued motor units with each stepwise increase in pulse-duration may contribute to minimizing muscle fatigue and low-frequency fatigue. The no-modulation protocol used a low-frequency and recruited a large motor unit population throughout the protocol, which may help to minimize muscle fatigue.

Chapter 2

EFFECT OF FREQUENCY AND PULSE DURATION ON MUSCLE FATIGUE DURING REPETITIVE ELECTRICAL STIMULATION

2.1 Abstract

Different combinations of stimulation frequency and intensity can generate a targeted force during functional electrical stimulation (FES). This study compared performance and muscle fatigue during repetitive stimulation with 3 different combinations of frequency and pulse duration that produced the same initial peak forces: Protocol #1 used 600-μs (long-pulse duration) and low-frequency, Protocol #2 used 30-Hz (medium-frequency) and medium-pulse duration, and Protocol #3 used 60-Hz (high-frequency) and short-pulse duration. Twenty- and 60-Hz pre- and post-fatigue testing trains were delivered at the pulse duration used by the fatiguing trains and at 600-μs pulse duration. The 60-Hz testing trains were used to evaluate muscle fatigue. The 20-Hz:60-Hz peak force ratio was used as a measure of low-frequency fatigue. The results showed that Protocol #1 produced the least decline in peak force and force time integral in response to the fatiguing trains, muscle fatigue, and low-frequency fatigue when the pulse duration was maintained at the level used by the fatiguing trains. Interestingly, Protocol #2 produced the least muscle fatigue and there were no differences in the levels of low-frequency fatigue across protocols when a comparable motor unit population was tested using 600-μs pulse duration. The results suggest that if the frequency and intensity are kept constant during

FES, using the lowest frequency and longest pulse duration may minimize fatigue. However, if the intensity is increased as the muscle starts to fatigue, as is done in most current FES systems, using a medium-frequency may maximize performance.

2.2 Introduction

Functional electrical stimulation (FES) uses electrical stimulation to generate functional movements in individuals with upper motor neuron paresis [50, 48]. FES can help individuals with paralysis due to upper motor neuron dysfunctions such as spinal cord injury, cerebral palsy, and stroke to regain the ability to stand [77], walk [1, 41], and grasp objects [51]. FES has proven to be effective for lower and upper extremity rehabilitation in individuals with hemiplegia following stroke [64], and for providing exercise alternatives for tetraplegic individuals through FES-induced rowing [22, 83] and cycling [32, 38]. For effective task performance during FES, it is necessary to maintain the level of muscle force required for generation of the FES-elicited movement. During repetitive stimulation, however, skeletal muscle force output declines as the muscle fatigues. The problem of muscle fatigue is further compounded by the fact that paralyzed muscles show greater fatigability than healthy muscle [30, 31]. Muscle fatigue is an important factor limiting the clinical use of FES [62].

During repetitive electrical stimulation, stimulation frequency and intensity are two primary parameters that can be modulated to control skeletal muscle force. Although numerous combinations of frequency and intensity can be used to generate the required muscle force during FES, most clinical FES systems use the minimum frequency that can generate a fused tetanic contraction in the muscle being stimulated and vary the intensity to produce the desired force [80, 73, 79]. The stimulation intensity is further increased as the muscle fatigues [80, 73, 79]. The rationale for using low frequencies in FES is based on the premise that higher frequencies cause greater fatigue [7, 29, 11]. Although several previous studies have shown that fatigue is a function of the stimulation frequency or the number of pulses [7, 52, 29, 11], these studies have often ignored the effect of either the differences in initial peak force or stimulation intensities on muscle fatigue [10, 66]. Interestingly, Russ and colleagues [66] recently showed that increasing the frequency or number of pulses did not affect the amount of muscle fatigue produced during repetitive isometric contractions if the initial force produced by the stimulation trains was controlled [66]. Another study showed that intermittent high-frequency stimulation produced less fatigue than low-frequency repetitive stimulation in able-bodied and spinal cord injured subjects [54]. Thus, previous literature does not provide conclusive evidence about the isolated effect of stimulation frequency on muscle fatigue.

Only a few studies have investigated the relationship between stimulation intensity and muscle fatigue. Binder-Macleod and colleagues tested the rate and amount of fatigue during repetitive stimulation of the human quadriceps muscle with trains at stimulation amplitudes that produced 20%, 50%, and 80% MVIC forces and found less fatigue during repetitive stimulation at higher compared to lower stimulation amplitudes [10]. In contrast, Godfrey and colleagues [33] recently showed greater declines in force due to fatigue at high (supra-maximal) compared to low (sub-maximal) stimulation intensities [33]. Thus, we also do not know which stimulation intensity levels can help to minimize fatigue when used for repetitive stimulation during FES.

Because the stimulation frequency [7, 52, 29, 11], intensity [10, 33], and force generated in response to electrical stimulation [66] can effect the amount of muscle fatigue produced during repetitive stimulation, it is difficult to isolate the effects of stimulation frequency versus intensity on muscle fatigue while controlling for the force generated in response to electrical stimulation. However, for FES applications, because the targeted force is determined by the task-requirements, it may not be important to isolate the effects of frequency versus intensity on muscle fatigue and performance, but to determine the combination of frequency and intensity that can generate the targeted muscle force while simultaneously minimizing muscle fatigue. It has been hypothesized, but not systematically tested that the combination of the lowest frequency and highest intensity may minimize fatigue when used for repetitive stimulation [12]. No previous study has attempted to systematically investigate the combination of stimulations frequency and intensity that can minimize fatigue while producing the desired force level during repetitive stimulation.

The purpose of this study was to determine which combination of stimulation intensity and frequency produces the least decline in force during repetitive electrical stimulation, for the same initial peak force. Both the amplitude and the duration of the stimulus pulses can be varied to modulate the stimulation intensity during electrical stimulation. For this study, we used stimulus pulse duration to vary the intensity because it was easier to control and provided a more consistent force response from the muscle compared to stimulation amplitude [35, 55]. Specifically, we compared the percentage decline in quadriceps femoris isometric muscle force produced during repetitive stimulation with trains consisting of 3 different combinations of stimulation frequencies and pulse durations that produced the same initial targeted peak force: Protocol #1 used trains with 600-μs pulse duration (long-pulse duration) and the frequency was varied for each subject to generate the targeted force (low-frequency); Protocol #2 used 30-Hz trains (medium-frequency) and the pulse-duration was varied for each subject to generate the targeted force (medium-pulse duration); and Protocol #3 used 60-Hz trains (high-frequency) and the pulse-duration was varied for each subject to generate the targeted force (short-pulse duration). The terms 'medium-pulse duration' and 'short-pulse duration' are operational definitions for the pulse durations used to generate the targeted force using 30-Hz and 60-Hz trains for Protocols #2 and #3, respectively. In addition, the aim of the study was to compare muscle performance during repetitive stimulation using the combinations of long-pulse duration and low-frequency (Protocol #1) versus short-pulse duration and high-frequency (Protocol #3). The third combination of medium-frequency and medium-pulse duration (Protocol #2) was introduced to include frequencies and intensities in the mid-range of the steep rising portions of the force-frequency and force-pulse duration curves, respectively. In addition, the frequency and pulse duration used during Protocol #2 are similar to the parameters commonly used in clinical FES systems [73, 24, 41] .

2.3 Materials and Methods

2.3.1 Subjects

Twelve healthy individuals (6 males + 6 females) aged 22-30 years participated in the study. The subjects had no history of lower extremity orthopedic, neurological, or vascular problems. The subjects were requested to refrain from strenuous exercise for at least 48 hours before the testing sessions. The subjects signed informed consent forms approved by the Human Subjects Review Board of the University of Delaware (See Appendix A).

2.3.2 Apparatus and Setup

The subjects were seated on an electromechanical force dynamometer (Kin-Com III 500-11, Chattecx Corp., Chattanooga, TN) with the back supported, hips flexed approximately to 75^o, and knees flexed at 90^o (Figure 2.1). Velcro straps were used to stabilize the subjects' upper trunk, waist, and thigh. Each subject's ankle was stabilized with a strap placed approximately 2 inches proximal to the lateral malleolus. The isometric force output of the quadriceps femoris muscle was recorded via a force transducer placed against the anterior aspect of the leg. The subjects could see a representation of the force recorded by the KinCom force transducer on a display screen.

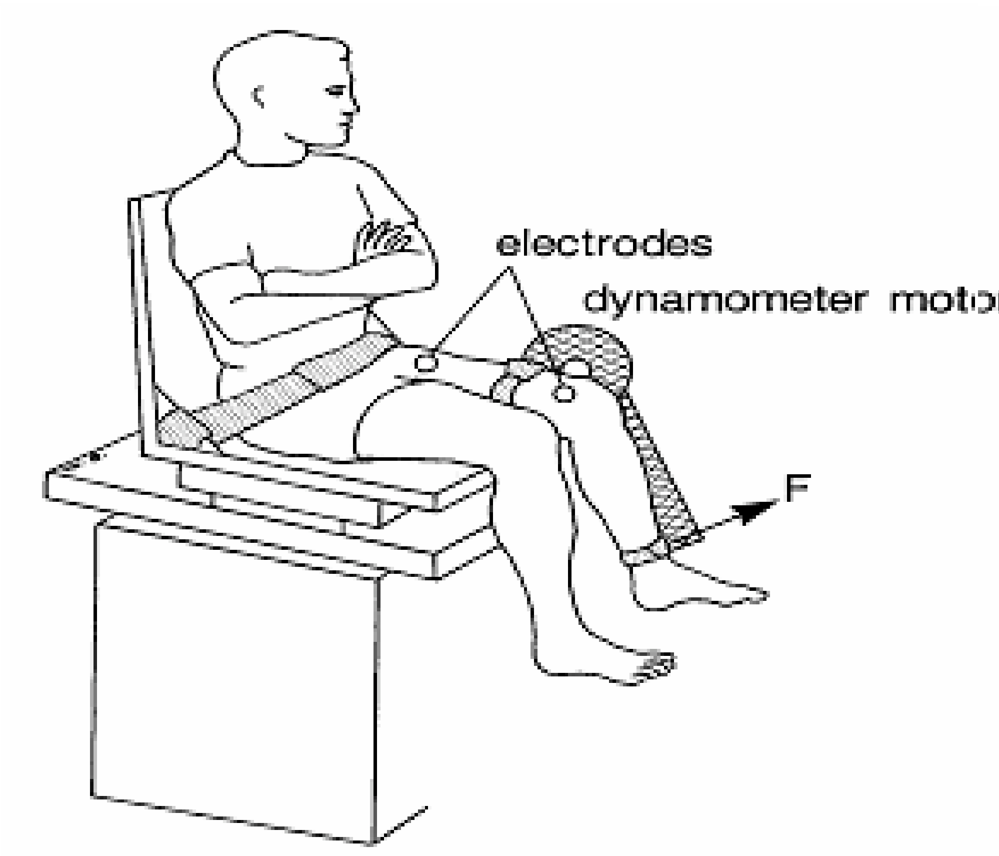


Figure 2.1: Schematic representation of the experimental setup used for testing.

Electrical stimulation was delivered via two self-adhesive surface electrodes (Versa-Stim, 76-mm x 127-mm, CONMED Corp., New York, USA). The proximal electrode (cathode) was placed over the upper thigh, covering the proximal portion of the rectus femoris and vastus lateralis muscles. The distal electrode (anode) was placed over the lower aspect of the thigh, covering the vastus medialis and distal portion of the rectus femoris. A Grass S8800 (Grass Instrument Company, Quincy, MA) stimulator with a SIU8T stimulus isolation unit was used to deliver the electrical stimulation. A personal computer equipped with a PCI-6024E data acquisition board and a PCI-6602 counter-timer board was used. A custom-made switch was connected in series with the stimulator to control the pulse duration. A custom-written LabVIEW program was used for data-acquisition, and to control the timing and the duration of the pulses.

2.3.3 Experimental Procedure

Each subject participated in 4 sessions, with a minimum of 48 hours separating consecutive sessions. At the start of the first session, subjects received an overview of the testing procedures, signed the informed consent form, and were trained to perform the maximal voluntary isometric contraction (MVIC) test. They were seated on the KinCom, surface electrodes were attached to the skin of the subjects' thigh and tested for appropriate placement (Figure 2.2). Next, the MVIC force was recorded using the burst superimposition technique [68]. During the MVIC test, the subjects attempted to produce as much knee extension force as possible. During the maximal voluntary contraction, an electrical stimulation train (amplitude: 130 mV, frequency: 100-Hz, pulse duration: 600-μs) was delivered to the quadriceps femoris muscle. This stimulation train or 'burst' was superimposed on the volitional contraction to ensure that the subjects were truly generating maximal force. If the electrical stimulation train increased the force by $\leq$10%, the subject's MVIC was recorded. If the electrical stimulation train increased the subject's force output by

$> 10\%$, the MVIC test was repeated after a 10-minute rest. If the subject failed to elicit a true MVIC within 3 repetitions, they were not tested on that day. For each subject, the MVIC value measured during the 1^{st}session was used to set the stimulation amplitude for all 4 sessions.

The remaining 3 sessions involved fatigue testing. The order of testing of the 3 fatigue protocols was randomized across subjects. Only one protocol was tested on each day. Each testing session consisted of 'fatiguing trains' and pre- and post-fatigue 'testing trains'. The 'fatiguing trains', consisting of stimulation trains of 3 different combinations of frequency and pulse duration repetitively delivered at the rate of 1 train every second, were used to fatigue the muscle and to assess the muscle's performance during the fatigue test. The 'testing trains', consisting of stimulation trains of 60-Hz and 20-Hz frequency and 2 different pulse durations, were delivered before (pre-fatigue) and after (post-fatigue) the fatiguing trains. The testing trains measured the decline in the force generating ability of the muscle. All fatiguing and testing trains were 300-ms long. However, due to differences in frequencies between the trains used for testing, the fatiguing trains for the each of the 3 protocols contained different number of pulses.

The following procedures were followed during the 3 fatigue sessions (Figure 2.2):

Set stimulation amplitude to generate 50% MVIC peak force. During each testing session, after applying the electrodes, the stimulation amplitude was set to produce 50% of the subject's MVIC force using 300-ms long, 60-Hz trains with 600-μs pulse duration. The 50% MVIC amplitude was used because it allowed a range of frequencies and pulse durations to be used to generate the target force of 20% MVIC.

Potentiation. Eleven trains (770-ms train duration, 14-Hz frequency, 600-μs pulse duration) were delivered with a 5-second rest time between trains to potentiate

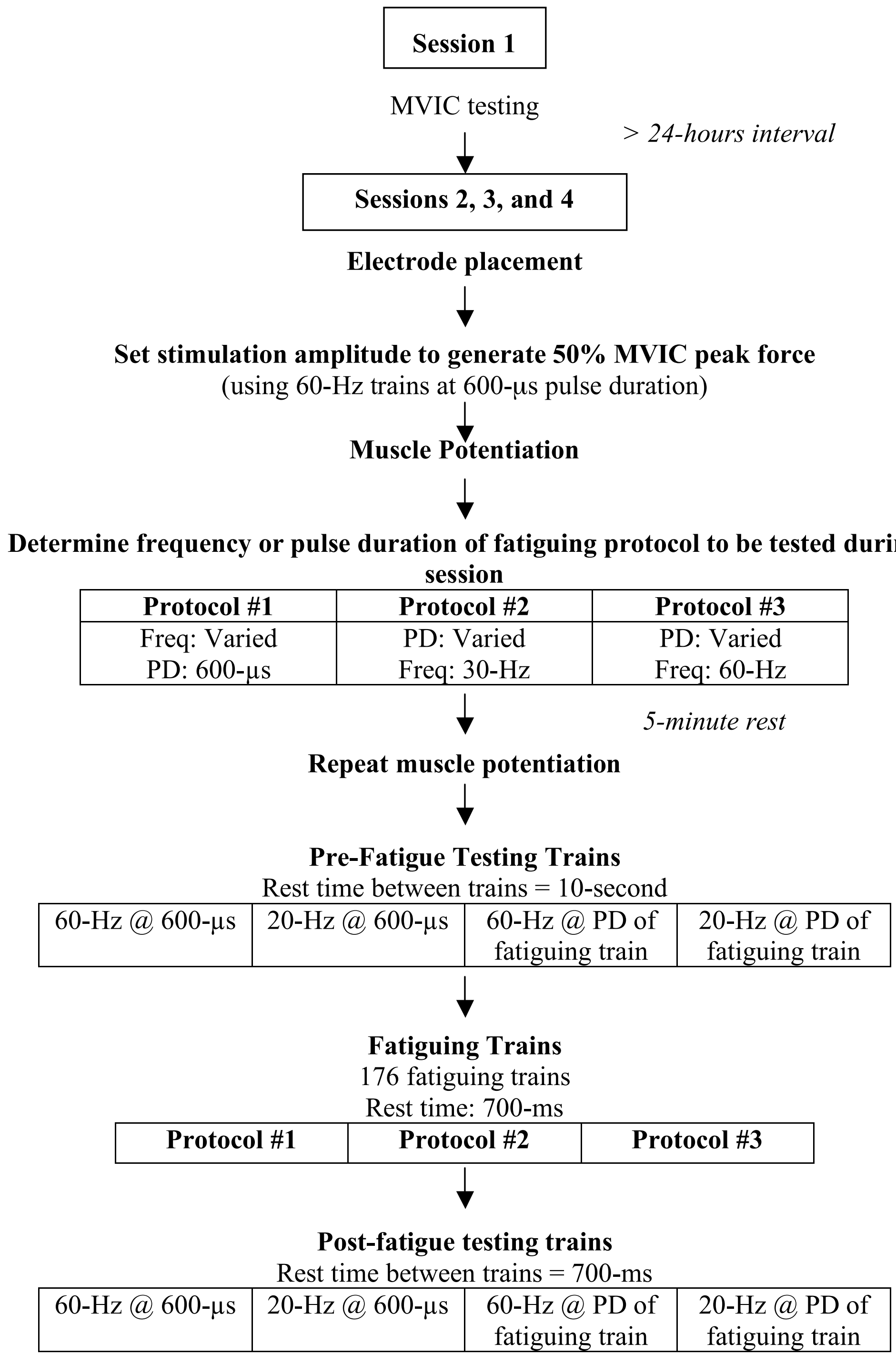


Figure 2.2: Flowchart showing the experimental protocol for the 4 sessions. Please see text for details.

the muscle [9].

Set frequency and pulse duration to generate 20% MVIC target peak force. Either the stimulation pulse duration or frequency of a 300-ms long train was varied to generate peak force equal to 20% of the subject's MVIC. The first trains of each of the 3 fatigue protocols generated 20% MVIC peak force.

Potentiation. Potentiation was repeated before the fatigue test to prevent the effects of potentiation from interacting with fatigue [9].

Pre-Fatigue Testing Trains. Sixty-Hz and 20-Hz trains were delivered at the same pulse duration as that used for the fatiguing trains for that session and also at 600-μs pulse duration.

Fatiguing Trains. After the potentiation and pre-fatigue testing trains had been delivered, 176 fatiguing trains were delivered at the rate of 1 train every second (train duration: 300-ms; rest time: 700-ms). Three different combinations of frequency and pulse duration were used during the 3 different fatigue protocols, as follows:

Protocol #1 - **Low-frequency, long-pulse duration (600-μs):** The pulse duration of a 300-ms long train was fixed at 600-μs and the frequency was set to produce 20% MVIC peak force.

Protocol #2 - **Medium-frequency (30-Hz), medium-pulse duration:** The frequency of a 300-ms long train was fixed at 30-Hz and the pulse duration was set to produce 20% MVIC peak force.

Protocol #3- **High-frequency (60-Hz), short-pulse duration:** The frequency of a 300-ms long train was fixed at 60-Hz and the pulse duration was set to produce 20% MVIC peak force.

Post-fatigue testing trains. At the end of the fatiguing trains, post-fatigue testing trains were delivered at the rate of 1 train every second to maintain the state of muscle fatigue.

2.4 Data Analyses

The decline in force generated in response to the fatiguing trains for each protocol was used as a measure of the 'muscle's performance' or the muscle's ability to maintain force output in response to the fatiguing trains. The % declines in peak force and force time integral between the first and last fatiguing train were calculated for each fatigue protocol. In addition, the sum of peak forces and force time integrals produced in response to $1^{st}$to 60^{th}, 61^{st} to 120^{th}, 121^{st} to 176^{th} and $1^{st}$to 176^{th} fatiguing trains were additional measures of muscle performance.

Because muscle fatigue was the primary focus of our study, it was important to define muscle fatigue in the context of this study. Muscle fatigue is a decline in the force generating ability of the muscle due to recent activity [78, 66]. We used the % decline in peak force between pre- and post-fatigue 60-Hz testing trains as a measure of the decline in the force-generating ability [78, 65]. The decline in peak force of the 60-Hz testing trains at the same pulse duration as the fatiguing trains provided a measure of the amount of muscle fatigue produced in the population of motor units recruited by the fatiguing trains during each fatiguing protocol. The decline in peak force of the 60-Hz testing trains at the 600-μs pulse duration provided a measure of the muscle fatigue produced within a comparable number of motor units as were recruited during the protocol that used the longest pulse duration (i.e., Protocol #1). In addition, the ratio of peak forces produced in response to 20-Hz versus 60-Hz testing trains (20-Hz: 60-Hz peak force ratio) was used as a measure of low-frequency fatigue [78, 65]. The 20-Hz:60-Hz peak force ratios were calculated at the beginning (pre-fatigue) and end (post-fatigue) of the fatigue protocols both for testing trains at the pulse duration of the fatiguing trains and at the 600-μs pulse duration .

2.4.1 Statistical Analyses

The percent decline in peak forces and force time integrals from the $1^{st}$to last fatiguing trains, sum of peak forces and force time integrals produced by 1^{st} to 176^{th}, $1^{st}$to 60^{th}, 61^{st} to 120^{th}, 121^{st} to 176^{th} fatiguing trains, % decline in peak force between pre- and post-fatigue 60-Hz testing trains at the pulse duration of the fatiguing trains, and the % decline in 60-Hz testing trains at 600-μs pulse duration were compared using one-way repeated measures ANOVAs. Pair-wise post-hoc comparisons using least squared difference (LSD) were performed only if the ANOVA showed significant differences. The pre- and post-fatigue 20-Hz:60-Hz peak force ratios for the 3 fatigue protocols were compared using 2-way (protocol X fatigue) repeated measures ANOVAs. In addition, peak forces produced in response to the 1^{st}fatiguing train of each protocol were compared using a repeated-measures one-way ANOVA to determine if the fatigue protocols produced similar initial peak forces. The significance level was set at $p \leq 0.05$.

2.5 Results

Data were collected on 12 healthy individuals (6 males + 6 females). All 12 subjects were able to successfully complete the MVIC testing during the 1^{st}session in 1-2 attempts. Table 2.1 provides detailed information about the subjects' age, gender, MVIC forces, and the stimulation parameters of the fatiguing trains (See Table 2.1). The frequency used for repetitive stimulation during Protocol #1 was 11.5±1.2-Hz. The pulse durations used for repetitive stimulation during Protocols #2 and #3 were 150±22-μs and 131±24-μs respectively. The peak forces produced in response to the fatiguing trains during the 3 fatigue protocols for a representative subject are shown in Figure 2.3. It is notable that though the 1^{st}fatiguing trains of the 3 protocols produced similar peak forces, the last fatiguing train of Protocol #1 produced the largest and the last fatiguing train of Protocol #3 produced the least peak force for this subject (Figure 2.3). The repeated measures ANOVA showed no

Table 2.1: The gender (M=male, F=female), age, and maximal voluntary isometric contraction (MVIC) force for individual subjects. The frequency (freq) and pulse durations (PD) used for repetitive stimulation during the 3 fatigue protocols are also listed.

Subject	Gender	Age	MVIC	Protocol #1		Protocol #2		Protocol #3	
		(years)	(N)	PD (μs)	Freq (Hz)	PD (μs)	Freq (Hz)	PD (μs)	Freq (Hz)
1	M	22	1012	600	11.8	148	30	138	60
2	M	26	881	600	11.7	178	30	164	60
3	M	27	1117	600	14	112	30	106	60
4	M	24	1231	600	10.8	182	30	166	60
5	M	24	1335	600	12	155	30	139	60
6	M	30	1277	600	13.1	180	30	78	60
7	F	23	449	600	10	123	30	121	60
8	F	24	798	600	10.6	148	30	137	60
9	F	23	775	600	12.4	147	30	128	60
10	F	22	969	600	10.6	141	30	129	60
11	F	27	549	600	10.4	147	30	135	60
12	F	26	1000	600	10.3	145	30	137	60
Average		***24.8***	***949.4***	***600***	***11.5***	***150***	***30***	***131***	***60***
Std. Dev.		***2.4***	***276.2***	***-***	***1.2***	***22***	***-***	***24***	***-***

significant difference in the initial peak force produced by the 3 fatigue protocols (F=1.09; p= 0.35). The average peak forces in response to the 1^{st}fatiguing trains were 21.2±1.7 % MVIC, 21.5±2.2 % MVIC, and 22.3±2.2 % MVIC for Protocols #1, #2, and #3 respectively.

2.5.1 Force Responses to the Fatiguing Trains

There were significant differences in % decline in peak force (F=30.08; p<0.01) and force time integral (F=16.83; p<0.01) between the first and last fatiguing trains among the 3 protocols (Figure 2.4). Protocol #1, consisting of fatiguing trains with long (600-μs) pulse duration and low-frequency, produced the smallest % decline in peak force (31.3±9.4%) and Protocol #3, consisting of fatiguing trains with high-frequency (60-Hz) and short-pulse duration, produced the largest % decline in peak force (51.3±7.5%) (Figure 2.4). The % declines in force time integrals of the fatiguing trains showed the same trend (Figure 2.4).

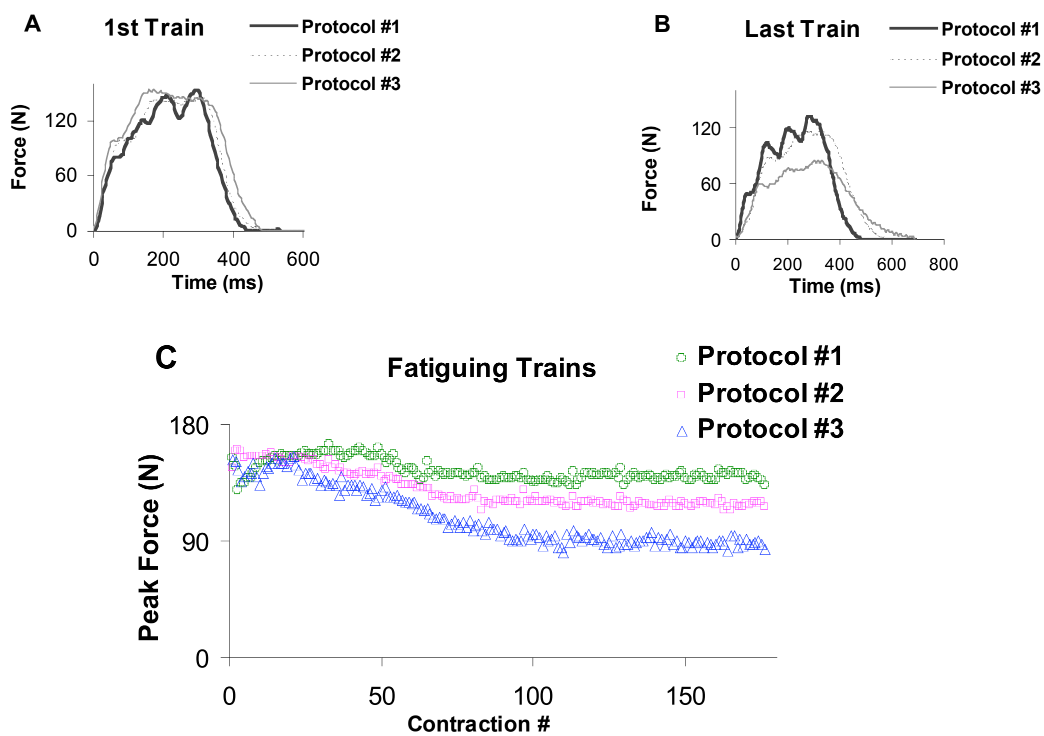


Figure 2.3: Raw force profiles of the first (A) and last (B) fatiguing trains of a representative subject for the 3 fatigue protocols. The peak forces produced in response to each fatiguing train during the 3 fatigue protocols for a representative subject(C).

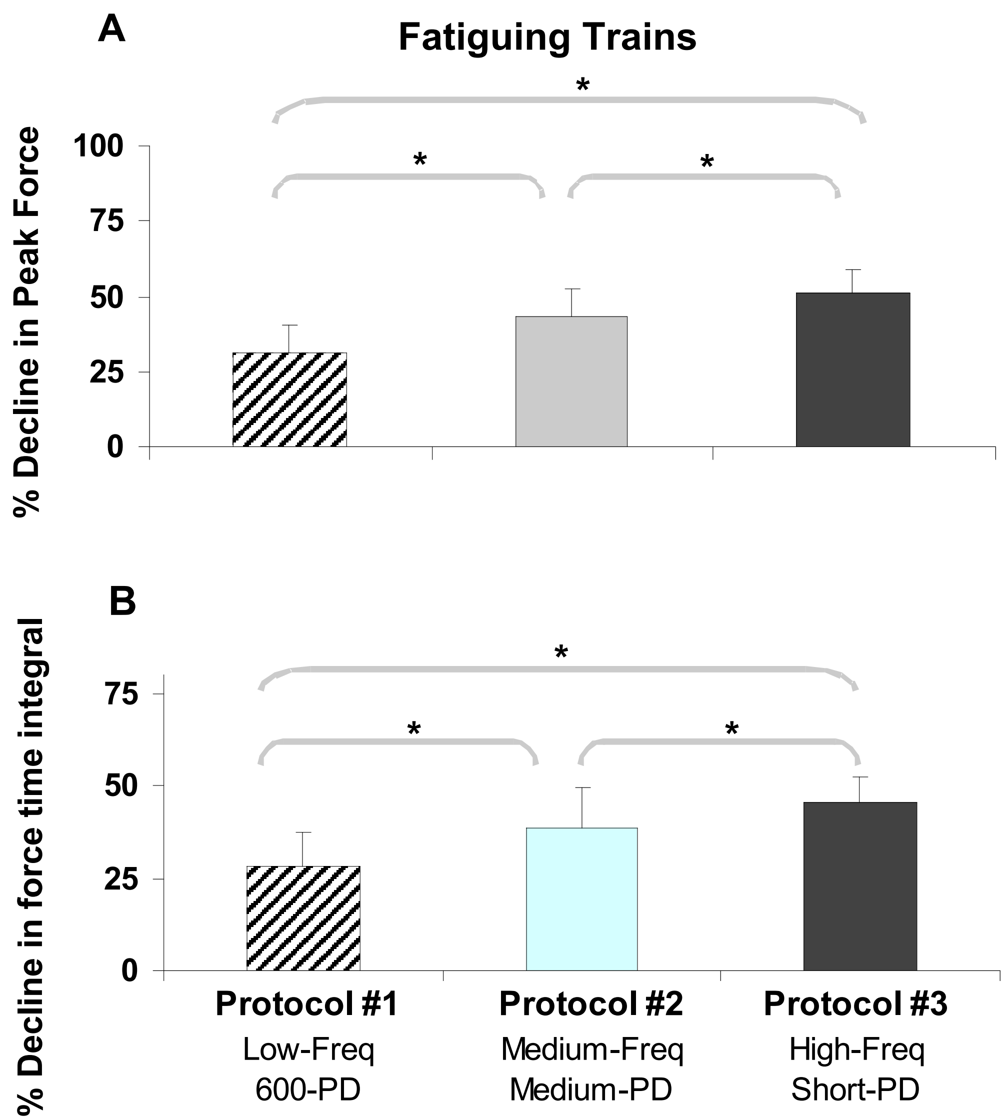


Figure 2.4: The percent decline in peak force (A) and force time integrals (B) produced between the first to the last fatiguing trains during the 3 fatigue protocols. PD = pulse-duration. * Significant differences between protocols ($p \leq 0.01$).

Protocol #1 produced the largest sum of peak forces and Protocol #3 produced the smallest sum of peak forces in response to the 60^{th} to 120^{th}, 120^{th} to 176^{th}, and $1^{st}$to 176^{th} fatiguing trains ($p<0.05$) (Figure 2.5). There was no significant difference in the sum of peak forces produced in response to the 1^{st} to 60^{th} fatiguing trains of the 3 fatigue protocols (Figure 2.5). Protocol #3 produced the largest sum of force time integrals and Protocol #1 produced the smallest sum of force time integrals in response to the $1^{st}$to 60^{th} fatiguing trains ($p<0.05$) (Figure 2.5). There were no significant differences in the sum of force time integrals produced in response to fatiguing trains during the remaining stages of the 3 fatigue protocols (Figure 2.5).

2.5.2 Force Responses of Testing Trains

Protocol #1 produced the smallest decline in peak force (21.5±9.5%), Protocol #2 produced an intermediate decline (27.4±8.2%), and Protocol #3 produced the largest decline (46.1±6.7%) in peak force in response to the 60-Hz testing train at the same pulse duration as the fatiguing trains ($F=77.23$; $p<0.01$) (Figure 2.6). Interestingly, for the 60-Hz testing trains at 600-μs pulse duration, Protocol #2, consisting of medium-frequency and medium-pulse duration fatiguing trains, produced significantly smaller declines in peak force (14.7±7%) than Protocol #1 (23.6±8.3%) or Protocol #3 (22.4±10.3%) ($F=6.40$; $p=0.01$) (Figure 2.6).

For testing trains at the pulse duration of the fatiguing trains, the 2-way repeated measures ANOVA (protocol X fatigue) showed significant effects of protocol ($F= 32.00$; $p<0.01$) and fatigue ($F=308.93$; $p<0.01$) on the 20-Hz:60-Hz peak force ratios (Figure 2.7). There was a significant interaction between protocol and fatigue ($F=35.32$; $p<0.01$). There were no significant differences in the pre-fatigue 20-Hz:60-Hz peak force ratios among the 3 protocols. Protocol #1 showed the largest post-fatigue 20-Hz:60-Hz ratio (0.61±0.07), and Protocol #3 showed the smallest ratio(0.37±0.11) (Figure 2.7). For testing trains at 600-s pulse duration, the 2-way

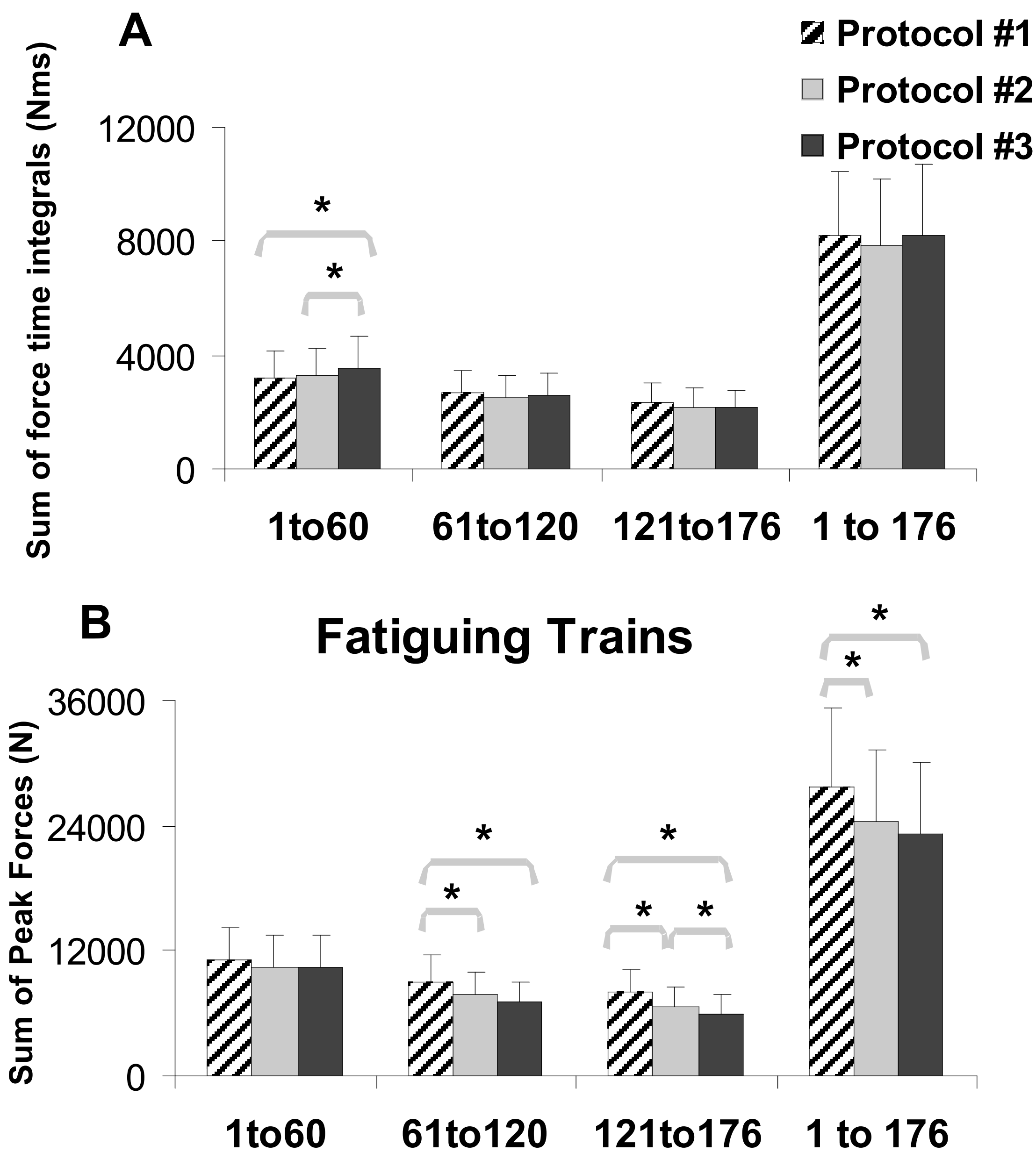


Figure 2.5: Sum of force time integrals (A) and peak forces (B) produced in response to the fatiguing trains. Peak forces and force time integrals were summated across the $1^{st}$to 60^{th}, 60^{th} to 120^{th}, 120^{th} to 176^{th}, and $1^{st}$to 176^{th} contractions. *Significant difference between fatigue protocols ($p \leq 0.05$).

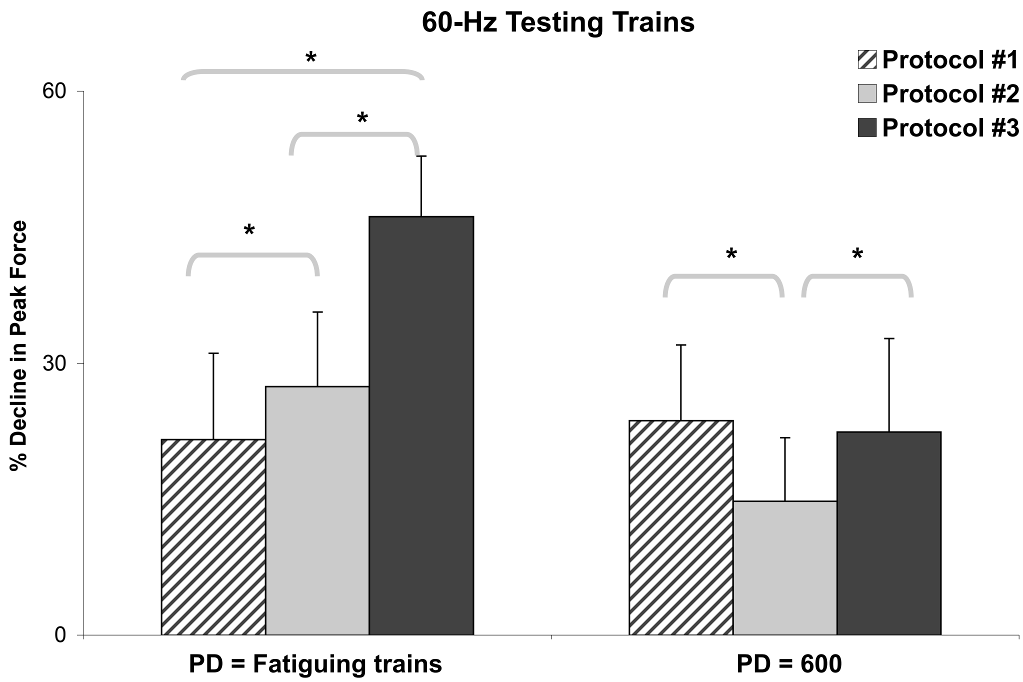


Figure 2.6: The % decline in peak forces between pre- and post-fatigue 60-Hz testing trains at the same pulse duration as used for the fatiguing trains (left) and at 600-μs pulse duration (PD) (right). * Significant difference between protocols ($p \leq 0.01$).

ANOVA showed a significant effect of fatigue ($F=66.95$; $p<0.01$), no significant effect of protocol ($F=0.03$; $p=0.97$), and no significant interaction between the effects of protocol and fatigue ($F=2.62$; $p=0.09$) (Figure 2.7).

2.6 Discussion

Our study compared the performance and muscle fatigue produced during repetitive stimulation using 3 different combinations of frequencies and pulse durations, and found that Protocol #1 produced the least muscle fatigue in the motor units recruited by the fatiguing trains (Figure 2.6). Of the 3 protocols tested, Protocol #1 used the lowest frequency (11.5 ± 1.2-Hz) and highest level of recruitment (600-μs pulse duration) to fatigue the muscle. Because Protocol #1 recruited the most motor units and all 3 protocols generated the same target peak force, the least amount of force was generated by each active muscle fiber during Protocol #1. Because ATP utilization by actin-myosin ATPase is proportional to force generation [70, 13, 59], we suggest that Protocol #1 resulted in the least ATP utilization by the actin-myosin ATPase per muscle fiber. In addition to the actin-myosin ATPase, the $Ca^{2+} - ATPase$ and $Na^{+} - K^{+}ATPase$ reactions in response to each action potential contribute to ATP utilization during muscle activation [37, 5, 36, 26]. Because Protocol #1 used the fewest number of pulses and generated the fewest action potentials, the least ATP utilization by the $Ca^{2+} - ATPase$ and $Na^{+} - K^{+}ATPase$ reactions also occurred during Protocol #1 [26, 52]. Because metabolic demand is related to muscle fatigue [20, 67, 81], we believe that Protocol #1 produced the least fatigue in the motor unit population recruited by the fatiguing trains because the lowest ATP consumption and lowest metabolic demand was placed on each muscle fiber during Protocol #1 among the 3 protocols tested.

In a recent study, Godfrey and colleagues [33] studied the effects of stimulation intensity on force production of thenar muscles in patients with spinal cord injury. In contrast to our present findings, Godfrey and colleagues [33] found greater

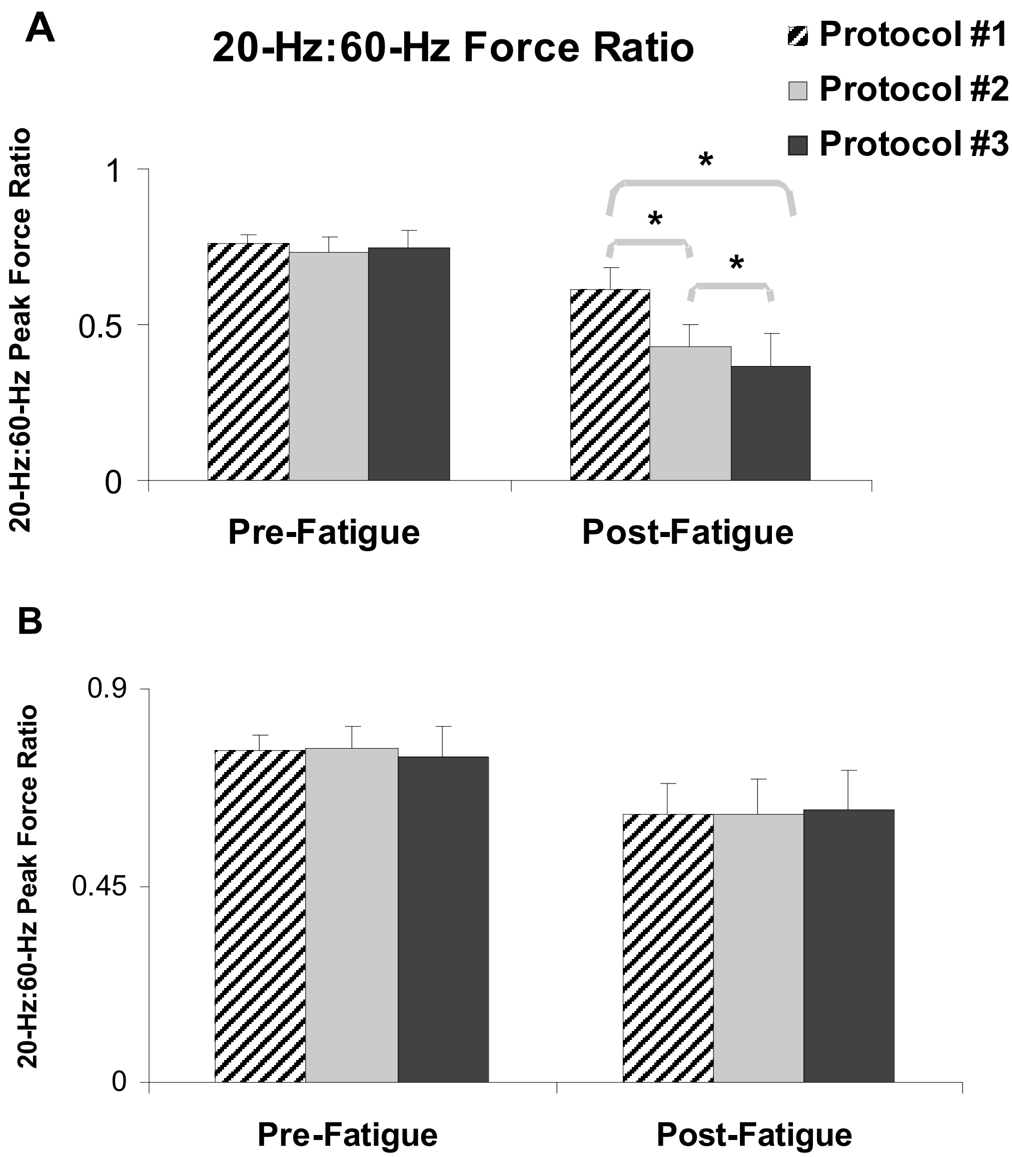


Figure 2.7: Ratios of peak forces produced in response to the pre- and post-fatigue 20-Hz versus 60-Hz testing trains (20-Hz:60-Hz peak force ratio) at the same pulse duration as used for the fatiguing trains (A) and at the 600-μs pulse duration (B) during the 3 fatigue protocols. *Significant differences between fatigue protocols ($p \leq 0.05$). All pre-fatigue 20-Hz:60-Hz peak force ratios were significantly different from the post-fatigue ratios ($p \leq 0.05$).

fatigue during stimulation at supra- compared to sub-maximal intensities. We believe that the differences in the findings of Godfrey and colleagues [33] versus our present study were due to methodological differences. Because Godfrey and colleagues [33] delivered both supra- and sub-maximal stimulation at the same frequency (40-Hz), different force levels were generated at the two intensities. The higher forces generated at supra- versus sub-maximal intensities probably contributed to the greater muscle fatigue observed by Godfrey and colleagues [33] at the supra-maximal intensities [66]. However, the differences between the results of our present study and those of Godfrey and colleagues [33] may also be due to differences in the subject populations and the muscle tested.

Protocol #1 also produced the least low-frequency fatigue in the motor units recruited by the fatiguing trains (Figure 2.7). Low-frequency fatigue is the result of impairment in excitation-contraction coupling that is thought to result from increased levels of intracellular Ca^{2+} during muscle activation [17, 18, 82, 25]. Intracellular Ca^{2+} concentrations have been shown to be directly related to the stimulation frequency[17]. The low-frequency used during Protocol #1 would, therefore, result in the lowest levels of intracellular Ca^{2+} and the least low-frequency fatigue among the 3 protocols [17, 18, 82].

Although Protocol #1 used low frequencies, whose performance would be most impaired by the effects of low-frequency fatigue among the 3 protocols, Protocol #1 produced the smallest decline in peak force and force time integral in response to the fatiguing trains (Figure 2.4) . Because muscle performance is a function of both the amount of muscle fatigue and the degree of low-frequency fatigue, we can infer that the impairment in force generation caused by low-frequency fatigue in response to the trains of Protocol #1 was outweighed by the lower levels of impairments in force generating ability during Protocol #1 compared to Protocols #2 and #3. In contrast to our present findings, Matsunaga and colleagues [54] showed lesser decline in force during repetitive stimulation at high- (100-Hz) versus low- (20-Hz) frequency activation. Compared to our study, the fatigue protocols tested by Matsunaga and colleagues [54] were of much longer durations (60-minutes versus 180-seconds in our study), used much lower duty cycles (1:15, 1:30 and 1:60 versus 1:2.3 in our study), and produced smaller percent declines in peak force (22.3±15.1% at 100-Hz versus 51.3±7.5% at 60-Hz in our study) [54]. Because muscle performance is a function of the extent of force fatigue and low-frequency fatigue produced by the fatiguing trains in the motor unit population recruited by the fatiguing trains, we believe that the results of Matsunaga and colleagues [54] can be explained by greater low-frequency fatigue and lesser muscle fatigue than presently observed in our study. That is, even if greater muscle fatigue was produced at 100-Hz than at 20-Hz in their study, low-frequency fatigue may have markedly compromised the muscles' performance at 20-Hz and resulted in the poorer performance at 20- versus 100-Hz.

The frequencies used during Protocols #1, #2, and #3 in our study were in the low, middle, and high ranges of the force-frequency curves, respectively. However, surprisingly, the pulse durations used during Protocols #2 (150±22-μs) and #3 (131±24-μs) only varied by 19-μs on average. A possible reason could be that the pulse durations used for Protocols #2 and #3 were in the steep rising part of the force versus pulse-durations curves. Thus, our results showed that although the difference in frequencies between Protocols #2 and #3 was relatively large (30-Hz versus 60-Hz), a relatively small difference in pulse-duration between Protocol #3 and Protocol #2 enabled the targeted 20% MVIC force to be reached for both the protocols.

The present findings could have implications for developing strategies for optimal activation of skeletal muscle during FES. FES is used to generate functional movements in patients with upper motor neuron lesions, such as spinal cord injury, hemiplegia following stroke, and cerebral palsy. This study represents the first step in a project whose long-term aim is to develop electrical stimulation strategies that can maximize FES performance. Initial testing on healthy individuals has helped us to identify hypotheses, which can then be tested on paralyzed muscles using fewer experimental sessions. Interestingly, consistent with out present findings, recent studies showed that for healthy subjects and subjects with spinal cord injury, starting at low- and later switching to high-frequencies produced better performance during repetitive non-isometric contractions than stimulation using either a low- or high-frequency alone [45, 46]. Starting repetitive stimulation with low-frequencies produced less muscle fatigue and switching to a higher stimulation frequency and allowed the stimulation to overcome the effects of low-frequency fatigue [45, 46]. Future studies will need to identify the best frequency and intensity of the initial trains as well as the strategies for modulation of frequency and intensity of the subsequent trains to maximize muscle performance during FES.

An interesting finding of this study was the difference in the responses to the testing trains when the pulse duration was maintained at the levels used to fatigue the muscle versus when a 600-μs pulse duration was used (See Figures 2.6 and 2.7). During Protocols #2 and #3, the post-fatigue testing trains at 600-μs pulse duration activated previously unrecruited motor units. The responses to the testing trains at 600-μs pulse duration were therefore the sum of the forces produced by the recruited and previously unrecruited motor units. Protocol #3 showed the most and Protocol #1 the least muscle fatigue and low-frequency fatigue when the pulse duration was maintained at the level used to fatigue the muscle (Figure 2.6). Thus, during FES applications, if the frequency and pulse duration are held constant during repetitive stimulation, using the lowest frequency and longest pulse duration may maximize performance. However, Protocols #3 and #1 showed comparable amounts of muscle fatigue and Protocol #2 showed the least muscle fatigue when tested at the 600-μs pulse duration (Figure 2.6). In addition, in response to testing trains at 600-μs pulse duration, there were no differences in the overall levels of low-frequency fatigue among the 3 protocols (Figure 2.7). This is an important finding because most current FES systems deliver a constant frequency and increase the intensity to increase muscle force output as the muscle fatigues [73, 24, 41]. Thus, for FES applications where intensity is increased as the muscle fatigues, a 'medium' frequency, similar to the frequency used in Protocol #2 of our study may maximize performance. The combination of frequency and pulse duration that minimizes muscle fatigue during FES may depend on whether or not modulation of frequency or intensity will be used as strategies during repetitive stimulation.

2.7 Conclusions

The present findings support the hypothesis that when the same initial peak force is generated using different combinations of frequency and pulse duration, and the frequency and pulse duration are kept constant throughout repetitive stimulation, repetitive stimulation with a long-pulse duration (600-μs) and low-frequency (11.5$\pm$1.2-Hz) (Protocol #1) would maximize performance by minimizing muscle fatigue. Interestingly, repetitive stimulation with a medium-frequency (30-Hz) and medium-pulse duration (150$\pm$22-μs) (Protocol #2) produced the least muscle fatigue when comparable motor unit populations were tested across protocols. These findings may have important implications for designing optimal stimulation strategies to use during FES. Specifically, the present results should help to design future studies involving patient populations where complex stimulation strategies that modulate both the frequency and pulse durations will be tested.

Chapter 3

EFFECTS OF STIMULATION FREQUENCY- VERSUS INTENSITY-MODULATION ON MUSCLE FATIGUE

3.1 Abstract

During functional electrical stimulation (FES), both the frequency and intensity can be increased to increase muscle force output and counteract the effects of muscle fatigue. Most current FES systems, however, deliver a constant frequency and only vary the stimulation intensity to control muscle force. This study compared muscle performance and fatigue produced during repetitive electrical stimulation using 3 different strategies - #1: constant pulse-duration and stepwise increases in frequency (frequency-modulation); #2: constant frequency and stepwise increases in pulse-duration (pulse-duration-modulation, and #3: constant frequency and pulse-duration (no-modulation). Surface electrical stimulation was delivered to the quadriceps femoris muscles of 12 healthy individuals and isometric forces were recorded. The percent change in the peak forces and force-time integrals generated between the 1^{st} and the last fatiguing trains were each used to assess muscle performance. The results showed that frequency-modulation showed better performance for both peak forces and force-time integrals in response to the fatiguing trains than pulse-duration-modulation. In addition, both of the modulation protocols showed better performance in response to the fatiguing trains than the no-modulation protocol. Although frequency-modulation is not commonly used during FES, clinicians should consider this strategy to improve muscle performance.

3.2 Introduction

The central nervous system achieves a precise and task-specific control of skeletal muscle forces by controlling the number of activated motor units (recruitment) and the firing frequency of the activated motor units (rate-coding) [44, 43]. In individuals with paralysis due to upper motor neuron dysfunction, functional electrical stimulation (FES) is used to substitute for the loss of voluntary motor control to enable patients to stand, walk, and grasp objects [50, 48]. Analogous to the two mechanisms of recruitment and rate-coding, stimulation intensity and frequency can be modulated to control muscle force output during FES. Although FES has immense potential application, it has not gained widespread popularity due to the limitations in current FES systems [62, 15, 58]. An important limitation that discourages FES users is the rapid onset of muscle fatigue [14, 2, 53, 60, 62]. Muscle fatigue may impede efficient task performance during FES by limiting the number of steps that FES users can perform or the number of minutes that FES users can stand or grasp an object [14, 2, 53, 60, 62].

Stimulation trains of different combinations of frequency and intensity can be used to generate the muscle force required to perform a functional task during FES. However, with repetitive activation, the muscle will fatigue and an increase in either the frequency or the intensity of stimulation will be required to enable the targeted muscle force to be maintained. Interestingly, although both the stimulation intensity and frequency can be modulated, most current FES systems deliver a constant frequency and only increase the stimulation intensity to increase force output as the muscle fatigues [57, 61, 73, 24]. Previous studies on animal muscles show that compared to modulating either the pulse-duration or frequency, a simultaneous modulation of both stimulation pulse-duration and frequency produces improved control of isometric torque during FES [19, 49]. However, no previous studies on human muscles have systematically compared the effects of increasing stimulation frequency versus stimulation intensity on the muscle fatigue produced during repetitive electrical stimulation. The purpose of this study, therefore, was to compare the muscle fatigue and performance during repetitive stimulation using trains consisting of #1: constant pulse-duration and stepwise increases in frequency (frequency-modulation); #2: constant-frequency and stepwise increases in pulse-duration (pulse-duration-modulation); and #3: constant-frequency and constant-pulse-duration (no-modulation).

During FES, single pulses are grouped together to form stimulation trains. The stimulation frequency within each train can be controlled by varying the inter-pulse interval. The stimulation intensity can be controlled by varying either the amplitude or the duration of each pulse within a train. In this study, we kept the stimulation amplitude constant during each testing session and varied pulse-duration to modulate the stimulation intensity. Previous studies have shown that modulating the pulse-duration to control intensity requires a smaller charge per stimulus to produce a particular force and allows a greater selectivity of recruitment thresholds compared to modulating the pulse-amplitude [35, 55].

3.3 Materials and Methods

3.3.1 Subjects

Twelve healthy individuals (6 males + 6 females) aged 22-30 years with no history of lower extremity orthopedic, neurological, or vascular problems participated in the study. The subjects were requested to refrain from strenuous exercise for at least 48 hours before testing. The subjects were informed about the testing procedures and signed informed consent forms approved by the Human Subjects Review Board of the University of Delaware (Appendix A).

3.3.2 Apparatus and Setup

The subject was seated on an electromechanical force dynamometer (KinCom III 500-11, Chattecx Corp., Chattanooga, TN), with the back supported, hips flexed to approximately 75^o, and knees flexed at 90^o. Velcro straps were used to stabilize the subject's upper trunk, waist, and thigh. The subject's ankle was stabilized with a strap, placed approximately 2 inches proximal to the lateral malleolus. The isometric force output of the quadriceps femoris muscle was recorded via a force transducer placed against the anterior aspect of the leg, about 2 inches proximal to the lateral malleolus. The subject could see a representation of the force recorded by the KinCom force transducer on a display screen.

Electrical stimulation was delivered via two self-adhesive surface electrodes (Versa-Stim, CONMED Corp., New York, USA, 76-mm x 127-mm). The proximal electrode was placed over the upper thigh, covering the proximal portion of the rectus femoris and vastus lateralis muscles. The distal electrode was placed over the lower aspect of the thigh, covering the vastus medialis and distal portion of the rectus femoris. A Grass S8800 (Grass Instrument Company, Quincy, MA) stimulator with a SIU8T stimulus isolation unit was used to deliver the electrical stimulation. A personal computer equipped with a PCI-6024E data acquisition board and a PCI-6602 counter-timer board was used for data-acquisition. A custom-made switch was connected in series with the stimulator to modulate the duration and timing of the pulses. A custom-written LabVIEW program was used for data-acquisition.

3.3.3 Experimental Procedure

Each subject participated in 4 sessions with a minimum of 48 hours separating consecutive sessions. During the 1^{st} session, subjects were familiarized with the testing procedures and equipment, their maximal voluntary isometric contraction (MVIC) forces were determined, and data were collected for plotting their force-frequency and force-pulse-duration curves. During the 2nd to 4th sessions, one of the 3 fatigue protocols was tested each day in random order. The following procedures were performed during the 4 sessions (Figure 3.1):

3.3.3.1 Initial Data-Collection (1^{st} Session)

At the start of the 1^{st} session, subjects were informed about the testing procedures, requested to sign the informed consent forms, and trained to perform the MVIC test.

3.3.3.1.1 MVIC Testing.

The burst superimposition technique was used to determine each subject's MVIC force [68]. During the MVIC testing, the subjects were asked to produce as much knee extension force as possible. While the subjects were generating knee extension force, a supra-maximal electrical stimulation train (amplitude: 130 mV, train duration: 100-ms, frequency: 100-Hz, pulse-duration: 600-μs) was delivered to the quadriceps femoris muscle. If the electrical stimulation train increased the muscle force by $<$10%, the subject's MVIC was recorded. If the electrical stimulation train increased the force output $\geq$10%, the MVIC testing was repeated after a 10-minute rest. If the subjects failed to complete the MVIC testing within 3 repetitions, they were not tested on that day.

3.3.3.1.2 Data-collection for force-frequency and force-pulse-duration curves.

The MVIC test was followed by a 10-minute rest. Next, the stimulation amplitude was set to elicit 50% of the subject's MVIC using a 300-ms long 100-Hz train at 600-μs pulse-duration. After setting the stimulation amplitude, a series of trains were delivered to collect data for plotting the subject's force versus frequency and force versus pulse-duration curves. The first 11 trains were 770-ms long 14-Hz trains with 600-μs pulse-duration, which were used to potentiate the muscle. Next,

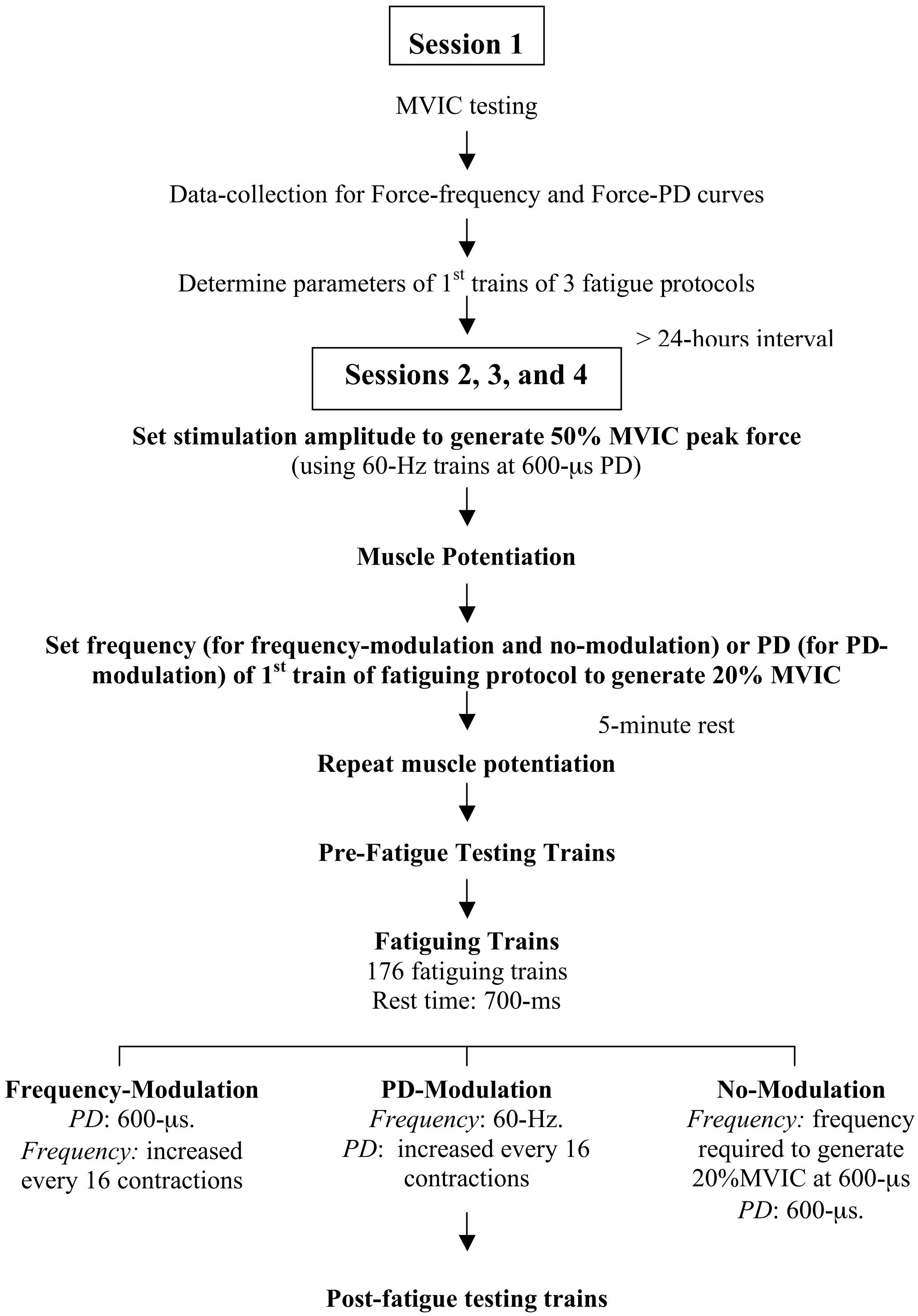


Figure 3.1: Flowchart showing the experimental protocol for the 4 testing sessions. (PD: pulse duration). See text for details.

twenty-two 300-ms long trains with 600-μs pulse-duration and frequencies ranging from 10- to 100-Hz were delivered in random order. Finally, twenty-two 300-ms long trains with 60-Hz frequency and pulse-durations varying from 100- to 600-μs were delivered in random order. There was a 5-second rest time between consecutive trains.

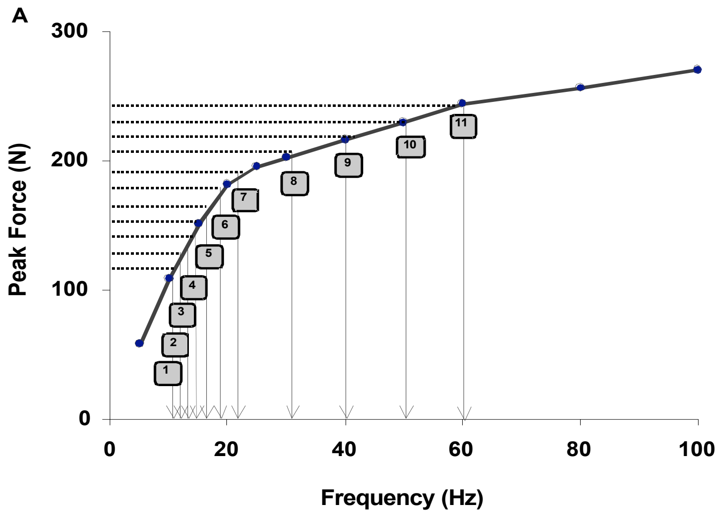
3.3.3.1.3 Determination of parameters of 1^{st} trains of the fatigue protocols.

The stimulation frequency that generated 20% MVIC peak force in response to a 300-ms long train at 600-μs pulse-duration was recorded, and used for the first 16 trains of the no-modulation and the frequency-modulation protocols. In addition, the stimulus pulse-duration that generated 20% MVIC peak force in response to a 300-ms long 60-Hz train was recorded and used for the first 16 trains of the pulse-duration-modulation protocol.

3.3.3.1.4 Determination of Steps for the Modulation-protocols

Steps for the frequency and pulse-duration-modulation protocols were custom-designed for each subject based on their force-frequency and force-pulse-duration curves respectively. First, the force-frequency and force-intensity curves were plotted. The frequency of the first 16 trains was set at the frequency of the 300-ms long train with pulses of 600-μs duration that generated 20% of the subject's MVIC, as explained above, to determine the steps for the frequency-modulation protocol. Following this, the portion of the y-axis (peak force) of the force-frequency curve between the 20% MVIC peak force and the peak force generated at 60-Hz was divided into 10 equal parts to obtain 11 equal 'force-steps' (Figure 3.2).

The frequencies corresponding to the peak forces at each of the force-steps were then recorded from the x-axis of the force-frequency curve. During frequency-modulation, the stimulus pulse-duration was fixed at 600-μs and the frequency was



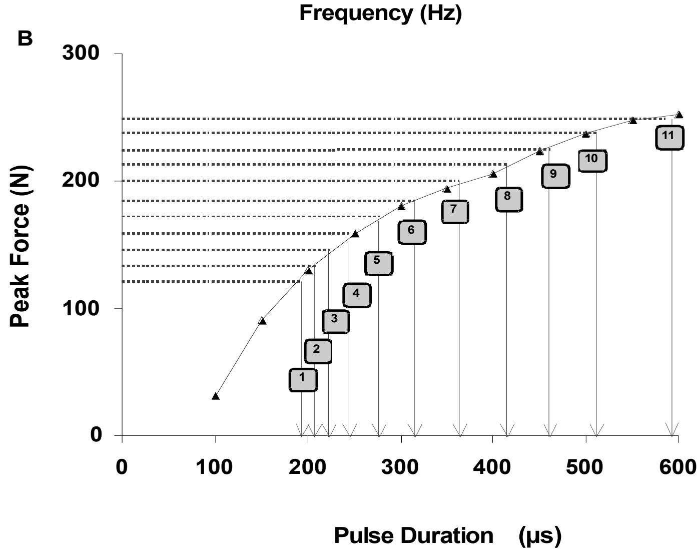


Figure 3.2: A schematic showing the peak force versus frequency (A) and peak force versus pulse duration (PD) (B) curves for a representative subject and the method used to determine steps for frequency-modulation (A) and PD-modulation (B). The horizontal shaded lines point to the 11 equal force-steps. The vertical arrows point to the corresponding frequency (A) and PD (B) steps used for modulation. Note that the frequency- and PD-steps were spaced on the curves such that each stepwise increase in frequency or PD caused an approximately equal increase in force output.

increased every 16 stimulation trains according to the 11 steps obtained as explained above. Similarly, 11 equal force steps for the pulse-duration-modulation protocol were determined using the force versus pulse-duration curve for each subject (Figure 3.2). During the pulse-duration-modulation protocol, the stimulation frequency was fixed at 60-Hz and the pulse-duration was increased every 16 contractions.

3.3.3.2 Fatigue Protocols (2nd to 4th Sessions)

One of the 3 fatigue protocols were tested in random order during each of the remaining 3 sessions. During each session, after electrode placement, the stimulation amplitude was set to produce 50% of the subject's MVIC force using 300-ms long, 60-Hz trains with 600-μs pulse-duration.

The electrical stimulation protocols for the 2nd to 4th sessions consisted of potentiation trains, pre-fatigue testing trains, fatiguing trains, and post-fatigue testing trains as explained below (Figure 3.1):

Potentiation trains. Eleven 770-ms long 14-Hz trains with 600-μs pulse-duration were delivered with a 5-second rest time between trains to potentiate the muscle [9].

Pre- and Post-Fatigue Testing Trains. Pair of 60- and 20-Hz testing trains and a twitch at 600-μs pulse-duration were delivered before and after the fatiguing trains to measure the force generating ability of the muscle. The rest time between pre-fatigue testing trains was 10-seconds. The rest time between post-fatigue testing trains was 1-second.

Fatiguing Trains. A total of 176 trains were delivered at the rate of 1 train every second to fatigue the muscle. The stimulation parameters of the fatiguing trains during the 3 fatigue protocols were as follows:

(1) **Frequency-modulation Protocol:** The pulse-duration was fixed at 600-μs. The frequency of the 1^{st} train was set to the frequency required to generate

20% MVIC peak force and the frequency of the 176^{th} train was 60 Hz. Stimulation frequency was increased stepwise every 16 contractions in 11 equal force steps.

(2) **Pulse-duration-modulation Protocol:**

The frequency was fixed at 60-Hz. The pulse-duration of the 1^{st} train was set to the pulse-duration required to generate 20% MVIC peak force and the pulse-duration of the 176^{th} train was 600-μs. Stimulus pulse-duration was increased stepwise every 16 contractions in 11 equal force-steps.

(3)**No-modulation Protocol:**

The pulse-duration was fixed at 600-μs. The frequency of the 1^{st} train was set to the frequency required to generate 20% MVIC peak force. Both the pulse-duration and the frequency were kept constant for all the trains in the protocol. This protocol was compared to the 2 modulation protocols because out of 3 combinations of frequency and pulse-duration tested in Specific Aim 1, repetitive stimulation with the combination of a low-frequency and 600-μs pulse-duration produced better performance in response to fatiguing trains compared to either a medium-pulse-duration and medium-frequency, or a high-frequency (60-Hz) and short-pulse-duration (See Chapter 2).

3.4 Data Analyses

The peak forces and force-time integrals in response to each stimulation train were calculated for the 3 fatigue protocols. The percent change in peak force and force-time integral from the first to the last fatiguing train were used as a measures of the muscle's 'performance' or its ability to generate force in response to the fatiguing trains. A positive percentage change indicated that the last fatiguing train generated greater force than the 1^{st} fatiguing train and a negative percentage change indicated that the last fatiguing train generated less force than the 1^{st} fatiguing train. In addition, the sums of the peak forces and force-time integrals produced in response to the 1^{st} to 176^{th}, 1^{st} to 60^{th}, 61^{st} to 120^{th}, and 121^{st} to 176^{th} fatiguing trains were used as additional measures of muscle performance. The percentage declines in peak force between the pre- and post-fatigue 60-Hz testing trains were used as a measure of muscle fatigue. The ratio of peak forces produced in response to 20-Hz versus 60-Hz pre- and post-fatigue testing trains (20-Hz:60-Hz peak force ratio) were used as measures of the degree of low-frequency fatigue in the muscle [65, 78].

3.4.1 Statistical Analyses

Each of the dependant variables, except the 20-Hz:60-Hz peak force ratios, were compared using one-way repeated measures ANOVAs. Pair-wise post-hoc comparisons were performed if significant differences were present. The 20-Hz:60-Hz peak force ratios were compared using 2-way (protocol X fatigue) repeated measures ANOVAs. The significance level was set at p=0.05.

3.5 Results

The average ages of the 12 subjects tested were 24.8 ± 2.4 years and their MVIC forces were 949.4 ± 246.2 N. Table 3.1 shows the frequencies and pulse-durations of the 1^{st} and last trains for the 3 fatigue protocols.

Table 3.1: The stimulation frequency and pulse duration (PD) of the 1^{st} and last trains for each of the 3 fatigue protocols. The frequency and PD of the 1^{st} trains were adjusted to generate 20%MVIC peak force. The frequency and PD was increased stepwise in 11 equal force steps from the 1^{st} to the last fatiguing train during frequency- and PD-modulation respectively.

		Freq(Hz)	PD (μs)
Frequency-Modulation	1^{st} Train	11.6 ± 1.5	600
	Last Train	60.0	600
PD-Modulation	1^{st} Train	60.0	131 ± 23
	Last Train	60.0	600
No-Modulation	1^{st} Train	11.3 ± 1.6	600
	Last Train	11.3 ± 1.6	600

The peak forces produced in response to each of the 176 fatiguing trains, and the forces generated in response to the 1^{st} and last fatiguing trains for a representative subject are shown in Figure 3.3. Similar to the force data shown in Figure 3.3, for 9 out of the 12 subjects tested, frequency-modulation produced larger peak forces in response to the last compared to the 1^{st} fatiguing trains (Figure 3.3) .

3.5.1 Fatiguing Trains (Measures of Muscle Performance)

Analyses of the percentage change in peak force between the 1^{st} and last fatiguing trains showed that the frequency-modulation protocol produced an increase in peak force (percent change = $15.5 \pm 28.7\%$), the pulse-duration-modulation produced a small decrease in peak force (percent change = $-6.2 \pm 20.3\%$), and the no-modulation protocol produced a large decline in peak force (percent change = $-31.2 \pm 9.4\%$) ($p<0.01$) (Figure 3.4). The percent changes in force-time integrals of the fatiguing trains showed a similar trend, however, both frequency- and pulse-duration-modulation protocols showed increases in the force-time integrals from the 1^{st} to the last fatiguing train (Figure 3.4). All of the differences between the protocols were significant ($p \leq 0.01$).

The frequency-modulation protocol produced the largest and the no-modulation protocol produced the smallest sum of the peak forces in response to the 1^{st} to 176^{th}, 1^{st} to 60^{th}, 61^{st} to 120^{th}, and 121^{st} to 176^{th} fatiguing trains ($p<0.01$) (Figure 3.5). Similarly, frequency-modulation produced the largest and no-modulation the smallest sum of the force-time integrals in response to the 1^{st} to 60^{th} and 121^{st} to 176^{th} fatiguing trains ($p<0.01$) (Figure 3.5). In response to 61^{st} to 120^{th} and 1^{st} to 176^{th} fatiguing trains, the no-modulation protocol produced the smallest sum of the force-time integrals, but there were no differences in the sum of the force-time integrals between frequency- and pulse-duration-modulation (Figure 3.5).

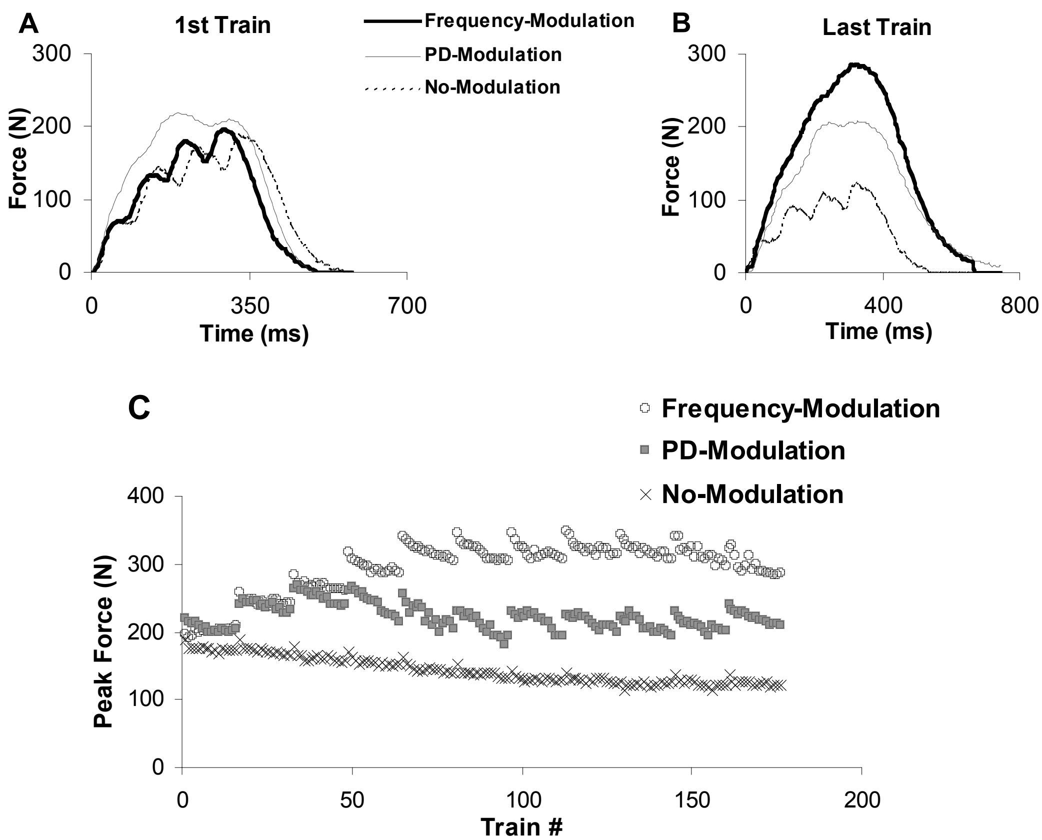


Figure 3.3: The peak forces (y-axis) produced in response to each fatiguing train (x-axis) during the 3 fatigue protocols (C) for a representative subject. Force responses of the 1^{st} (A) and last (B) fatiguing trains for each of the 3 fatigue protocols. Note that for the modulation protocols, the force in response to the last train is either equal to or greater than the force in response to the 1^{st} train. In contrast, the no-modulation protocol causes a decline in force from the 1^{st} to the last train.

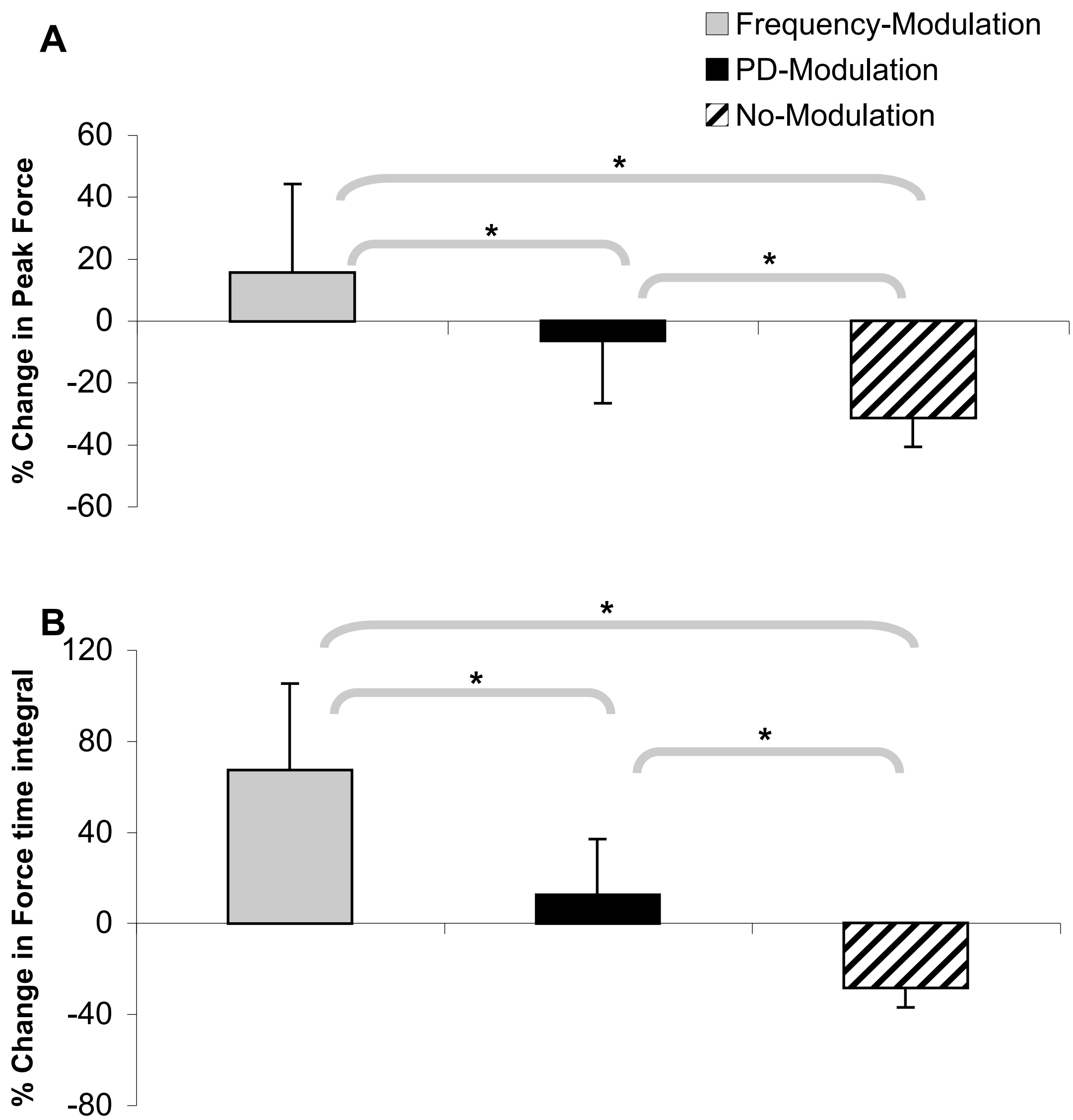


Figure 3.4: The percent change in peak force (A) and force time integrals (B) between the first and the last fatiguing trains during the 3 fatigue protocols. Note the positive percentage change in peak force for the frequency-modulation protocol (A) and the positive percentage change in force time integral for frequency-modulation and PD-modulation protocols (B). *Significant differences between protocols ($p \leq 0.01$).

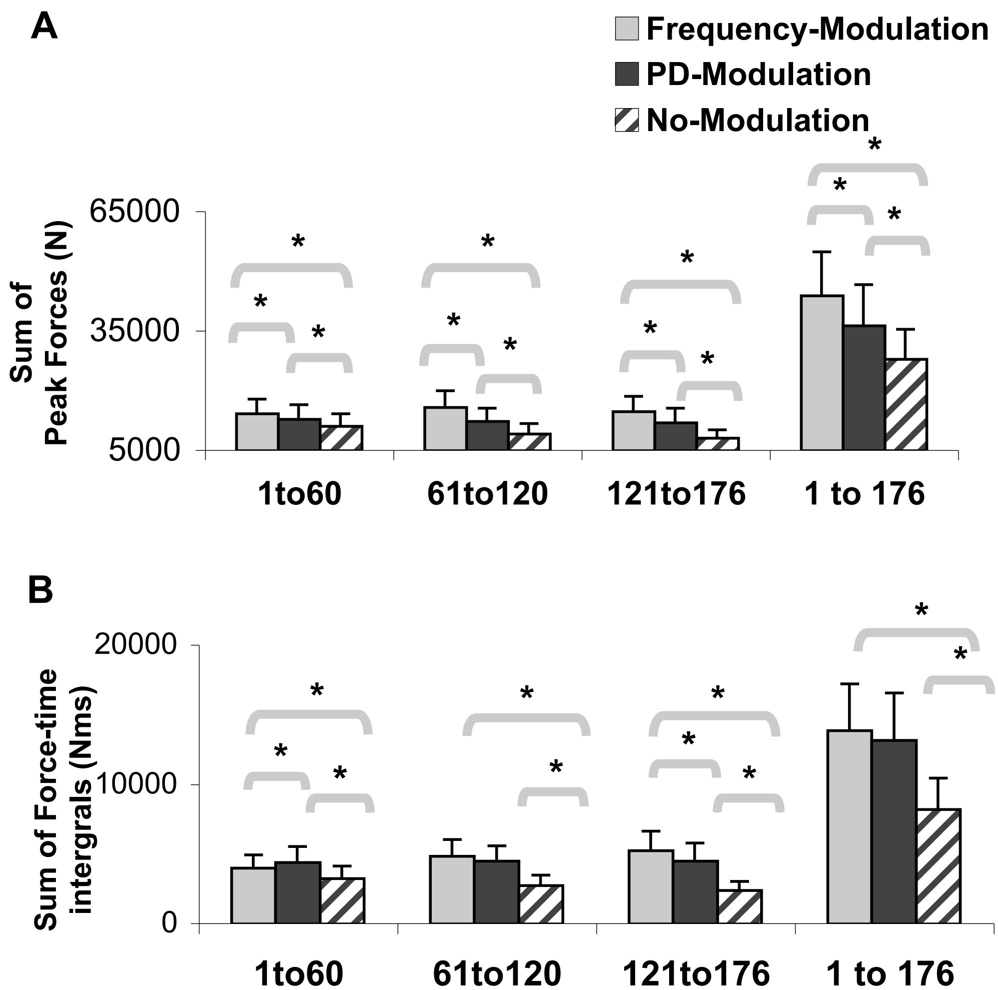


Figure 3.5: Sum of peak forces (A) and force time integrals (B) generated in response to the 1^{st} to 60^{th}, 61^{st} to 120^{th}, 121^{st} to 176^{th}, and 1^{st} to 176^{th} fatiguing trains during the 3 fatigue protocols. *Significant difference between fatigue protocols (p≤0.01).

3.5.2 Testing Trains (Measures of Muscle Fatigue)

The no-modulation protocol produced a significantly smaller decline in peak force of the 60-Hz testing trains (23.6±8.4%) compared to both the frequency-modulation and the pulse-duration-modulation protocols ($p<0.01$) (Figure 3.6). There was no significant difference in percentage decline in peak force of the 60-Hz testing trains between the frequency-modulation (46.6±9.4%) and the pulse-duration-modulation (48.0±10.6%) protocols ($p=0.47$) (Figure 3.6).

The 2-way ANOVA for the 20-Hz:60-Hz peak force ratios showed significant effects of protocol ($F=22.8$, $p<0.01$), fatigue ($F=312.8$, $p<0.01$), and a significant interaction ($F=50.7$, $p<0.01$) (Figure 3.6). There were no differences in the pre-fatigue 20-Hz:60-Hz peak force ratios across protocols ($F=0.37$, $p=0.70$). The no-modulation protocol showed the smallest post-fatigue 20-Hz:60-Hz peak force ratios ($p<0.01$). There were no differences between frequency- and pulse-duration-modulation protocols in the post-fatigue 20-Hz:60-Hz peak force ratios ($p=0.77$) (Figure 3.6). For all 3 protocols, post-fatigue ratios were smaller than pre-fatigue 20-Hz:60-Hz ratios (all $p<0.01$) (Figure 3.6).

3.6 Discussion

An interesting finding of the present study was that, although the last 16 fatiguing trains were identical (60-Hz with 600-μs pulse-duration) and similar amounts of muscle fatigue and low-frequency fatigue (Figure 3.6) were observed, the frequency-modulation protocol produced better performance in response to the fatiguing trains than the pulse-duration-modulation protocol (Figure 3.4). One possible explanation for these findings is that the pulse-duration-modulation protocol may have produced greater high-frequency fatigue than the frequency-modulation protocol.

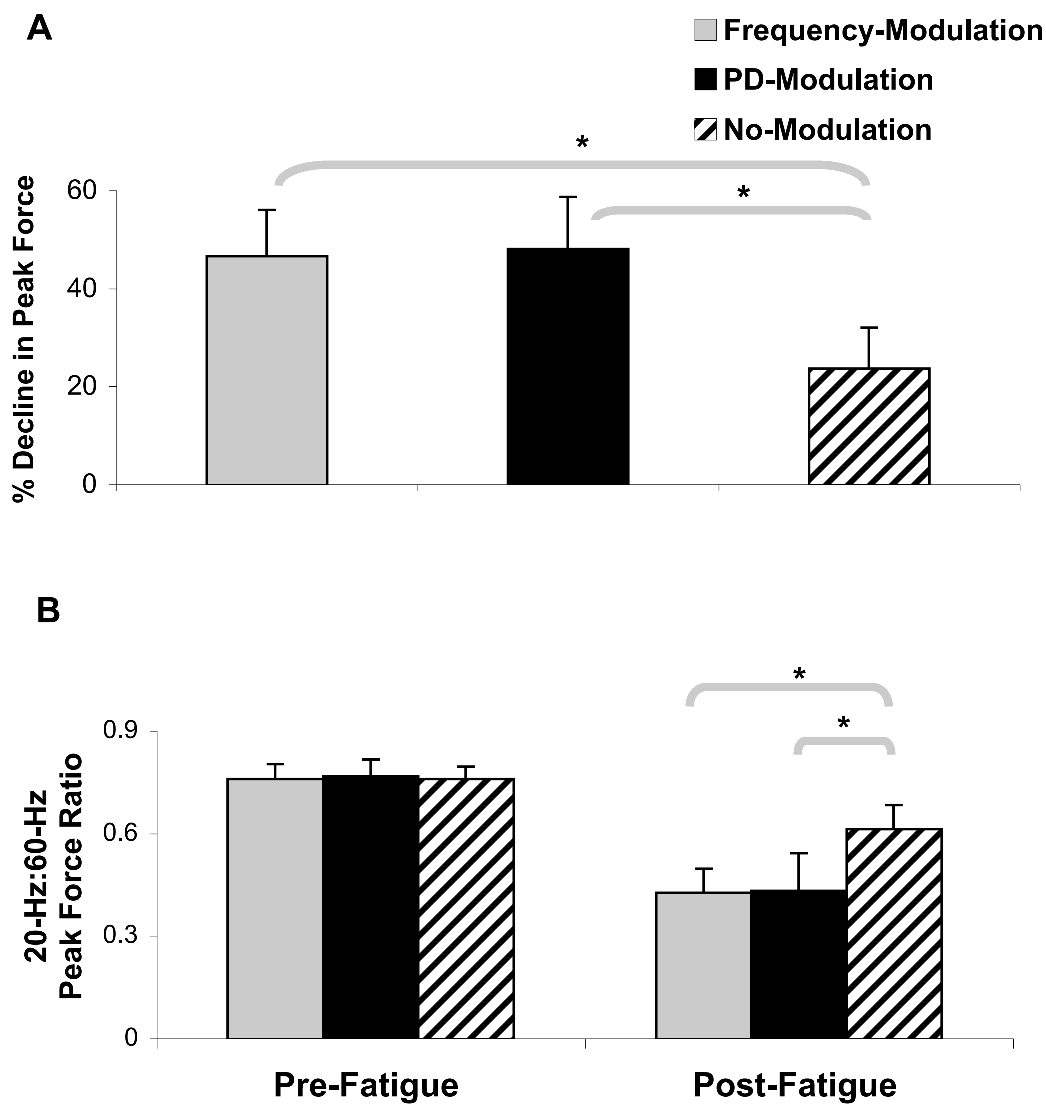


Figure 3.6: The percent decline in peak force between pre-fatigue and post-fatigue 60-Hz testing trains (A) and the pre- and post-fatigue 20-Hz: 60-Hz peak force ratios (B) for the 3 fatigue protocols tested. There were significant differences between the pre- and post-fatigue 20-Hz: 60-Hz peak force ratios for each of the 3 fatigue protocols ($p<0.01$). *Significant differences between protocols ($p\leq0.05$.

High-frequency fatigue is characterized by the selective loss of force after repetitive stimulation at high frequencies and a rapid recovery on reducing the stimulation frequency [42]. The frequency-modulation protocol involved stepwise increases of the stimulation frequency from low (11.6±1.5-Hz) to high (60-Hz); in contrast, a relatively high stimulation frequency (60-Hz) was used throughout the pulse-duration-modulation protocol. The use of high-frequency trains throughout the pulse-duration-modulation protocol may have caused a greater degree of high-frequency fatigue [42], thereby resulting in poorer muscle performance of the pulse-duration-modulation protocol. Furthermore, because a twitch separated the last fatiguing train and the post-fatigue 60-Hz testing train during the experimental protocols, we believe that there would have been time for recovery from high-frequency fatigue, which may explain why the 60-Hz testing trains did not show any differences in the levels of muscle fatigue between the 2 modulation protocols (Figure 3.6). We compared the peak forces generated in response to the last fatiguing trains and the post-fatigue 60-Hz testing trains during the frequency- and pulse-duration-modulation protocols to see if such recovery actually occurred (Figure 3.7). A comparison of the relative increases in peak forces from the last fatiguing train to the post-fatigue 60-Hz testing train during the 2 modulation protocols showed greater recovery for the pulse-duration-modulation protocol and supported our suggestion that a greater level of high-frequency fatigue produced by the pulse-duration-modulation protocol may have contributed to its poor performance (Figure 3.7). Unfortunately, because we did not collect electromyography data, we were unable to test for the degree of high-frequency fatigue produced by each protocol.

Although the no-modulation protocol produced the least muscle fatigue and low-frequency fatigue (Figure 3.6), both modulation protocols showed better performance in response to the fatiguing trains than the no-modulation protocol (Figure

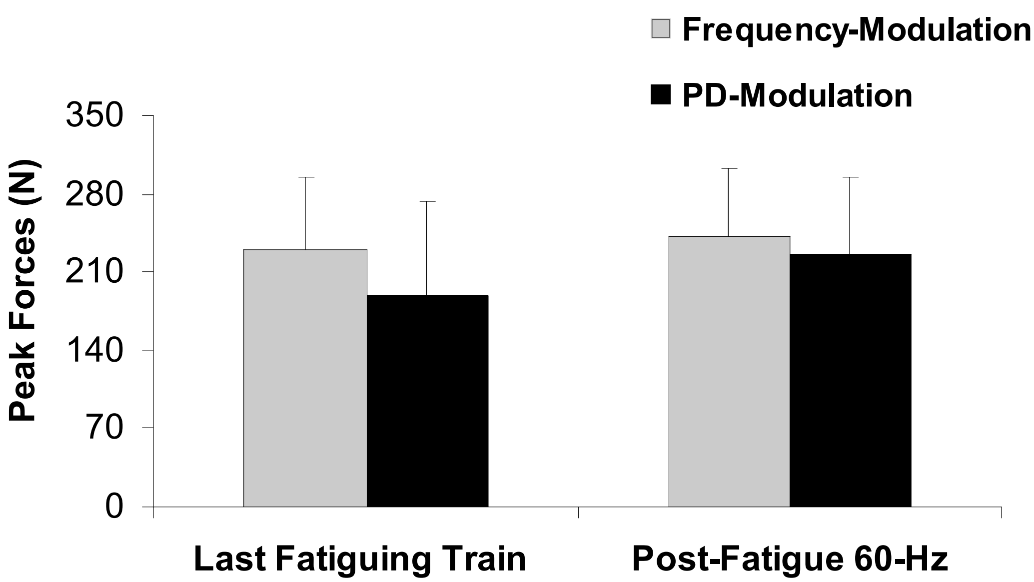


Figure 3.7: Peak forces generated in response to the last fatiguing train (left) and the post-fatigue 60-Hz testing train (right) during the frequency-modulation and the pulse-duration (PD)-modulation protocol. Both the last fatiguing train and the post-fatigue testing trains were 60-Hz trains with 600-μs PD. Note the larger increase in peak force from the last fatiguing train to the post-fatigue 60-Hz testing train during the PD-Modulation than the frequency-modulation protocol.

3.4). Thus, as the levels of muscle fatigue and low-frequency fatigue increased, increases in the level of temporal and spatial summation of muscle forces produced by the frequency- and pulse-duration-modulation protocols, respectively, resulted in improved muscle performance. The no-modulation protocol maintained a lower stimulation frequency (11.3±1.6-Hz) than either the pulse-duration- (60-Hz throughout) or the frequency-modulation protocol (stepwise increase from 11.6±1.5-Hz to 60-Hz), which may have contributed to the lower muscle fatigue [10, 52, 26] and low-frequency fatigue [17, 18, 82] (Figure 3.6). Low-frequency fatigue is characterized by the selective loss of force at low- versus high-frequencies during fatigue or recovery from fatigue [25, 71, 17]. Low-frequency fatigue causes a rightward shift in the force-frequency curve, due to which higher frequencies are needed to produce comparable forces to the pre-fatigued state [11, 8, 75, 28]. Thus, low-frequency fatigue, as well as overall muscle fatigue, would cause an attenuation of performance in response to the low-frequency trains (11.3±1.6-Hz) used during the no-modulation protocol. However, during the latter half of the frequency-modulation protocol, and throughout the pulse-duration-modulation-protocol, the muscle was stimulated with frequencies in the high-frequency range of the force-frequency curve (Figure 3.2). This high-frequency stimulation would overcome the effects of low-frequency fatigue [25, 65], and contribute to the better performance of the 2 modulation protocols compared to the no-modulation protocol.

Previously, Graupe and colleagues showed that the stochastic modulation of the inter-pulse intervals within stimulation trains decreased the rate of muscle fatigue of the quadriceps femoris muscle compared to stimulation at a constant frequency on a single subject [34]. In contrast, Thrasher and colleagues recently showed that random modulation of frequency (mean: 40-Hz), amplitude (mean: 75% maximal tetanic force), and pulse-duration (mean: 250-μs) by ±15% of their mean values every 100-ms did not effect the rate of fatigue during isometric contractions of the tibialis anterior and quadriceps femoris muscles of 7 spinal cord injured subjects [76]. Our present study is the first to show improvement in isometric muscle performance using stepwise increases in stimulation frequency during repetitive stimulation. In addition, Kebaetse and Binder-Macleod recently showed that for healthy subjects and subjects with spinal cord injury, starting at a low- and later switching to a high-frequency stimulation produced better performance during repetitive non-isometric contractions than stimulation using either a low- or high-frequency alone [45, 46]. These findings regarding frequency-modulation may have important implications for clinical applications of FES because most current FES systems deliver a constant frequency and only increase the stimulation intensity to increase muscle force [57, 61, 73, 24].

During FES, muscle force must repetitively reach a targeted level to enable efficient task performance. The stepwise increases in frequency and pulse-duration in our study caused the peak force to rise above the 20% MVIC targeted force level (Figure 3.3). This overshoot of force may have caused greater metabolic energy expenditure [13, 59, 70], and produced greater muscle fatigue [20, 67, 81] than would have been produced if the targeted force was not exceeded. A better strategy would have been to only increase the stimulation frequency or pulse-duration to the level needed to produce the targeted force with minimal overshoot. Future studies will use predictive mathematical models that account for changes in the force-frequency curve due to fatigue to determine the appropriate frequency and pulse-duration steps required to generate the targeted force [23].

3.7 Conclusions

During repetitive electrical stimulation, increasing the stimulation frequency (frequency-modulation) produced better isometric skeletal muscle performance in response to the fatiguing trains compared to the strategy of increasing intensity (pulse-duration-modulation). Although frequency-modulation is not a strategy currently used in FES, clinicians and researchers in the field of FES should consider incorporating frequency-modulation as a strategy for skeletal muscle force control in FES systems. Future work is needed to develop stimulation strategies that can minimize fatigue and improve skeletal muscle performance during FES applications. These strategies may involve combining frequency- and pulse-duration-modulation to maximize muscle performance.

Chapter 4

CONCLUSION

4.1 Outcomes Related to Specific Aims and Hypotheses

Specific Aim 1: To compare the muscle fatigue and isometric performance in response to repetitive surface electrical stimulation with three different combinations of frequencies and pulse-durations that were each selected to produce the same initial peak force: #1 low frequency, long-pulse duration (600-μs); #2 medium frequency (30-Hz), medium pulse duration; and #3 high frequency (60-Hz), short-pulse duration.

Hypothesis 1.1: Protocol #1 will produce the best and Protocol #3 the poorest isometric muscle performance in response to the fatiguing trains.

The results supported this hypothesis. Protocol #1, consisting of fatiguing trains of a low-frequency and long-pulse-duration, produced the smallest (31.3±9.4% and 28.2±8.9% for peak force and force-time integrals respectively) and Protocol #3, consisting of fatiguing trains of a high-frequency and short-pulse-duration, produced the largest (51.3±7.5% and 45.4±6.8% for peak force and force-time integrals respectively) % decline in response to the fatiguing trains.

Hypothesis 1.2: Protocol #1 will produce the least and Protocol #3 the most muscle fatigue in response to the testing trains.

The results supported this hypothesis when muscle fatigue was assessed using testing trains at the pulse duration of the fatiguing trains. However, a surprising finding was that when muscle fatigue was assessed using testing trains at 600-μs pulse-duration, Protocol #2, consisting of fatiguing trains of medium-frequency (30-Hz) and medium-pulse-duration produced the least muscle fatigue.

Hypothesis 1.3: Protocol #1 will produce the least and Protocol #3 the most low-frequency fatigue.

Similar to Hypothesis 1.2, the results supported this hypothesis when low-frequency fatigue was assessed using testing trains at the pulse duration of the fatiguing trains. However, when low-frequency fatigue was assessed using testing trains at 600-μs pulse duration, there were no differences in the amount of low-frequency fatigue produced at the end of the 3 protocols.

4.2 Implications of Outcomes from Specific Aim 1

The findings from Specific Aim 1 showed that when the same initial peak force was generated using different combinations of frequency and pulse duration, and the frequency and pulse duration were kept constant throughout repetitive stimulation, repetitive stimulation with a low-frequency (11.5$\pm$1.2-Hz) and long-pulse duration (600-μs) (Protocol #1) maximized isometric muscle performance by minimizing muscle fatigue in the motor unit population recruited by the fatiguing trains. In addition, the differences in the observed levels of muscle fatigue and low-frequency fatigue measured using testing trains at the pulse duration used to fatigue the muscle versus testing trains at 600-μs pulse duration may have important clinical implications for FES. The results suggest that during FES applications, if the frequency and pulse duration are held constant during repetitive stimulation, repetitive stimulation using the lowest frequency and longest pulse duration (similar to Protocol #1) may maximize performance by minimizing muscle fatigue. In contrast, during FES applications, if the intensity is increased as the muscle fatigues, repetitive stimulation with a 'medium' frequency, similar to the frequency used in Protocol #2 of Specific Aim 1 may minimize muscle fatigue. The optimum combination of frequency and pulse duration during FES may therefore depend on whether or not the strategies of modulation of frequency or intensity will be during repetitive stimulation.

Specific Aim 2: To compare the muscle fatigue and isometric performance of the healthy human quadriceps femoris muscles during repetitive surface electrical stimulation with a frequency-modulation protocol, a pulse-duration-modulation protocol, and a no-modulation protocol.

Hypothesis 2.1: The no-modulation protocol will show poorer performance in response to the fatiguing trains compared to the frequency- or pulse duration-modulation protocols.

The results supported this hypothesis. The frequency- modulation, pulse-duration-modulation, and no-modulation protocols produced +15.5±28.7%, -6.2±20.3%, and -31.2±9.4% change in peak forces, and +67.2±38.1%, +12.6±24.3% and -28.2±8.9% change in force-time integrals from the 1st to the last fatiguing train, respectively.

Hypothesis 2.2: The frequency-modulation protocol will show better performance than the pulse-duration-modulation protocol.

The results supported this hypothesis. The frequency-modulation protocol produced +15.5±28.7% and +67.2±38.1% change in peak forces and force-time integrals from the 1st to the last fatiguing trains, and the pulse-duration-modulation protocol produced -6.2±20.3% and +12.6±24.3% change in peak forces and force-time integrals from the 1st to the last fatiguing trains.

Hypothesis 2.2: There will be differences in the amount of muscle fatigue and low-frequency fatigue produced among the 3 protocols.

The results for the comparison between the no-modulation and the two modulation protocols supported this hypothesis. The no-modulation protocol produced lesser muscle fatigue and low-frequency fatigue compared to either of the 2 modulation protocols. However, the hypothesis was not supported for the comparison between the frequency- and pulse-duration-modulation protocols. There were no differences in the amount of muscle or low-frequency fatigue produced between the frequency- and pulse-duration-modulation protocols.

4.3 Implications of Outcomes from Specific Aim 2

The most interesting finding from Specific Aim 2 was that although similar amounts of muscle fatigue and low-frequency fatigue were observed, the frequency-modulation protocol produced better performance in response to the fatiguing trains than the pulse-duration-modulation protocol. This is important because most current FES systems deliver a constant frequency and only increase the stimulation intensity to increase muscle force [73, 74, 41, 51, 64, 79]. In addition, although the no-modulation protocol produced the least muscle fatigue and low-frequency fatigue, both the modulation protocols showed better performance in response to the fatiguing trains than the no-modulation protocol. The results suggest that although frequency-modulation is not a strategy currently used in FES, clinicians and researchers in the field of FES should consider incorporating frequency-modulation as a strategy for skeletal muscle force control in FES systems. Future work is needed to develop electrical stimulation strategies that can minimize fatigue and improve skeletal muscle performance during FES applications.

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Appendix A

INFORMED CONSENT FORM

Project Title:

Effects of modulation of frequency and intensity on the fatigability of skeletal muscle during electrical stimulation

Principal Investigator: Stuart Binder-Macleod, PT, PhD
Secondary Investigators: Trisha Kesar, PT, LiWei Chou, MS.

You are being asked to participate in a research project to find the effects of different kinds of electrical stimulation methods on the forces produced by the muscles in the front of your thigh. **Twelve healthy individuals** will participate in the study. The testing will be of no benefit to you. However, the findings of this study may help to develop strategies to improve performance of paralyzed muscles of individuals with stroke, spinal cord injury and other neurological disorders during electrical stimulation.

Your identity will remain confidential and will not be revealed in any publications resulting from this work. Your data will be the property of this laboratory and will be on secured computers that can be accessed by laboratory personnel only. Following completion of this project (approximately five years), the data will be destroyed or transferred to a long-term storage medium, such as a CD, and stored in a secured closet in Dr Binder-Macleod's office.

Participation in this study is totally voluntary. You may withdraw from the study at any time without any consequences. **You will be asked to participate in 8 testing sessions over the time period of approximately a month. At least 48 hours will separate any two consecutive sessions. You will be requested to refrain from strenuous physical activity for at least 24 hours prior to testing**. **Each testing session will last no more than 1 hour. You will be paid $20 for each testing session.**

Procedures

First Testing Session

(i) Familiarization with Setup:

You will be familiarized with the setup and the procedures. For the testing, you will be seated on a machine that measures muscle forces and surface electrodes will be attached over the skin on your thigh. Before we start testing, we will deliver a low level electrical current through the electrodes to acquaint you with the sensation of the stimulation and to test for placement of electrodes.

(ii) Testing your Maximal Force:

Following this, we will determine the maximum force that you can generate from your knee muscle. For this, we will ask you to kick as hard as you can by contracting the muscle in the front of your thigh as strongly as possible. While you are contracting your muscle, a burst of current will be delivered to your muscle to see if you are truly producing a maximal force from your knee muscle. If you are not able to produce a maximal contraction in three attempts, the testing will not be continued.

(iii) Testing (Non-fatigue):

After determining your muscle's maximal force generating ability, we will deliver a series of bursts of current (1 every 10-seconds). When electric currents are being delivered to your muscle, you may feel a "prickly" sensation on your skin or feel as if your muscle is being squeezed, but you should not feel pain. Each burst of current will be slightly different; although some bursts will make your muscle work a little harder than the others, you will only feel slight differences in sensation between each burst.

Seven Subsequent Sessions (Fatigue Testing)

In each of the 7 subsequent sessions, a series of 180 bursts of current will be delivered to your muscle at the rate of 1 per second. Each session will test a slightly different burst. **All you will have to do during stimulation is to try to relax your muscle as much as possible.** During the fatigue testing, your muscle will feel tired, and some fatigue tests may be more uncomfortable than the others. After the fatigue test, we will also ask you to mark on a scale an estimate of the extent of pain/fatigue experienced by you during the testing.

If you wish to stop the procedure at any time for any reason, please let us know and we will stop the testing immediately.

Possible Risks

Although the procedures to which you will be exposed are safe, some subjects do report some muscle soreness for about 2 days after testing that is similar to the muscle soreness that you might feel if you lift weights or exercise vigorously after a long lay off. During some tests you may feel pain in your thigh muscles similar to what you may experience when bicycling hard up a long hill. The sensation of pain and fatigue will subside soon after the end of the stimulation. Although the possibility of injury, such as muscle strains and tears, injury of the kneecap and injury to the bones of the leg does exist, it is highly unlikely. The potential for equipment malfunction is also present, which might result in burns to the skin. However, the equipment used is highly reliable and the prolonged exposure necessary to cause the risk of skin damage is highly unlikely during this experiment.

Contact information - If you have any questions about this research project, you may contact **Dr Stuart Binder-Macleod (831-8046).** For questions related to your rights as a human subject, please contact the University of Delaware Human Subjects Review Board (**T.W. Fraser Russell at 831-2136**).

INFORMED CONSENT FORM
Effects of modulation of frequency and intensity on the fatigability of skeletal muscle during electrical stimulation

By signing this informed consent, I certify that I have chosen to participate in this study. The investigators have explained the purpose of the study, described the risks associated with my participation, and have defined what is expected of me as a subject. The investigators have answered all of my questions about the procedures to my satisfaction.

- I have never been treated for any **knee problems** such as, but not limited to ligament injury, meniscus injury, fractures or muscle tears involving the leg to be tested.
- I have never had **blood vessel disease** involving either my arteries or veins of my leg, such as, but not limited to, blood clots, or blockage of the blood vessels.
- I have no imposed **limitations in activity** due to heart disease or uncontrolled high blood pressure.
- I have never had **cancer.**
- I have never had any known **neurological disorders** or **muscle diseases** such as, but not limited to multiple sclerosis, nerve injury, polio, muscular dystrophy or myotonia.
- Lastly, in the event of physical injury resulting from these research procedures, the investigators will provide me emergency medical treatment. If I require additional medical treatment, it is my responsibility to seek additional medical care and to pay all expenses for any medical treatment received.

______________________________ ______________________________

Subject's Signature Date Witness

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Subject's Name