COMPARISON OF THREE TYPES OF NEUROMUSCULAR ELECTRICAL STIMULATION ON FATIGABILITY IN THE QUADRICEPS MUSCLES

by

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ABSTRACT

Neuromuscular electrical stimulation (NMES) can produce contractions of paralyzed muscles to improve function and reduce secondary complications for individuals with a spinal cord injury (SCI). NMES is typically delivered through a single pair of electrodes over a muscle belly at a stimulation frequency of ~40 Hz, which in this thesis will be referred to as conventional NMES, and its' benefits are limited by rapid contraction fatigability. Therefore, the present study compared three NMES approaches including NMES over the femoral nerve (NERVE), NMES rotated between four electrodes over the muscle belly (sequential NMES; SEQ) and hybrid NMES (HYBRID) which combines NERVE and SEQ NMES to determine which type of NMES produces the most fatigue resistant contractions of the quadriceps muscle. In addition, we assessed variability between consecutive contractions and discomfort (Visual Analogue Scale; VAS) associated with the stimulation. Fourteen healthy human participants (10 males and 4 females; 27±8 years) were recruited. The 3 types of NMES were tested in different sessions on separate days. Each session incorporated a fatigue protocol consisting of 180 contractions (0.3s "on", 0.7s "off"; 40 Hz). Fatigability was quantified as the decrease in evoked torque over time. There were no differences in contraction fatigability between the three NMES types; torque declined by 17±34%, 30±12%, and 31±17% for NERVE, SEQ and HYBRID, respectively. SEQ resulted in the least variability between successive contractions (2.9±2.9 %). NERVE produced the most variability between contractions and least discomfort (VAS = 23±14 mm). As there was no difference in the amount of fatigability

between the three protocols, and NERVE and SEQ have resulted in less fatigability than conventional NMES, we suggest that all three types of NMES studied in this thesis produced less contraction fatigability than is typical of conventional NMES. As there was no clear difference in fatigability between the three protocols, and given the practical issues with delivering NMES over the femoral nerve, which affects both the NERVE and HYBRID protocols in the present study, we suggest SEQ to be incorporated into clinical practice. SEQ has consistently shown to reduce fatigability compared to conventional NMES (Bergquist, Babbar, Ali, Popovic, & Masani, 2016; Downey, Bellman, Kawai, Gregory, & Dixon, 2015; Maneski, Malesevic, Savic, Keller, & Popovic, 2013; Nguyen, Masani, Micera, Morari, & Popovic, 2011; Popovic & Malesevic, 2009; Sayenko, Nguyen, Hirabayashi, Popovic, & Masani, 2015; Sayenko, Nguyen, Popovic, & Masani, 2014), is easy to apply and the torque generated is consistent between evoked contractions, hence it could be easily incorporated in clinical settings.

PREFACE

This thesis is an original work by Francisca Claveria-Gonzalez. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name "Fatigue of the quadriceps muscle during electrical stimulation", Pro00059140, 7/29/2016.

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LIST OF ABBREVIATIONS

NMES	neuromuscular electrical stimulation
NERVE	neuromuscular electrical stimulation applied over the femoral nerve
SEQ	neuromuscular electrical stimulation rotated between four electrodes over the muscle belly
HYBRID	hybrid neuromuscular electrical stimulation
Conventional NMES	Neuromuscular electrical stimulation delivered through two electrodes over the muscle belly at 40 Hz
FES	Functional electrical stimulation
MUs	motor units
MVC	maximal voluntary contraction
EMG	electromyography
H-reflexes	Hoffman's reflex
SCI	spinal cord injury
Rm ANOVA	repeated measures analysis of variance

CHAPTER 1: GENERAL INTRODUCTION

1.1 Preface

People with spinal cord injury (SCI) frequently report secondary complications such as type II diabetes, osteoporosis, and cardiovascular disease as consequence of physical inactivity (Chih-Wei et al., 2011; Deley, Denuziller, & Babault, 2015; Kocina, 1997; Shields, 2002). One way to minimize these complications is through the use of neuromuscular electrical stimulation (NMES). NMES can produce contractions of paralyzed muscles to increase functionality and prevent or diminish secondary complications of a SCI. However, the major problem with NMES is the rapid development of fatigability which is due to the non-physiological way that contractions are produced by electrical stimulation. Contraction fatigability is defined as a significant decrease in torque over repeated contractions. Consequently, the effectiveness of NMES is limited. In this thesis, three types of NMES were compared to determine which would reduce fatigability the most when stimulating the quadriceps muscles. One type was NERVE, which was applied over the femoral nerve trunk in the femoral triangle. Another was sequential stimulation (SEQ), which consisted of rotating stimulation pulses between multiple electrodes over the muscle belly. Both of these types of NMES have been shown to reduce fatigability compared to the way NMES is typically delivered (hereafter called conventional NMES), which is delivered through two electrodes over the muscle belly at a stimulation frequency of ~40 Hz(Bergquist, Wiest, Okuma, & Collins, 2014; Popovic & Malesevic, 2009). The work described in this thesis is the first to test a new type which we have called

"HYBRID" NMES, because it combines features of SEQ and NERVE. We hypothesized that HYBRID would reduce fatigability to a greater extent than NERVE and SEQ. In addition to fatigability, contraction variability and discomfort were also measured during each of type of NMES.

The first chapter of this thesis provides a review of the literature relevant to my MSc research. Chapter 2 describes my MSc research in which the main goal was to compare three types of NMES for reducing fatigability. Chapter 3 is the General Discussion in which I summarize the findings, describe clinical implications of the findings, discuss limitations, significance of the work, and suggest future directions for this project. Appendix 1 provides a brief summary of a pilot experiment I conducted as part of my MSc that is related to the work described in Chapter 2.

1.2 Neuromuscular electrical stimulation (NMES)

1.2.1 History

In 1791, Luigi Galvani, an Italian physician and physicist discovered "animal electricity." Galvani showed that two frog legs attached by the sciatic nerves contracted vigorously when electrical current was applied to one of the legs. Based on his findings, Galvani hypothesized that animal tissues are endowed with an intrinsic electricity involved in nerve conduction which generates muscular contractions (Piccolino, 1998; Verkhratsky, Krishtal, & Petersen, 2006). After the death of Galvani (1798), his nephew, Giovanni Aldini, continued the experiments on animal electricity. He was the first to produce motor responses by stimulating mammalian brains (cerebellum and corpus callosum) (Parent, 2004; Verkhratsky et al., 2006), as well as the first to use transcranial electrical stimulation to treat patients with mental disorders (Parent, 2004). Finally, Du Bois-Reymond known as the founder of electrophysiology, confirmed Galvani's theory about the electrical nature of nerve signals through his work "Researches on Animal Electricity" in 1848 (Finkelstein, 2015).

Continuing with this work was Guillaume Duchenne who developed the era of the modern electrotherapy (Cambridge, 1977). He used electricity to treat patients with facial paralysis, and to develop functional mappings of the muscles in people with and without a neuromuscular disorders (Parent, 2005). The first report of Functional Electrical Stimulation (FES) occurred in 1961 by Wladimir Liberstone who developed the first electrical stimulator to prevent foot drop in patients with hemiplegia. In this study, FES not only improved gait during its' application, but also improved muscle function even when the stimulation was off. This marked the beginning of the modern era of neurorehabilitation with functional electrical stimulation (FES) (Liberson, Holmquest, Scot, & Dow, 1961). Finally, the first time that FES was applied on paraplegic patients was in 1963, where surface stimulation was applied over the quadriceps and gluteus muscles to facilitate standing position for a brief period of time (Kralj, 1989). This work motivated continual research efforts to improve FES applications for people with spinal cord injury.

1.1.2 Contemporary use of NMES

NMES involves applying electricity to an intact nerve to produce action potentials in axons generating muscle contractions (Ho et al., 2014). It can be applied in a variety of manners: using surface electrodes over the skin; percutaneous intramuscular electrodes, which are considered as a precursor to fully implanted systems; or implanted electrodes for long-term functional improvement on systems. In the clinic, surface NMES is the most commonly used and is typically delivered through two electrodes on the skin over a muscle. NMES is also referred to as FES when is applied associated to functional activities or exercise to recover or improve a function lost due to an injury or disease (Ho et al., 2014; Peckham & Knutson, 2005; Pereira, Mehta, McIntyre, Lobo, & Teasell, 2012). NMES can assist in vital body function such as standing (Gillette et al., 2008), walking (Street, Taylor, & Swain, 2015), grasping (Gan et al., 2012), diaphragmatic functioning (Gorman, 2000), and bladder and bowel voiding (Creasey et al., 2001). In addition, NMES can be used for exercise applications such as cycling with legs, cycling with legs and arms (Hunt, Fang, Saengsuwan, Grob, & Laubacher, 2012; Perret, Berry, Hunt, Donaldson, & Kakebeeke, 2010; Raymond, Davis, Fahey, Climstein, & Sutton, 1997), and rowing (Taylor, Picard, & Widrick, 2011), which seek to improve cardiovascular conditioning and prevent muscular atrophy (Deley et al., 2015; Hamid & Hayek, 2008). The focus of this thesis is on these types of NMES-exercise applications.

1.1.3 How NMES generates muscle contractions

A muscle is comprised of several muscle fibers which are innervated by motor axons. A single motor axon and all the muscle fibers innervated by it is referred to as a motor unit (MU). During NMES, contractions are not generated by direct activation of muscle fibers. Instead contractions are generated by activation of the motor axons. This is because the charge threshold to activate axons is lower than that required to activate muscle fibers (Peckham & Knutson, 2005). Commonly, to produce a contraction, NMES is applied over intact nerves. However, it can also be applied over denervated muscles, but a higher current is needed to activate denervated muscles than muscles with intact nerves. In this thesis, we studied the activation of muscles in individuals with intact nerves.

1.1.3.1 Biophysics

NMES depolarizes axons by causing movement of ions through ion channels in the axonal membrane. At rest, the inside of the axonal membrane is negatively charged compared to the outside. When the stimulation is on, the negatively charged ions (anions) move from the cathode towards the anode, and the positive ions (cations) move from the anode to the cathode. Under the cathode, anions in the extracellular space are repelled from the electrode towards the membrane, and cations are attracted towards the cathode, thus, the membrane becomes more positive (depolarized). In contrast, under the anode, cations in the extracellular space are repelled. Consequently, under the anode becomes more negative compared to the inside (hyperpolarized). If the stimulation intensity is large enough to achieve the depolarization threshold, voltage-gated sodium and potassium channels open and action potentials are generated. Action potentials will propagate in different directions: orthodromic (normal direction); and antidromic (opposite direction) along the motor and sensory axons. Orthodromic signals travel along the motor axon to the muscle fibers and along the sensory axon to the spinal cord, and antidromic signals travels along the motor axon to the cell body and along the sensory axon to the receptors (Gersh, 1992).

1.1.3.2 Pathways

Traditionally, when NMES is applied over the muscle belly, it generates contractions predominantly through peripheral pathways, by the depolarization of motor axons. When contractions are generated through peripheral pathways, MUs discharge synchronously, time-locked to each stimulation pulse (Bickel, Gregory, & Dean, 2011; Gorgey, Black, Elder, & Dudley, 2009). This synchronous MU discharge is represented in the electromyographic (EMG) signal as motor M-waves, as shown in Figure 1-2. In addition to peripheral pathways, NMES can also generate contractions through central pathways, by the depolarization of sensory axons. These central pathways recruit MUs via reflex pathways that travel through the spinal cord and back to the muscle (Bergquist, Clair, Lagerquist, et al., 2011) as shown in Figure 1-1A. Support for the idea that central pathways contribute to contractions during NMES has been provided by experiments where NMES was applied before and during a peripheral nerve block that disconnected the stimulation site and the spinal cord. In these experiments, before the nerve block, contractions were large because central pathways were involved; however, during the nerve block the amplitude of the contractions was reduced because only peripheral pathways were contributing (Collins, Burke, & Gandevia, 2001, 2002; Lagerquist, Walsh, Blouin, Collins, & Gandevia, 2009). Thus, during NMES contractions can be produced by peripheral and central pathways, and the contribution of central pathways can increase the torque generated through peripheral pathways (Bergquist, Clair, Lagerquist, et al., 2011; Bergquist, Wiest, & Collins, 2012; Collins et al., 2001, 2002; Lagerquist et al., 2009). Central pathways recruit MUs in two different ways. One of them, and the most commonly described, is recorded in the EMG signal as a "Hoffmann" or H-reflex (Collins 2007). MUs recruited through H-reflex pathways discharge synchronously relative to the stimulation pulses, like M-waves, but at a longer latency as shown in Figure 1-1A. H-reflexes have been shown to contribute to contractions produced by NMES in the triceps surae and quadriceps muscles (Bergquist, Clair, & Collins, 2011; Bergquist et al., 2012). Another way of central contribution is "asynchronous activity," which results in a MU discharge that is not time-locked to each stimulus pulse, similar to voluntary contractions. In the EMG, asynchronous activity can be observed as an increase in baseline activity between the M-wave and the H-reflex (Bergquist, Clair, Lagerquist, et al., 2011), as is shown in Figure 1B. The strength of these central contributions during NMES depends on the muscle, parameters and location of the stimulation applied and it variates between individuals (Baldwin, Klakowicz, & Collins, 2006; Bergquist, Clair, Lagerquist, et al., 2011; Bergquist et al., 2012; Bergquist et al., 2014).

1.1.4. Stimulation parameters

During electrically-evoked contractions, parameters such as frequency, stimulation intensity and pulse duration will impact neuromuscular responses to NMES. Frequency of stimulation refers to how many pulses per second (Hz) are delivered to the muscle and increases in stimulation frequency results in higher force production, whereby after approximately (~) 60 Hz, torque does not increase anymore and plateaus (Bickel et al., 2011; Gregory, Dixon, & Bickel, 2007). Stimulation intensity refers to the quantity of current delivered by each pulse, and is typically measured in milliamperes (mA). When the current is enough to depolarize the membrane to threshold, an action potential is generated, and torque is produced. By modulating intensity, the number of MUs recruited can be controlled, where high intensities recruit more motor units and increase muscle torque (Gorgey, Mahoney, Kendall, & Dudley, 2006). Pulse duration is the span of time that current is being delivered per each pulse of stimulation; the commonly used parameters are 0.3 - 0.6 milliseconds (ms) (Doucet, Lam, & Griffin, 2012). Longer pulse duration can produce a desired torque using less current than shorter pulse duration, and increments in force production have been observed with increments in pulse duration of up to 600 μs (Bickel et al., 2011; Gorgey et al., 2006).

1.1.5 SCI and NMES

A SCI is one of the most devastating lesions of the nervous system. It can cause mild to severe neurological deficits that result in paralyzed muscles and reduced functionality (Bickenbach & International Spinal Cord, 2013; Chih-Wei et al., 2011). In 2013, the World Health Organization reported approximately 250,000 – 500,000 new cases of a SCI emerge per year worldwide (Bickenbach & International Spinal Cord, 2013). In Canada, the estimated prevalence in 2010 was 86,000, and the incidence was approximately 4,300 new cases (Farry & Baxter, 2011). Most people with a SCI have a life expectancy that is getting closer to that of people without a disability (World Health Organization & The International Spinal Cord Society, 2013). However, due to their limited or lack of voluntary movement, they live a very sedentary life. This sedentary lifestyle produces changes in muscle, such as disuse atrophy resulting in a decline in muscle mass. Therefore, muscle fibers become weaker, and convert to a fast-fatigable phenotype, causing muscles of people with a SCI to fatigue quickly (Duffell et al., 2008). Also, individuals with a SCI have a decrease in bone mineral density (Kocina, 1997) and tend to be overweight (Wong et al., 2015). Other secondary complications include type II diabetes, pressure ulcers, and cardiovascular disease (Duffell et al., 2008; Gerrits, de Haan, Sargeant, Dallmeijer, & Hopman, 2000; Martin Ginis et al., 2008). Cardiovascular disease is the major cause of death in this population, with a rate of mortality about 2.3 times greater than able-bodied individuals (Dyson-Hudson & Nash, 2009; Kocina, 1997)

Exercise with NMES has been used as a therapeutic tool to prevent and/or reduce secondary complications of a SCI. In the cardiovascular system, NMES can: increase the cross sectional area of arteries and increase density of capillaries improving blood inflow to the legs (Gerrits, de Haan, Sargeant, van Langen, & Hopman, 2001); increase peak oxygen uptake and ventilation by 20%-35% (Hooker, Scremin, Mutton, Kunkel, & Cagle, 1995); and reduce cardiovascular risks (Chih-Wei et al., 2011). In addition, in the musculoskeletal system, NMES can increase muscle endurance and power (Griffin et al., 2009), increase muscle mass (Skold et al., 2002) and increase and/or maintain the number of fatigue-resistant types fibers (Andersen, Mohr, Biering-Sorensen, Galbo, & Kjaer, 1996; Crameri, Cooper, Sinclair, Bryant, & Weston, 2004). Furthermore, NMES can increase muscle-to-adipose tissue ratio (Pacy et al., 1988), reduce spasticity (Krause, Szecsi, & Straube, 2008), and reverse or reduce the loss of bone mineral density (Chen et al., 2005; Lai et al., 2010). Lastly, training with NMES has also shown positive results in improving insulin sensitivity and preventing the insulin resistance syndrome (Griffin et al., 2009; Mohr et al., 2001).

1.1.6 Limitations

For people with a SCI to improve cardiorespiratory fitness and muscle strength, guidelines recommend at least 20 minutes of moderate to vigorous aerobic exercises twice per week and three sets of strengthening exercises for each major functioning muscle group, at a moderate to vigorous intensity, twice per week (Ginis et al., 2017; SCI Action Canada, 2011). A major barrier to compliance with these recommendations using NMES is contraction fatigability. Hence, people with a SCI cannot exercise at sufficient intensities or for long enough durations to optimise the physiological benefits of NMES (Maffiuletti, 2010; Martin, Sadowsky, Obst, Meyer, & McDonald, 2012). Fatigability is mainly related to the non-physiological way that MUs are recruited during NMES, whereby MUs recruited in random order with respect to type and the same motor units are recruited synchronously at unphysiologically high rates. Discomfort is another limitation to the benefits of NMES because it restricts the number of people who can participate in NMES training and the intensities at which people can train (Lai, Domenico, & Strauss, 1988). Discomfort during NMES is a consequence of the stimulation of afferents from nociceptors, and it is most closely related to the amount of current delivered (Maffiuletti, 2010). Finally, another factor needs to be considered regarding NMES, although less well studied than fatigue and discomfort, is variability between consecutive contractions (Baldwin et al., 2006; Bergquist et al., 2016; Bergquist et al., 2012). High variability between contractions has been associated with contractions produced through central pathways and when NMES is applied over the nerve trunk versus over the muscle belly (Baldwin et al., 2006; Bergquist et al., 2012). Such variability in the size of contractions produced by NMES will have implications for performed functional tasks and may negatively impact performance during FES-based exercise programs.

1.2 Discomfort

Discomfort during NMES has been associated with stimulation of pain fibers in the skin or muscle such as A delta fibers (mechanical and thermal information), and C fibers (mechanical, thermal and chemical information); resultant ischemia from muscle contraction; metabolite accumulation and/or musculotendinous stress from isometric muscle contractions (Delitto, Strube, Shulman, & Minor, 1992; Matthews, Wheeler, Burnham, Malone, & Steadwarde, 1997).

Typically, to achieve benefits from NMES such as improving exercise tolerance and muscle strength, the stimulation intensity needs to be sufficient to evoke relatively strong contractions (Delitto et al., 1992; SCI Action Canada, 2011; Vivodtzev et al., 2012; Vivodtzev et al., 2014). For example, a three-week NMES training of isometric contractions in the quadriceps muscles showed that training at higher intensities resulted in greater strength gains (~49%) than training at low intensities (Lai et al., 1988). However, current has been directly associated with discomfort, since as stimulation intensity increases, more nociceptors in the skin and in the muscle would be activated in addition to muscle fibers, resulting in more discomfort (Delitto et al., 1992; Forrester & Petrofsky, 2004; Naaman, Stein, & Thomas, 2000). Therefore, subjects with intact or at least partial sensation such as those with an incomplete SCI or stroke may not be able to tolerate increments in stimulation intensity, limiting the benefits of NMES training.

Stimulation parameters such as pulse duration, electrode size and location of stimulation have been also studied as contributors to discomfort during NMES. A study by Liebano et al (2013) compared discomfort produced by NMES at three different pulse durations (400, 700, and 1000 μs) over the quadriceps muscle, and found longer pulse durations produced greater discomfort. This was explained by comparing the strength-duration curves of sensory, motor and pain fibres. With long pulse durations, the strength of the stimulus required to activate motor and pain fibres were similar; hence, longer pulse stimuli increase the likelihood of discomfort (Alon, Allin, & Inbar, 1983). Conversely, another study in the quadriceps muscles found that with pulse durations of 200 μs, individuals were better able to tolerate the stimulation to produce greater amounts of torque than when using shorter pulse durations (50 μs) (Scott, Causey, & Marshall, 2009). Expanding on this, Scott et al. (2014) reported that 500 μs stimulation pulse widths allowed participants to generate greater amounts of torque and tolerate higher levels of current than 200 μs in the same muscle. These findings were attributed to a greater amount of MUs recruited during 500 μs than during 200 μs. However, it is unknown whether the difference in torque and discomfort found between 50 and 200 μs, and between 200 and 500 μs would result in clinical differences in patient outcomes such as strength gain. These inconsistent outcomes found in pulse duration and discomfort may be explained by differences in previous experience with electrical stimulation, body composition of the populations studied, and the combination of stimulation parameters such as frequency and duty cycle used in each of these studies. Thus, more research is needed to determine the effect of pulse duration on discomfort and its clinical implications in patient’s outcomes during NMES-based therapy.

Regarding electrode size and its impact on discomfort, results are still ambiguous. A study by Patterson and Lockwood (1991) compared different electrode sizes from 20 to 60 cm^2, and found that larger electrode sizes produced less pain than smaller ones when used to stimulate the quadriceps muscle to generate 25 %MVC. Similar results were shown in the gastrocnemius muscle, where less discomfort and also greater force was found using electrodes sizes of 20.25 and 40.3 cm^2 compared to 2.25 and 9.0 cm^2 (Alon, Kantor, & Ho, 1994). On the contrary, another study in the gastrocnemius muscle compared two electrodes of different areas (19.63 and 38.48 cm^2), found the smaller electrodes were less uncomfortable (Lyons, Leane, Clarke-Moloney, O'Brien, & Grace, 2004). These studies however used different stimulation parameters such as pulse duration, frequency, pulse waveform, and duty cycle and electrode locations, complicating their comparison. Thus, it is still unknown what the optimal electrode size is to produce less discomfort during NMES-based therapy.

Finally, another factor related to discomfort is stimulation site (nerve trunk vs muscle belly), which is relevant for the work described in this thesis. Few studies have compared discomfort between stimulation over the nerve and over the muscle. To produce the same amount of torque, stimulation over the nerve requires less current than stimulation over the muscle because when the nerve is stimulated, axons are bundled together and located directly under the stimulating electrodes (Bergquist, Clair, Lagerquist, et al., 2011). On the other hand, when stimulation is applied over the muscle belly, motor axons are spread out throughout the muscle, thus more current is required to activate the same amount of motor axons as nerve stimulation (Okuma, Bergquist, Hong, Chan, & Collins, 2013). Therefore, less discomfort is expected during stimulation over the nerve than during conventional NMES (Bergquist et al., 2012; Naaman et al., 2000). In the tibialis anterior muscle, less discomfort was found by applying stimulation over the common peroneal nerve than the muscle belly (Naaman et al., 2000). Similarly, also in the tibialis anterior muscle, Wiest et al (2017) found that ~63% less discomfort was produced during stimulation over the nerve compared to conventional NMES to generate a 30% MVC. Conversely, in the quadriceps muscles other studies have reported opposite results, finding conventional NMES more comfortable than stimulation over the femoral nerve trunk (Martin, Millet, Martin, Deley, & Lattier, 2004; Place, Casartelli, Glatthorn, & Maffiuletti, 2010). The higher level of discomfort during nerve stimulation compared to muscle stimulation found in these studies was associated with the higher current density generated in small electrodes size. However, other studies have not found a relation between current density and discomfort (Martinsen, Grimnes, & Piltan, 2004; Turi et al., 2014).

For people with no sensation, such as those with complete SCI, discomfort during NMES will not be an issue; however, autonomic dysreflexia may be induced during NMES in people with high levels of injury, above the major splanchnic outflow (about T6) (Matthews et al., 1997). Autonomic dysreflexia is a syndrome that occurs when a nociceptive signal below the level of the lesion triggers a sympathetic discharge which causes hypertension, piloerection, sweating and anxiety (Ashley et al., 1993; Matthews et al., 1997). During NMES therapy sessions, it is important to avoid triggering autonomic dysreflexia because if not controlled properly, autonomic dysreflexia can cause serious health complications (Erickson, 1980).

1.3 Fatigability during conventional NMES

As mentioned earlier, during conventional NMES MUs are recruited differently than during voluntary contractions, which is the main cause of fatigue; therefore, these differences are described in the following.

1.3.1 Recruitment of motor units during voluntary contractions

On the basis of physiological properties, MUs can be divided in two groups: slow-twitch and fast-twitch (Burke, 1967). Fast-twitch MUs, hereinafter referred to as fast-fatigable MUs, are larger in size, produce a large amount of force, but fatigue rapidly. On the other hand, slow-twitch MUs, hereinafter referred to as fatigue-resistant MUs, are smaller in size, produce lower amounts of force output and fatigue less (Bickel et al., 2011; Burke, 1967; "Neuroscience in the 21st century from basic to clinical," 2013). During voluntary contractions, MUs are recruited in a stereotypical order, whereby small fatigue-resistant MUs are recruited first, and progressively as force increases larger fast-fatigable MUs are recruited. This orderly recruitment, first described by Henneman, means that for a given net input, a larger excitatory postsynaptic potential will be generated in small MUs than large ones, thus small MUs reach threshold for producing action potentials sooner than large MUs (Henneman, 1957).

Once recruited, MU discharge rates increase as contraction amplitude increases, avoiding unnecessary high discharge rates during low to moderate contractions (Bigland & Lippold, 1954). In general, MUs discharge between < 10 to ~ 40 Hz during MVCs (Barss et al., 2017; Bellemare, Woods, Johansson, & Bigland-Ritchie, 1983; Bickel et al., 2011). In addition, to produce smooth contractions with low frequencies, MUs discharge at different times relative to each other in an asynchronous pattern. This asynchronous pattern produces fused, tetanic contractions at relatively low, metabolically-efficient, discharge rates which also help to diminish fatigability. Furthermore, during prolonged voluntary contractions, MUs cyclically alternate their activity which allows newly recruited MU to replace a previously fatigued unit (Westgaard & de Luca, 1999).

1.3.2 Recruitment of motor units during electrically evoked contractions

During conventional NMES, MUs are recruited randomly with respect to type (Bickel et al., 2011). Contrary to contractions being generated by synaptic input to motor neurons as occurs during voluntary contractions, the random recruitment of MUs during conventional NMES is due to the direct activation of motor axons under the stimulating electrodes. Theoretically, large diameter axons are activated at lower stimulation amplitudes than small axons due to their lower axial resistance (Enoka, 2002). If the diameter of the axon were the only factor determining the activation of MUs during NMES, MUs would be recruited in a reversed order during electrically-evoked contractions than voluntary contractions. However, when axons are recruited by stimulating electrodes over the skin, both axon diameter and distance from the stimulating electrodes determine which MU is recruited first. Accordingly, action potentials may be initiated in small diameter axons located closer to the electrode at a lower current than larger axons located farther away (Grill et al., 1995). As a result, during conventional NMES, superficial MUs closer to the electrode are recruited first, while progressively deeper MUs are recruited as stimulation intensity is increased (Mesin, Merlo, Merletti, & Orizio, 2010; Okuma et al., 2013; Rodriguez-Falces, Maffiuletti, & Place, 2013; Vanderthommen et al., 2000). In the quadriceps muscles this spatial recruitment during conventional NMES has lead to a preferential recruitment of fast-fatigable fibers because they predominate in superficial parts of the muscle (Lexell, Downham, & Sjöström, 1986; Vanderthommen et al., 2003).

Contrary to voluntary contractions when MUs discharge asynchronously from one and other, during conventional NMES, motor axons are recruited synchronously with each stimulation pulse and MU discharge is time-locked to each stimulation pulse. Due to the synchronous nature of MU activation during NMES, higher firing rates are needed to generate fused contractions than during voluntary contractions of similar amplitude (20Hz – 40Hz), which produces higher metabolic demands over the muscle (Vanderthommen et al., 2003; Wegrzyk et al., 2015). Thus, fatigability is greater and occurs more rapidly than during voluntary contractions due to the random recruitment of MUs and higher MU discharge rates during NMES (Gregory & Bickel, 2005b; Kiernan, Lin, & Burke, 2004; Maffiuletti, 2010; Vanderthommen et al., 2003).

1.3.3 Mechanisms of contraction fatigability

Fatigability can be induced by impairments of one or several physiological processes from the site of stimulation to deep within the muscle (Enoka & Duchateau, 2008; Martin et al., 2016). These mechanisms include that contribute to fatigability during NMES include failure in excitation-contraction coupling, neuromuscular transmission failure, decreased axonal excitability and decreased availability of metabolic substrates. Impairments of excitation-contraction coupling has been attributed to reduced Calcium ($Ca^{+)}$ release from the sarcoplasmic reticulum, decreased sensitivity of troponin to Ca^{+}, failure in the conduction of the action potential through the T-tubules, and reduction of Ca^{+} reuptake by the sarcoplasmic reticulum (Jones, Howell, Roussos, & Edwards, 1982; Keeton & Binder-Macleod, 2006). This mechanism of fatigability has been called low frequency fatigue, and is characterized by a greater loss of force when tested at low frequencies compared to high frequencies, and by a slow recovery over hours to days (Jones et al., 1982). Low frequency fatigue has been frequently assessed in people with and without a SCI (Cometti, Babault, & Deley, 2016; Keeton & Binder-Macleod, 2006; Mahoney, Puetz, Dudley, & McCully, 2007). This mechanism of fatigability has been assessed by recording the torque evoked at low (10 – 20 Hz) and high (80 – 100 Hz) frequencies and calculating a ratio of the force production with 10 or 20 Hz frequency stimulation to that of the 80-Hz or 100-Hz stimulation. Then, low frequency fatigue is quantified by measuring changes in this ratio before and after a fatigue protocol – a decrease in ratio being interpreted as greater low frequency fatigue (Keeton & Binder-Macleod, 2006; Mahoney et al., 2007).

Neuromuscular transmission impairment is another mechanism that contributes to fatigability during NMES (Jones, 1996). Neuromuscular transmission failure can include impairments at the neuromuscular junction or propagation of the action potential along the sarcolemma. It can be caused from a depletion of neurotransmitters stores (Sieck & Prakash, 1995), or an accumulation of potassium and depletion of sodium ions in the extracellular space along the muscle membrane, resulting in failed excitation and propagation of action potentials along the membrane (Jones, 1996; Jones, Bigland-Ritchie, & Edwards, 1979). Fatigability associated with this mechanism has been characterized as a loss of force at preferentially high stimulation frequencies and rapid force recovery (Jones, 1996).

More recently, it has been suggested that decreased axonal excitability can also contribute to contraction fatigability during NMES (Martin et al., 2016; Matkowski, Lepers, & Martin, 2015). Decreased axonal excitability has been associated with overactivity of the sodium-potassium pump due to an accumulation of Na^+ intracellularly (Burke, Kiernan, & Bostock, 2001). As a result of this decrease in axonal excitability, the number of recruited MUs declines, which causes a reduction in torque-production (Papaiordanidou, Stevenot, Mustacchi, Vanoncini, & Martin, 2014).

Failure of metabolism related-processes can also contribute to fatigability during NMES. The lack of energy supply (Adenosine triphosphate, ATP) to meet energy demands, and the accumulation of metabolites such as H^+, Mg-ADP, and Pi have been identified as the metabolic factors associated with decline in force (Enoka & Stuart, 1992). Vanderthommen et al. (2003) found that for isometric contractions of the same intensity, a higher energy demand was produced during NMES delivered at 50 Hz compared to voluntary contractions. Finally, details of the task such as intensity, duration and contraction type (isometric or isokinetic) can influence the relative contribution of each of these mechanisms to contraction fatigability (Enoka & Stuart, 1992).

1.3.4 Stimulation parameters and fatigability

Stimulation frequency, intensity, and pulse duration can influence muscle performance and affect fatigability during electrically-evoked contractions (Bickel et al., 2011). During voluntary contractions to maintain a force output with low fatigability, a large number of MUs discharge at relatively low frequencies (Bellemare et al., 1983; Bickel et al., 2011). Conversely, during NMES, a smaller number of MUs discharge at higher frequencies (30 – 50 Hz) than during voluntary contractions of the same intensity. These high MU discharge rates result in high metabolic demands over the muscle, leading to rapid contraction fatigability (Bickel et al., 2011; Gorgey et al., 2006; Gregory et al., 2007). Interestingly, stimulating at very high frequencies (above 80 Hz), has been shown to increase the contribution of central pathways, which could lead to MUs being synaptically recruited thereby decreasing fatigability (Bergquist, Clair, & Collins, 2011; Collins et al., 2001; Dean, Yates, & Collins, 2007). For example, Collins et al. (2001) found that by stimulating the triceps surae muscle at 100 Hz, an additional force to that produced through peripheral pathways was generated through central pathways. Similarly, larger H-reflex amplitudes and larger contractions were observed during stimulation trains of 20 Hz after brief periods of 100 Hz stimulation than during constant stimulation at 20 Hz in the soleus muscle (Bergquist, Clair, Lagerquist, et al., 2011; Lagerquist & Collins, 2010). Interestingly, this central recruitment of MUs produced by high stimulation frequencies donot reduce fatigability in comparison to contractions generated mainly through peripheral pathways (Martin et al., 2016). For example, Martin et al. (2016), found no difference in fatigability between stimulation protocols at 20 Hz and 100 Hz, despite the larger H-reflex amplitudes observed at 100 Hz.

The impact of stimulation intensity on fatigability is still uncertain. For example, a study by Gorgey A et al. (2009) found no difference in fatigability at several stimulation amplitudes, while another study reported greater fatigability at higher intensities (20 vs 50% MVC) (Binder-Macleod, Halden, & Jungles, 1995). In addition, stimulation intensity can also determine the relative contribution of central pathways to electrically-evoked contractions. By applying NMES at high stimulation amplitudes, antidromic signals from the motor axons would collide with signals coming back from the spinal cord via sensory axons, limiting central contribution. Therefore, when the direct motor response is maximal (maximum M-wave), signals generated by input from sensory axons (H-reflexes) disappear (Pierrot-Deseilligny & Mazevet, 2000). Consequently, the amplitude delivered during NMES needs to be low enough to avoid antidromic block, but sufficiently high to produce functional contractions (Bergquist, Clair, Lagerquist, et al., 2011).

The effect of pulse duration on fatigability is still not well established. For instance, Gorgey et al. (2006) found that a NMES protocol with 150 μs produced the same amount of fatigability than a NMES protocol with a longer pulse duration of 450 μs in the quadriceps muscles. Conversely, another study also in the quadriceps muscles described a higher fatigability during a NMES protocol of 200 μs at 50 Hz compared to 500 μs at 20 Hz. However, fatigability was mainly attributed to the higher frequency of stimulation applied (Gregory et al., 2007). Although the relationship between pulse duration and fatigability is still not clear, changes in pulse duration can affect the relative recruitment of sensory and motor axons during electrically-evoked contractions. The use of short pulse durations (0.06 – 0.4 ms) recruits MUs mainly through peripheral pathways by activating motor axons (Grill & Mortimer, 1996). On the other hand, longer pulse durations (0.5 to 1 ms) can result in the preferentialrecruitment of MUs through central pathways by activating sensory axons (Veale, Mark, & Rees, 1973; Wegrzyk et al., 2015). For example, Collins et al. (2001) found that contractions could be generated in part through reflex pathways when NMES was delivered at 100 Hz using 1 ms pulse durations. This preferential recruitment of sensory axons with longer pulse durations is due to sensory axons having a lower rheobase and longer strength-duration time constant compared to motor axons (Bostock & Rothwell, 1997; Burke et al., 2001; Panizza et al., 1998). Rheobase refers to the minimal stimulus intensity necessary to activate the axon given an infinitely long pulse duration, while the strength-duration time constant is the rate at which the threshold to generate an action potential decreases as the pulse duration increases (Burke et al., 2001). The smaller rheobase and longer strength-duration time constant of sensory axons relative to motor axons are attributed to a greater density of persistent sodium channels (~2.5 vs 1% of all sodium channels, respectively) which have a lower threshold for activation compared to the typical transient sodium channel (Bostock & Rothwell, 1997; Burke et al., 2001). As described in the next section, generating contractions via reflex pathways through the spinal cord has potential for reducing contraction fatigability during NMES.

1.4 Approaches to reduce fatigability during NMES

Several strategies have been developed to reduce fatigability of contractions produced by NMES. In this thesis, we investigated three NMES approaches that were designed to reduce fatigability during NMES by incorporating physiological principles that minimise fatigability during voluntary contractions. Specifically, these approaches were designed to recruit motor units in their normal physiological order and within their physiological range of discharge frequencies.

1.4.1 Stimulation over the nerve trunk and recruitment of fatigue-resistant MUs

Stimulation over the nerve trunk for muscles such as triceps surae (Bergquist, Clair, & Collins, 2011) and quadriceps (Bergquist et al., 2012) can recruit MUs through central pathways. As was mentioned in Section 1.1.3.2, with stimulation of sensory axons, MUs can be recruited in order, as during voluntary contractions, where fatigue-resistant MUs are recruited first (Henneman's size principle). A study by Bergquist et al. (2014) found that NMES over the tibial nerve produced less fatigability when contractions where evoked, at least in part, through central pathways than when contractions were generated only through peripheral pathways. In particular, when NMES generated contractions in part through H-reflexes, torque decreased only 39%, but when contractions where generated only through peripheral pathways, torque decreased ~70%. However, only 50% of the participants (n=4) generated contractions with contribution of H-reflexes (Figure 1-2). Thus, even though stimulation over the nerve can generate contractions through central pathways, the contribution of central pathways can be highly variable between individuals (Wegrzyk et al., 2015).

When NMES is applied over the muscle belly, the depolarisation of motor axons recruits superficial MUs preferentially (See Section 1.4.2). However, when NMES is applied over a nerve, MUs recruited by the depolarisation of motor axons are evenly distributed throughout the muscle, regardless of the amplitude of the stimulation (Okuma et al., 2013; Rodriguez-Falces et al., 2013). In the quadriceps muscles, fatigue-resistant MUs are mainly located in deeper portions of the muscles and fast-fatigable MUs are mainly located in the surface (Lexell et al., 1986). Therefore, when NMES is applied over the femoral nerve trunk (hereinafter refer to as NERVE), likely a greater amount of fatigue-resistant MUs would be activated during NERVE than during conventional NMES. Recently in our laboratory, we compared NERVE with conventional NMES over the quadriceps muscles during a fatigue protocol of 170 contractions 0.3 sec “on”, 0.7 sec “off” (at 40 Hz frequency of stimulation and 1 ms of pulse duration) in ten healthy participants (Lou-Claveria, unpublished). During conventional NMES, torque dropped by 46% from the beginning (first five contractions) to the end (last five contractions) of the fatigue protocol, while during NERVE there was no significant drop in torque. Hence, fatigability was not produced during NERVE which may be related to a greater activation of fatigue-resistant fibers through both central (H-reflex) and peripheral (M-wave) pathways.

1.4.2 Sequential NMES and reduction of MUs discharge rates

Given the relationship between high stimulation frequencies and contraction fatigability (Vanderthommen et al., 2003; Wegrzyk et al., 2015) (see Section 1.3.2), a variety of strategies have been developed to reduce MU discharge rates during NMES. One of these approaches is Sequential NMES (Nguyen et al., 2011), also called Multimodal or Asynchronous NMES (Popovic & Malesevic, 2009). Sequential NMES (SEQ) consists of rotating stimulation pulses between different electrodes over the muscle belly. For example, by rotating stimulus pulses between four electrodes, a net frequency of 40 Hz can be delivered to the whole muscle, but only 10 Hz would be delivered at each stimulation site. The rationale is that different MUs will be recruited by each stimulation site, thus MUs will discharge at lower frequencies than during conventional NMES delivered at the same net frequency. Sayenko et al (2014) demonstrated that during SEQ different M-waves were produced at each stimulation site in the gastrocnemius muscle suggesting a different set of MUs alternatively recruited under each stimulation site. Thus, SEQ reduces the frequency of stimulation at each stimulation site and also crudely mimics the asynchronous discharge pattern of MUs that occurs during voluntary contractions. Furthermore, compared to conventional NMES, SEQ has consistently reduced fatigability in individuals with no neurological impairment (Bergquist et al., 2016; Downey, Tate, Kawai, & Dixon, 2014; Maneski et al., 2013) and in those with a SCI (Malesevic, Popovic, Schwirtlich, & Popovic, 2010; Popovic & Malesevic, 2009; Sayenko et al., 2015). In people with a SCI, SEQ has been shown to reduce fatigability in the knee-extensors and flexors, and planti- and dorsi-flexors compared to conventional NMES (Downey et al., 2015; Malesevic et al., 2010; Nguyen et al., 2011; Popovic & Malesevic, 2009; Sayenko et al., 2015).

1.4.3 New NMES type: HYBRID

Given the promising results shown previously for NERVE and SEQ in reducing fatigability, we developed a new type of NMES that we called hybrid NMES (HYBRID) which combines NERVE and SEQ. HYBRID brings together the main features of NERVE and SEQ by rotating stimulation pulses between three electrodes over the quadriceps muscles and one over the femoral nerve, with the goal of reducing fatigability more than either modality alone. By rotating pulses at low frequency across four electrodes, distinct MUs would be recruited from each stimulation site, lowering MU discharge rates, and the portion of MUs recruited via central pathways could be augmented by the femoral nerve stimulation.

1.5 Summary

As described in the preceding sections, NERVE and SEQ have been shown to reduce fatigability compared to conventional NMES. Presently, no research has compared NERVE and SEQ on fatigability. During NERVE, contractions can be generated in part through central pathways, whereby MUs are recruited according to their physiological order, with fatigue-resistant MUs being recruited first (Bergquist et al., 2012). In addition, during NERVE, MUs located both superficial and deep portions of the muscle can be recruited (Okuma et al., 2013). Since, fatigue-resistant MUs are mainly located in the deeper portions of the quadriceps muscles (Lexell et al., 1986) more fatigue-resistant MUs can be activated during NERVE than during conventional NMES.

SEQ, on the other hand, involves the rotation of stimulation pulses between multiple electrodes over a muscle belly. The idea is that different MUs will be recruited at each stimulation site, reducing MU discharge rates compared to conventional NMES delivered at the same net frequency which reduces fatigability (Bergquist et al., 2016; Downey et al., 2015; Ibitoye, Hamzaid, Hasnan, Abdul Wahab, & Davis, 2016; Malesevic et al., 2010; Maneski et al., 2013; Popovic, Malesevic, & Popovic, 2009; Sayenko et al., 2015).

Due to the effectiveness of NERVE and SEQ on reducing fatigability, HYBRID was developed to combine these two NMES types. HYBRID combines NERVE and SEQ by rotating stimulation pulses over different portions of the muscle and over the femoral nerve. My MSc research was designed to compare key outcome measures between these three type of NMES when they were used to generate contractions of the quadriceps muscles, the main muscles stimulated for NMES rehabilitation (Bax, Staes, & Verhagen, 2005). This study represents a first step towards implementing novel NMES types into rehabilitation with the aim of reducing contraction fatigability. This project was conducted on non-injured participants as a proof of concept. The results will help develop a knowledge-base upon which to establish best practices for using NMES to generate contractions of the quadriceps muscles for rehabilitation after injury or disease of the nervous system.

1.6 Thesis Objectives

The main goal of my MSc research was to determine which type of NMES (NERVE, SEQ or HYBRID) reduces fatigability to the greatest extent during stimulation of the quadriceps muscles.

I hypothesized that HYBRID would produce less fatigability than NERVE and SEQ due to the combination of reduced MU discharge rates, recruitment of MUs in both superficial and deep portions of the quadriceps and the recruitment of MUs through central pathways.

Secondary goals included assessing the variability in torque between consecutive contractions and the discomfort associated with each NMES type as both are important when considering practical applications of NMES.

I hypothesised that NERVE would produce more variability in torque between consecutives contractions. This hypothesis was based on the results of previous work showing a high variability in torque between contractions during stimulation over the nerve (Baldwin et al., 2006; Bergquist et al., 2012).

I also hypothesised that SEQ would produce the most discomfort and NERVE would produce the least discomfort. This hypothesis was based on previous work indicating that stimulation over a nerve requires less current and produces less discomfort than stimulation over a muscle (Bergquist, Clair, & Collins, 2011; Bergquist et al., 2012).

1.7 Figures

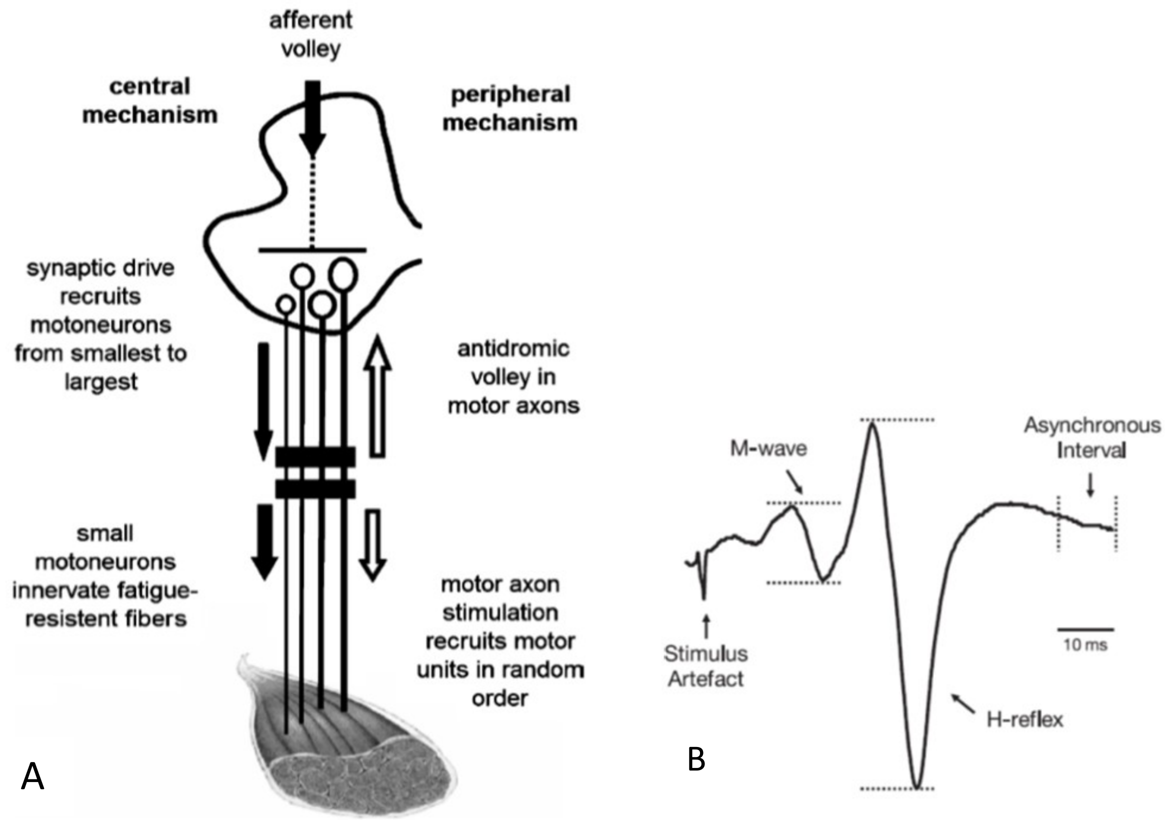

Figure 1-1 A. Schematic showing central and peripheral pathways that contribute to electrically-evoked contractions. Motor units (MUs) can be recruited by the depolarization of motor axons (peripheral mechanism), and can be seen in the electromyographic (EMG) signals as M-waves (B). MUs can also be recruited by the depolarization of sensory axons (central mechanism), and in the EMG can be seen as H-reflexes (B). In addition, antidromic signals from the motor axon (signals along the motor axons to the spinal cord) are shown. Figures extracted from Collins 2007 and Bergquist et al 2012.

[redacted]

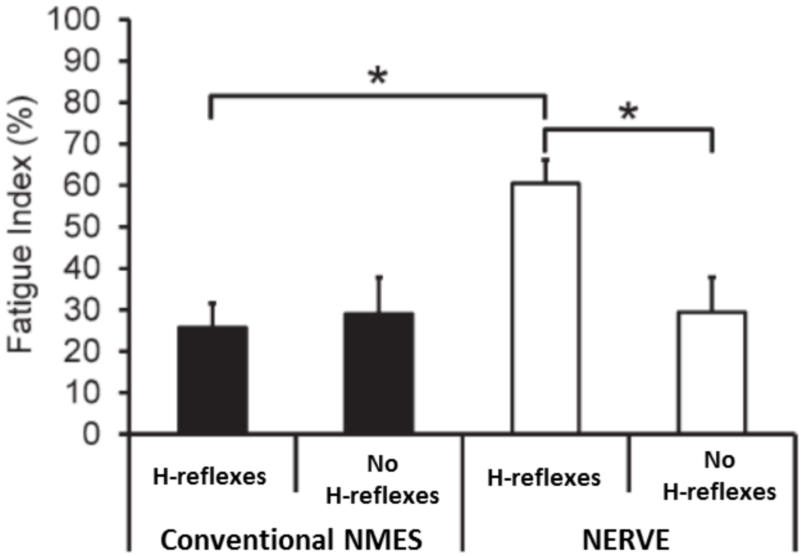

Figure 1-2. Difference between fatigue index (greater fatigue index means lower contraction fatigue) between individuals who elicited H-reflexes and individuals who did not during conventional NMES and NERVE. Extracted from Bergquist et al 2014.

CHAPTER 2: COMPARISON OF THREE TYPES OF NEUROMUSCULAR ELECTRICAL STIMULATION ON FATIGABILITY IN THE QUADRICEPS MUSCLES

2.1 Introduction

After a spinal cord injury (SCI), secondary complications due to inactivity include muscle atrophy, loss of bone mineral density, and decreased cardiovascular fitness. In fact, cardiovascular disease is the major cause of mortality in this population (Chih-Wei et al., 2011; Deley et al., 2015; Kocina, 1997; Shields, 2002). One approach to combat inactivity is neuromuscular electrical stimulation (NMES), which produces contractions of paralyzed muscles by the activation of nerve branches (Deley et al., 2015; Sheffler & Chae, 2007). In this way, NMES can provide opportunities for cardiovascular conditioning, prevention of muscular atrophy and reduced bone mineral density through exercise (Deley et al., 2015; Hamid & Hayek, 2008). Most commonly the stimulation is applied through two electrodes over the muscle belly at a stimulation frequency of ~ 40 Hz, which hereafter will be referred to as conventional NMES. During NMES-exercise applications where reversing deconditioning is the primary focus, the quadriceps muscles group is most commonly stimulated (Bax et al., 2005; Ibitoye et al., 2016). Unfortunately, rapid contraction fatigability is currently a major limitation to the widespread use and potential benefits of NMES-based rehabilitation programs (Bickel et al., 2011; Ibitoye et al., 2016; Maffiuletti, 2010).

Contraction fatigability is the progressive loss of torque generated by a muscle over time and occurs in large part due to the non-physiological way contractions are evoked during NMES (Enoka & Duchateau, 2008). Unlike voluntary contractions, NMES recruits motor units (MUs) in a random order (Gregory & Bickel, 2005b; Jubeau, Gondin, Martin, Sartorio, & Maffiuletti, 2007). As a result, fewer fatigue-resistant MUs are recruited than during voluntary contractions of a similar amplitude (Bickel et al., 2011; Gregory & Bickel, 2005b). In addition, unlike voluntary contractions whereby MUs discharge asynchronously from one and other, during NMES MUs discharge synchronously, time-locked to each stimulation pulse (Gregory & Bickel, 2005a). Therefore, to produce fused contractions of sufficient amplitude, higher discharge frequencies are needed than during voluntary contractions of equal amplitude (Bickel et al., 2011; Vanderthommen et al., 2003). During NMES, the random MU recruitment and higher discharge frequencies increase metabolic demand, and compromise neuromuscular transmission, leading to rapid contraction fatigability (Bickel et al., 2011; Jones et al., 1979; Maffiuletti, 2010; Vanderthommen et al., 2003). Contraction fatigability restricts the duration of NMES sessions, limiting the benefits of NMES-based programs.

Many approaches have been developed to reduce fatigability during NMES. Some have been designed to mimic the natural MU recruitment order that occurs during voluntary contractions, whereby fatigue-resistant fibers are recruited first. During conventional NMES, superficial MUs closest to the stimulation electrode are preferentially recruited (Vanderthommen et al., 2000). However, when stimulation is applied through surface electrodes over a nerve trunk (nerve stimulation), MUs recruited by the depolarisation of motor axons are evenly distributed throughout the muscle (Okuma et al., 2013; Rodriguez-Falces et al., 2013). Since fatigue-resistant fibers are preferentially located deeper in the quadriceps muscle (Lexell et al., 1986), there is likely a higher activation of fatigue-resistant fibers during stimulation over the femoral nerve than during conventional NMES. Accordingly, preliminary data from our lab suggests that there is less fatigability during stimulation over the femoral nerve than during stimulation over the quadriceps muscle belly (Lou, Claveria-Gonzalez, Barss, & Collins, unpublished). Throughout this chapter, NMES over the femoral nerve will be referred to as NERVE. During conventional NMES contractions are generated predominantly through peripheral pathways, by the depolarization of motor axons. Conversely, during NERVE, contractions can be also produced through central pathways, as sensory axons are depolarized and MUs are recruited synaptically via reflex pathways through the spinal cord (Bergquist et al., 2012). This recruits MUs in their natural order with fatigue-resistant MUs recruited first (Bergquist, Clair, Lagerquist, et al., 2011). MUs recruited via central pathways are represented in the surface EMG as H-reflexes (Bergquist, Clair, Lagerquist, et al., 2011; Bergquist et al., 2012; Collins et al., 2001) or asasynchronous activity that is not time-locked to each stimulus pulse (Bergquist, Clair, Lagerquist, et al., 2011). Contractions evoked in part through central pathways can reduce fatigability, however, not all individuals produce contractions through central paths during NMES (Bergquist et al., 2014; Wegrzyk et al., 2015).

Another way to reduce fatigability during NMES is by reducing MU discharge rates (Bickel et al., 2011; Gorgey et al., 2006; Maffiuletti, 2010). Therefore, researchers have explored distributing stimulus pulses between multiple electrodes over the muscle belly or splitting stimulation pulses between the muscle belly and nerve trunk (Lou, Bergquist, Aldayel, Czitron, & Collins, 2017). Sequential NMES (SEQ) (Popovic & Malesevic, 2009), also referred to as "multi-pad electrode NMES" (Nguyen et al., 2011) or "asynchronous NMES" (Downey et al., 2014) involves the rotation of stimulation pulses across multiple electrodes over different portions of a muscle group. The rationale is that different MUs will be recruited by each stimulation site, thus MUs will discharge at lower frequencies than during conventional NMES delivered at the same net frequency. In this way, these distributed NMES approaches crudely mimic the asynchronous firing that occurs during voluntary contractions (Nguyen et al., 2011; Popovic & Malesevic, 2009). SEQ has consistently been shown to reduce fatigability compared to conventional NMES in the quadriceps muscle and has been highlighted as a primary candidate for clinical translation (Barss et al., 2017; Downey et al., 2015; Popovic & Malesevic, 2009; Sayenko et al., 2015).

Given that NERVE and SEQ reduce fatigability compared to conventional NMES, we developed a novel type of stimulation coined hybrid NMES (HYBRID) which combines features of NERVE and SEQ with the goal of reducing fatigability to a greater extent than either modality alone. HYBRID involves rotating stimulation pulses between three electrodes over the muscle belly and one over the nerve trunk.

The addition of the nerve electrode allows for the activation of MUs in deep portions of the quadriceps muscles and increases chances of recruiting MUs via central pathways (Bergquist et al., 2014). Thus, by rotating pulses of stimulation between the four electrodes during HYBRID, we anticipate that distinct MUs from superficial and deep portions of the muscle will be recruited, resulting in less overlap than during SEQ (i.e. less MUs activated by more than one stimulation site), and some MUs will be recruited via central paths.

While contraction fatigability is the primary outcome variable within the current study, variability in torque between consecutive contractions and discomfort were also measured as they can also limit the benefits of NMES-based programs. Previously, stimulation over the nerve has resulted in a higher variability in torque production compared to muscle stimulation (Baldwin et al., 2006; Bergquist et al., 2012). This high variability between consecutives contractions could limit the practical application of NMES for functional tasks. Discomfort during NMES prevents individuals from participating in NMES-based training programs or from training at contraction intensities sufficiently high to induce physiological adaptations (Lai et al., 1988). Discomfort during NMES is a consequence of the stimulation of nociceptive afferents and is most clearly related with the amount of current used (Maffiuletti, 2010). In the quadriceps muscles, discomfort between nerve and muscle stimulation has not been compared. However, for the tibialis anterior muscle, NMES over the common peroneal nerve required less current and caused less discomfort than NMES over the muscle belly (Naaman et al., 2000). Furthermore, Bergquist et al. (2012) showed that to stimulate the quadriceps muscles, ~70% less current was required for NERVE compared to conventional NMES, and that participants only reported discomfort during conventional NMES, but not during NERVE.

The main objectives of this study were to compare fatigability, contraction variability and discomfort between SEQ, NERVE, and HYBRID of the quadriceps muscles group. It was hypothesized that HYBRID would result in less contraction fatigue than NERVE and SEQ due to the combination of reduced MU discharge rates, recruitment of MUs in both superficial and deep portions of the quadriceps and the recruitment of MUs through central pathways. It was also hypothesized that NERVE would produce the most variability between contractions, based on previous studies in which high variability was found during nerve stimulation (Baldwin et al., 2006; Bergquist et al., 2012). Finally, it was hypothesized that SEQ would result in greater discomfort than HYBRID, with NERVE producing the least discomfort, due to in previous work stimulation over a nerve required less current and produces less discomfort than stimulation over a muscle (Bergquist, Clair, & Collins, 2011; Bergquist et al., 2012).

2.2 Methods

2.2.1 Participants

Seventeen participants with no known neurological or musculoskeletal impairment volunteered for the present study. Of these seventeen three were unable to tolerate the stimulation intensity required to evoke 15% MVC and were thus excluded from the study. Therefore, data from fourteen participants (10 males and 4 females) aged 28.3 ± 9.1 were included in the analysis for this study.

After providing written informed consent, participants took part in three experimental sessions each lasting ~1.5 to 2 hours. One type of NMES (NERVE, SEQ or HYBRID) was delivered during each session, and each experimental session was separated by at least 48 hrs. The order of the NMES sessions was randomized for each participant. This study was approved by the Human Research Ethics Board at the University of Alberta.

2.2.2. Experimental setup

Participants were seated in the chair of a Biodex dynamometer (System 3, Biodex Medical System, Shirley, New York). All procedures were performed on the right leg with the hip and knee maintained at ~120° and ~85° respectively. The right leg was secured to the dynamometer above the ankle with center of rotation of the knee aligned with the axis of rotation of the dynamometer.

Electromyography

Surface electromyography (EMG) was recorded from the vastus medialis (VM) and vastus lateralis (VL) muscles, as shown in Figure 2-1. The skin was exfoliated with sand paper and cleaned with alcohol swabs. Adhesive electrodes (7.76 cm2; Tenby Medical, Canada) were placed in a bipolar configuration parallel to the predicted path of the muscle fibers with ~ 1 cm inter-electrode distance, and a reference electrode over the knee. The electrodes were placed according to SENIAM guidelines (Hermens & Freriks, 2007). EMG data were amplified 500 times and band-pass filtered at 10 to 1000 Hz (NeuroLog System; Digitimer, Welwyn Garden City, UK).

Neuromuscular electrical stimulation (NMES)

Stimulating electrodes were placed on the quadriceps muscle belly and/or femoral nerve trunk depending on the experimental session. Electrical stimulation in the form of monophasic square-wave pulses was delivered via a Digitimer DS7AH stimulator (Digitimer, Weylwyn Garden City, UK), with a constant current. This device was used for all experiments.

For NERVE**,** one cathode (1.25 cm round; Richmar; Chattanooga, US) was placed over the femoral nerve approximately in the middle of the femoral triangle as shown in Figure 2-1B with the anode (2x3.5 cm; Richmar, Chattanooga, US) placed over the gluteal fold. To improve the stability of stimulation to the nerve, participants wore a fall harness (MSA Safety Incorporated, Edmonton, Alberta, Canada) that was modified to provide pressure over the cathode and hold it in place. Depending on the participant, the time required to place the cathode in the optimal position and adjust the harness to produce sufficient pressure over the cathode to generate a desired contraction could take more than 15 minutes.

For SEQ, four electrodes were placed over the quadriceps muscles. As demonstrated in Figure 2-1C, electrode M1 targeted the motor points of the vastus medialis (VM), M2 and M3 vastus lateralis (VL), and M4 rectus femoralis (RF), (Botter et al., 2011). To deliver SEQ a custom-build stimulation distributor was connected to the Digitimer DS7AH stimulator. SEQ was delivered without a common anode, and instead the anode and cathode rotated among the four electrodes. This configuration was selected based on the results of a pilot experiment conducted in 8 participants in which fatigability was not different between this anode and cathode rotation configuration (See appendix), and the more commonly used configuration using a common anode (Malesevic et al., 2010; Popovic & Malesevic, 2009). HYBRID was accomplished by rotating stimulus pulses between one electrode over the femoral nerve and three over the muscle belly. As is shown in Figure 2-1D, M1 was placed between VM and VL and electrodes M2 and M3 targeted the motor points of VL and RF, respectively. M1 was positioned approximately a third of the distance between the anterior superior iliac spine to the center of the patella, covering similar muscle portions as M2 and M1 during SEQ. A cathode was positioned over femoral nerve with an anode over gluteal fold in the same manner as NERVE. In a similar fashion to SEQ, the cathode and anode rotated between the three electrodes over the muscle, while the femoral nerve had a unique anode over the gluteal fold.

2.2.3. Experiment procedures

Recruitment Curve

At the beginning of each experimental session A recruitment curve was constructed from responses to 30 stimulation pulses applied to the femoral nerve. M-waves and H-reflexes recorded during each recruitment curve were quantified peak-to-peak and normalized to each participant's single largest M-wave from the recruitment curves collected on the same day (Mmax).

Maximal Voluntary Contractions

At the beginning of each session, participants performed two isometric knee extension MVCs, extending the knee against the arm of the dynamometer with their maximal force for 3-5 s. If the peak torque produced during 2 consecutive MVCs was not within ~10% of each other, a third MVC was performed. These MVCs were separated from each other by 1 minute. Participants received verbal encouragement to perform maximally and visual feedback of their torque on a computer monitor. The MVC torque was quantified over a 0.3 s window centered on the peak during each MVC, and the MVC value was calculated as the average of 2 MVCs within 10% of each other. These MVCs were used to normalize torque for each participant and set target levels for each of the fatigue protocols.

Setting stimulation amplitudes

Five minutes after collecting MVCs, the stimulation amplitude was determined by delivering trains of NMES for 0.3 s at 40 Hz until the target amplitude of 15 to 20% of each participant's MVC was reached. For SEQ, the same current was delivered to each electrode with no attempt being made to produce the same amount of torque at each electrode. However, when the same current was delivered to each electrode during HYBRID, the nerve electrode produced much higher torque than the muscle electrodes. Therefore, in order to produce a similar amount of torque at each stimulation site, we reduced the current delivered to the nerve until the torque evoked by the nerve produced ~ 5 to 8 %MVC. Similar to SEQ, the amount of torque produced by each of the three muscle electrodes was not matched during HYBRID. The chosen stimulation amplitude was then maintained for the duration of the experiment.

Pre- and post-fatigue testing trains

Prior to and immediately after the fatigue protocols, a series of trains were delivered at the previously chosen stimulation intensity, as is shown in Figure 2-1A. Initially, one train was delivered at 40 Hz for NERVE, while for SEQ and HYBRID one train at 10 Hz was sent to each electrode. During these trains, the torque generated, and the current delivered to each electrode was measured. Subsequently to potentiate the muscle, the participants were asked to perform one MVC that was followed by 2 one-second trains at 20 Hz. Immediately after potentiation, 2 trains one-second long were delivered at 10 and at 100 Hz, to assess low frequency fatigue (see below, page 47) (Bergquist et al., 2016; Mahoney et al., 2007).

Fatigue protocol

The fatigue protocol consisted of 180 isometric contractions (0.3 sec on/0.7 sec off) produced by NMES delivered at 40 Hz at the previously chosen stimulation intensity. During each fatigue protocol, participants were asked to remain relaxed and received frequent reminders to not voluntarily contract.

Discomfort

To assess discomfort, a Visual Analogue Scale (VAS) was used in a similar fashion as has been done in previous work (Broderick, Kennedy, Breen, Kearns, & G, 2011; Clarke Moloney, Lyons, Breen, Burke, & Grace, 2006). The VAS consists of a 100-mm horizontal line upon which participants draw a line to represent the level of discomfort with respect to the labels at each end of the scale. The left end indicates "no pain" and the right end indicates "maximal tolerable pain". Participants were asked to evaluate discomfort half-way through the fatigue protocol immediately after contraction 90.

2.2.4 Data Acquisition and Analyses

Data were sampled at 5000 Hz using custom-written Labview software (National Instruments, Austin, TX) and stored on hard drive for subsequent analysis that was conducted using custom-written Matlab software (The Mathworks, Natick, MA). Torque for each contraction during the fatigue protocol was assessed as the region around the peak averaged over 0.3 s and normalized to each participant's MVC. During the fatigue protocol, contractions were binned by averaging 6 successive contractions (e.g. bin 1 = contraction 1 to 6, bin 2 = contraction 7 to 12), resulting in 30 bins. The following variables of interest were measured:

Contraction Fatigability

Contraction fatigability was assessed by measuring the percent change in torque from the beginning to the end of the fatigue protocol. Percent change in torque was assessed from the first six contractions (bin 1) to the last six contractions (bin 30) of the fatigue protocol and calculated according to the equation below.

$$\%\,Change = \frac{Bin\,30\ - Bin\,1}{Bin\,1} \times 100$$

A more negative value represents a greater decline in torque, indicative of greater contraction fatigability.

H- reflex amplitudes

H-reflexes generated by the last pulse of each train during the fatigue protocol were quantified peak-to-peak and normalized to Mmax to determine whether a participant's contractions were evoked in part through central pathways. The following criteria were used to determine if H-reflexes were present (Bergquist et al., 2014): (1) during the M-H recruitment curve, a peak-to-peak amplitude greater than 2% at the H-latency of ~25 and 50ms, and (2) at least 10% of the contractions during the fatigue protocol presented H-reflexes with a peak-to-peak amplitude greater than 2%. During SEQ and HYBRID noise in the EMG data limited our ability to measure M waves and H-reflexes during the fatigue protocol. Therefore, H-reflexes were only evaluated during NERVE.

Contraction Variability

The variability in torque between successive was quantified using a formula proposed by Bergquist et al. (2016). A trendline was formed by fitting the peak torque of the 180 contractions of the fatigue protocol (observed values) with a sixth order polynomial (predicted values) for each participant. The residuals represent the differences between the observed values and the trendline. The absolute values of the residuals were then divided by the corresponding predicted values and the variability is represented by the average of these ratios and reported as a percentage. Thus, variability was calculated as:

$$\text{Variability} = \left(\frac{\sum_{i=1}^{n=180} \frac{|residual(x_i)|}{trendline(x_i)}}{n} \right) \times 100$$

Low frequency fatigue ratio

Low frequency fatigue is defined as a greater loss of force at low (10 – 40 Hz) vs high stimulation frequencies (Keeton & Binder-Macleod, 2006). Low frequency fatigue was quantified by measuring changes in the ratio of peak torque generated by the average of 2 one-second 10 Hz testing train, and the average of 2 one-second 100 Hz testing trains, before and after the fatigue protocol. For SEQ and HYBRID, low frequency fatigue was assessed for each stimulation channel individually and then the ratios for each electrode were averaged to obtain a single representative value for each NMES type. A decline in this ratio means more low frequency fatigue, and it was calculated as:

$$\frac{10}{100} Hz\ Ratio = (10/100\ \ Hz\ ratio\ After)/(10/100\ \ Hz\ ratio\ Before)$$

Percent change in torque during pre- and-post trains

The percent change in torque between NMES trains delivered before and after the fatigue protocol at 40 Hz for NERVE, and at 10 Hz for SEQ and HYBRID was compared between NMES types. To compare the percent change in torque between NERVE and both SEQ and HYBRID, the percent change per electrode during SEQ and HYBRID was averaged. In addition, for SEQ and HYBRID the decline in torque produced at each electrode for each NMES type was assessed.

Current intensity and current density

Current is the factor primarily associated with discomfort (Delitto et al., 1992; Naaman et al., 2000); therefore, the amount of current delivered at each electrode was measured using a current probe (mA 2000 Noncontact Milliammeter; Bell Technologies, Orlando, Florida). In addition, current density was assessed to account for the differences in electrode size between muscle and nerve stimulation during NERVE, SEQ and HYBRID as it has also been associated with discomfort (Martinsen et al., 2004). Current density was calculated as:

$$\text{Current density} = \frac{Current\ (mA)}{electrode\ size\ (cm^2)}$$

Discomfort

Discomfort was assessed by measuring with a ruler the distance in millimetres between the mark made by the participant and the start of the line on the left side.

2.2.5 Statistical Analyses

Each variable was first calculated within a participant and then grouped for statistical analysis. Statistical analyses were performed using IBM SPSS software (IBM, Armonk, New York)). All data were entered into a database on a password protected computer in the Human Neurophysiology Laboratory at the University of Alberta. All data were normally distributed as determined using a Shapiro-Wilk test. For all statistical tests, the significance level was set at $\alpha = 0.05$. To determine the influence of NMES type (NERVE, SEQ, HYBRID) on percent decline in torque, variability of contractions, low frequency fatigue ratio, current, current density and percent reduction in torque during pre and post trains separate 1 x 3 ($NMES_{type}$) rm ANOVA test were conducted. To determine whether there was a significant decline in torque from the beginning (bin 1) to the end (bin 30) of the fatigue protocol a 2 (TIME) x 3 ($NMES_{type}$) ANOVA test was conducted. For current, due to inconsistencies in the measurement of one participant during the NERVE condition, data were analyzed for 13 participants. The measurement of current density was calculated per electrode for SEQ and HYBRID and then averaged. For SEQ and HYBRID a 1 x 4 rm ANOVA was conducted to identify differences in the percent reduction in torque between pre-and post-trains. For HYBRID, data were analyzed for 13 participants as quantification of one participants torque data were not possible. In addition, a 1 x 4 (electrode) rm ANOVA was conducted to determine any baseline differences in evoked torque between each electrode for SEQ and HYBRID at the chosen stimulation intensity. In addition, a linear regression analysis was performed to determine the relationship between current and discomfort (VAS). For all statistical analyses, Fisher's Least Significant Difference pairwise comparisons were employed where appropriate to test significant main effects and interactions. In addition, for rm ANOVA, whenever sphericity assumption was violated, a Greenhouse-Geisser correction was utilized. Participants' descriptive statistics are reported as mean ± 1 standard deviation.

2.3 Results

2.3.1 Torque during the fatigue protocol

Data recorded from a single participant during each of the 3 NMES fatigue protocols are shown in Figure 2-2. Torque recorded during the first 6 (bin 1) and last 6 (bin 30) contractions are shown in panel A and the peak torque generated during each of the 180 contractions of all three fatigue protocols are shown in panel B. Across the three NMES types, the mean torque at the beginning of the fatigue protocol (bin 1) was 20±1.2 %MVC (range 19-22 %MVC) for this participant. In contrast, during NERVE, torque was variable from one contraction to the next and declined very little from the beginning to the end of fatigue protocol. During both SEQ and HYBRID torque was relatively stable from one contraction to the next and tended to decline more rapidly over the first half of the fatigue protocol than the latter half.

Torque recorded during the NERVE, HYBRID and SEQ fatigue protocols averaged across the group of 14 participants is shown in Figure 2-3. Panel A shows torque for each of the 30 bins over the duration of the fatigue protocols for all three types of NMES. Torque recorded at the beginning of the fatigue protocols (bin 1; leftmost dashed box) was not significantly different between NMES types (1x3 rmANOVA, $F_{(2,26)} = 1.553$, $p = 0.230$) and was on average 17.2±3.5 %MVC (range 16.5 – 18.8 %MVC). To assess differences in decline in torque, during the fatigue protocol between NMES types, calculated as a percent change from bin 1 to bin 30, a 3 ($NMES_{type}$) x 1 (TIME) rmANOVA was run. There were no significant differences in the decline in torque across NMES types, ($F_{(1.3,17.4)} = 1.735$, $p = 0.208$; Panel B). On average, torque declined 26.3± 8.0 % (range 17.1 – 31.1 %) across NMES types. To assess whether there was a significant decline in torque during each fatigue protocol a 3 ($NMES_{type}$) x 2 (TIME) rm ANOVA was run. There was a significant main effect of TIME ($F_{(1,13)} = 69.28$, $p < 0.001$), with ~5 %MVC decline in torque from bin 1 to bin 30. There was no main effect of $NMES_{type}$ ($F_{(2,26)} = 3.949$, $p = 0.061$) which was on average 14.9±2.9 %MVC (range 13.8±2.9 – 16.8±2.9). No interaction was found between $NMES_{type}$ and TIME ($F_{(2,26)} = 0.628$, $p = 0.541$). This indicates that there was a significant decline in torque during each fatigue protocol that did not differ between NMES types (data not shown).

Based on the criteria outlined in the Methods, four of the fourteen participants generated contractions in part through H-reflexes during NERVE. These individuals are indicated by open circles in Figure 2-3B. During the NERVE fatigue protocols, torque declined 5.9±23 % when contractions were generated in part via H-reflexes and torque declined 21.5±36 % when no H-reflexes were produced. Statistical analyses were not performed on these data due to the small sample size.

Variability in the torque produced between successive contractions for the group is shown in Figure 2-4. The 1 x 3 ($NMES_{type}$) rmANOVA indicated a significant main effect of $NMES_{type}$ ($F_{(2,26)} = 14.559$, $p < 0.001$). Post-hoc tests identified significant differences between all three NMES types with NERVE generating contractions that varied the most (8.6±4.0 %) while SEQ generated contractions that varied the least (3.0±3.0 %).

For SEQ and HYBRID, the peak torque produced when the stimulation was delivered at 10 Hz to each electrode individually before the fatigue protocol was compared. During SEQ prior to the fatigue protocol, there was no main effect of electrode site on torque ($F_{(3,39)} = 0.369$, $p = 0.77$) which was on average 5.5±1.8 %MVC (range 5.01±1.7 – 5.7±2.1) (see Figure 2-5A). During HYBRID prior to the fatigue protocol, there was a main effect in the torque produced by the four electrodes ($F_{(3,36)} = 4.67$, $p = 0.007$). Post-hoc tests indicated the torque generated by electrode M3 was less than that for electrodes N (4.8±2.2 vs 8.4±2.6 %MVC, $p = 0.002$) and M2 (4.8±2.2 vs 6.9±1.7 %MVC, $p = 0.012$), as is shown in Figure 2-5B.

2.3.2 Pre- and Post-measurements

There was no significant interaction between TIME and $NMES_{type}$ for MVCs performed before and after each fatigue protocol ($F_{(2,26)} = 1.891$, $p = 0.127$; data not shown). There was, however, a main effect of TIME ($F_{(1,13)} = 5.28$, $p = 0.039$) indicating that when pooled across NMES type there was a significant reduction in peak voluntary force production after the fatigue protocol. On average, across NMES types, torque produced during the MVCs decreased 10.9±5.1% from before to after the fatigue protocols. There was no main effect of $NMES_{type}$ ($F_{(2,26)} = 0.474$, $p = 0.628$) indicating that there was a similar reduction in MVC torque between the sessions involving the different NMES types.

Figure 2-6 shows group data for 10/100 Hz ratio, and the change in torque between NMES trains delivered before and after the fatigue protocols. Panel A indicates the level of low frequency fatigue, expressed as the 10/100 Hz ratio, (lower ratio means greater low frequency fatigue) for the three NMES types. For SEQ and HYBRID the 10/100 Hz ratios calculated for each electrode were averaged. There was a main effect of $NMES_{type}$ for the 10/100 Hz ratio ($F_{(2,26)} = 3.966$, $p = 0.031$). Post-hoc comparisons indicate the 10/100 Hz ratio was higher during NERVE ($p = 0.009$) and HYBRID ($p = 0.035$) compared to SEQ, while there was no difference between NERVE and HYBRID ($p = 0.65$). For SEQ and HYBRID, 10/100 Hz ratios were assessed for each electrode site and compared within that NMES type (data not shown). During SEQ (n = 14) there was no difference in 10/100 Hz ratio between electrodes ($F_{(3,39)} = 0.794$, $p = 0.5$), and was on average 0.8±0.2 (range 0.71 – 0.8). During HYBRID (n = 13), there was a significant difference in 10/100 Hz ratio between electrodes ($F_{(3,36)} = 2.815$, $p = 0.053$). Post-hoc analysis indicated low frequency fatigue was greater for the M2 electrode than the N electrode (0.77±0.2 vs 1.5±1.1, $p = 0.042$).

Group data for the percent change in torque between trains of each type of NMES delivered immediately before (pre), and within the first minute after (post-) the fatigue protocols are shown in Figure 2-6B. For NERVE, the percent change was calculated between trains delivered at 40 Hz before and after the fatigue protocols. For SEQ and HYBRID, the percent change was calculated from trains delivered at 10 Hz at each electrode site individually, and the average percent change across the four electrode sites was calculated. There was a significant main effect of $NMES_{type}$ on the percent change in torque between the pre-and post-trains ($F_{(2,26)} = 7.930$, $p = 0.002$). Torque declined less during NERVE than SEQ ($p = 0.004$) and HYBRID ($p = 0.015$), with no difference between SEQ and HYBRID ($p = 0.684$). Accordingly, during NERVE torque recovered faster than during SEQ and HYBRID.

Group data for the percent change in peak torque between the pre and post trains delivered at each electrode site during SEQ are shown in Figure 2-6C. There were no significant differences in the amount of torque decline between the four electrodes ($F_{(3, 39)} = 0.55$, $p = 0.186$) which was on average -33.9±15.5 %, range -37.3 – -29.0 %. For HYBRID, there was a significant difference in percent change between the four electrodes ($F_{(3, 36)} = 8.171$, $p < 0.001$; n = 13, see methods), as depicted in Panel D. Post-hoc analysis indicated torque declined less for the N electrode than the M1 ($p = 0.01$), M2 ($p = 0.016$), and M3 ($p = 0.003$) electrodes. Hence, torque recovered faster in the N electrode than in the muscle electrodes (M1, M2, M3) during HYBRID.

2.3.3 Current and discomfort

Current delivered during the fatigue protocols for each NMES type averaged across 13 participants is shown in Figure 2-7A. There was a significant main effect of $NMES_{type}$ on the current delivered during each fatigue protocol ($F_{(2,24)} = 15{,}407$, $p <$

0.001). Post-hoc analysis indicated all 3 NMES types were different from each other with SEQ requiring the most current (32.5±12.2 mA) and NERVE the least (16.4±4.4 mA). In addition, there was a main effect of $NMES_{type}$ on current density ($F_{(1.08,)}$ = 15,407, $p < 0.001$), whereby current density was highest during NERVE presented (2.04±0.5 mA/cm^2), followed by HYBRID (0.9±0.3 mA/cm^2), and SEQ (0.7±0.2 mA/cm^2) (data not shown).

Figure 2-7B shows group data (n = 14) for the VAS scores used to assess discomfort during the fatigue protocols for each type of NMES. There was a significant main effect of $NMES_{type}$ on the VAS scores ($F_{(2,26)}$ = 15,244, $p < 0.001$). Post-hoc comparisons indicate NERVE produced less discomfort than SEQ ($p < 0.001$) and HYBRID ($p = 0.005$), with no difference between SEQ and HYBRID ($p = 0.058$).

Figure 2-7C shows the linear regression between discomfort and current. A significant linear regression was found between VAS scores and current ($F_{(1,37)}$ = 10.038, $p = 0.003$), with an R^2 of 0.213 which indicates that ~20% of the discomfort can be predicted by the amount of current delivered. According to Cohen's classification, the correlation between current and discomfort was moderate.

2.4 Discussion

This study was designed to compare contraction fatigability, variability and discomfort between SEQ, NERVE and HYBRID. Although there were no differences in fatigability between the three NMES types, SEQ generated contractions with the least variability, and NERVE produced contractions with the least discomfort.

Fatigability

The decline in torque during the fatigue protocols was not different between the three NMES types and on average torque decreased by ~26%. Although fatigability was not different between the three types of NMES, each resulted in less fatigability than has been previously reported during conventional NMES (Lou et al., Unpublished). In a study conducted in our lab, using a similar fatigue protocol as in the present study, torque produced by the quadriceps declined almost twice as much during conventional NMES (46%) as during NERVE, SEQ and HYBRID in the present study (Lou et al., p. unpublished). Furthermore, several studies have directly compared SEQ with conventional NMES and found SEQ to reduce fatigability (Bergquist et al., 2016; Downey et al., 2015; Maneski et al., 2013; Nguyen et al., 2011; Popovic & Malesevic, 2009; Sayenko et al., 2015; Sayenko et al., 2014). For example, torque declined ~30% less during SEQ than during conventional NMES of the quadriceps muscles of individuals with a SCI (Downey et al., 2015; Nguyen et al., 2011; Sayenko et al., 2015). Although HYBRID has not been previously compared with conventional NMES, the decline in torque between NERVE, SEQ and HYBRID was not different. Accordingly, these three NMES types are equally effective in reducing fatigability compared to conventional NMES.

Given that NERVE (Bergquist et al., 2014) and SEQ (Bergquist et al., 2016; Downey et al., 2015; Maneski et al., 2013; Nguyen et al., 2011; Popovic & Malesevic, 2009; Sayenko et al., 2015; Sayenko et al., 2014) were previously shown to reduce fatigability compared to conventional NMES, it was hypothesized that an approach that combined NERVE and SEQ (i.e. HYBRID) would reduce fatigability even further. It was anticipated that HYBRID would reduce MU discharge rates, recruit MUs in both superficial and deep portions of the quadriceps muscles and recruit of MUs via central pathways and that this would result in less contraction fatigability than either NERVE or SEQ. However, this hypothesis was not supported by the data. Although it is uncertain why HYBRID did not result in less contraction fatigability, we suggest some possible explanations and different HYBRID configurations to address these issues. The amount of overlap between stimulation sites may have been similar between HYBRID and SEQ because the nerve electrode during HYBRID may not have recruited a population of MUs that were as distinct from those recruited from the electrodes over the muscle as expected. Similar overlap would result in similar MU discharge rates between SEQ and HYBRID, and accordingly no difference in fatigability. During HYBRID one electrode was located over the nerve trunk and three electrodes were located over the muscle belly instead of four as during SEQ. A potential solution may be to position four electrodes over the muscle belly in addition to the nerve electrode to increase the number of different MUs recruited per contraction and reduce the stimulation frequency delivered to each stimulation site. Another possible explanation for similar levels of fatigability may be that HYBRID recruited few fatigue-resistant MUs. It is important to highlight that despite the greater stimulation frequencies delivered per electrode during NERVE than during SEQ and HYBRID, whereby the 40 Hz delivered to the cathode during NERVE where divided by four electrodes during SEQ and HYBRID, contraction fatigability was similar between the three NMES. This may be explained by a great activation of fatigue-resistant MUs during NERVE. Therefore, perhaps if during HYBRID the ratio of stimulation over the femoral nerve trunk versus over the muscle belly were greater (i.e. higher frequency of stimulation per contraction over the nerve electrode), a greater amount of fatigue-resistant MUs would be recruited, but at a lower frequency than during NERVE. Accordingly, fatigability would be reduced during HYBRID compared to NERVE and SEQ. However, these are only theoretical explanations and future research is needed to assess MU overlap between stimulation sites, and the type of MUs recruited during each of these NMES approaches.

Fatigability can be induced by impairments of one or several physiological processes from the site of stimulation to deep within the muscle (Enoka & Duchateau, 2008; Martin et al., 2016). In the present study, failure of excitation-contraction coupling was assessed because it has been suggested to contribute more to contraction fatigability in people with a SCI than able-bodied individuals (Mahoney et al., 2007). Such a failure has been attributed to reduced conduction of the action potential through the T tubules, decreased Ca^+ release from the sarcoplasmic reticulum, decreased sensitivity of troponin to Ca^+, and/or a reduction of Ca^+ reuptake by the sarcoplasmic reticulum (Jones et al., 1982; Keeton & Binder-Macleod, 2006). A failure of excitation-contraction coupling is also known as low frequency fatigue as it is characterized by a greater loss in force when tested at low than at high frequencies (Keeton & Binder-Macleod, 2006). Thus, low frequency fatigue is often assessed by the change in ratio of force produced at low frequencies (10 Hz) to that produced at high frequencies (100 Hz), whereby a lower ratio suggests greater low frequency fatigue. In the present study, there was a smaller 10/100 Hz ratio after the fatigue protocol during SEQ than during NERVE and HYBRID, suggesting that failure of excitation-contraction coupling contributed more prominently to fatigability during SEQ than during the other NMES fatigue protocols. In addition, low frequency fatigue is also characterised by long recovery times (Jones, 1996; Keeton & Binder-Macleod, 2006). In the present study, this recovery was assessed by comparing the relative decline in torque within one minute after the fatigue protocol for each NMES type (Figure 2-6B). The torque was not different at the end of the fatigue protocol, but it was higher one minute following the fatigue protocol during NERVE compared with SEQ and HYBRID, suggesting a faster recovery time. During HYBRID, the amount of torque generated by each electrode before and within one minute after the fatigue protocol was also compared. Similarly, the torque produced in the nerve electrode recovered faster than the torque produced by the muscle electrodes. Thus, when stimulation was applied over the muscle belly, failure of excitation-contraction coupling mechanism contributed more to fatigability when stimulation was applied over the nerve in the quadriceps muscles. This may be related to a greater activation of fatigue-resistant MUs during nerve stimulation as stimulation over the femoral nerve can activate those fatigue-resistant MUs which are mainly located in deep portions of the quadriceps muscles (Knight & Kamen, 2005; Lexell et al., 1986). Fatigue-resistant MUs are less susceptible to low frequency fatigue than fast-fatigable MUs (Mahoney et al., 2007; Powers & Binder, 1991; Rijkelijkhuizen, de Ruiter, Huijing, & de Haan, 2003). Given that low frequency fatigue is more prevalent during SEQ, strategies may be introduced to minimise the effects of low frequency fatigue. For example, incorporating high frequency bursts in between the electrically elicited contractions can increase muscle performance in people with a SCI (Kebaetse & Binder-Macleod, 2004; Kebaetse, Lee, Johnston, & Binder-Macleod, 2005; Keeton & Binder-Macleod, 2006). Another strategy consists of using variable-frequency trains which has previously shown to increase force production once low frequency fatigue has been induced (Binder-Macleod & Russ, 1999).

Contraction variability

Stimulation over a nerve trunk generally results in more variability in torque between contractions than during conventional NMES for both the quadriceps (Bergquist et al., 2012) and triceps surae muscles (Baldwin et al., 2006). In the present study, NERVE produced the most variability in torque between successive contractions, and SEQ generated contractions with the least variability. During NERVE this inconsistency in torque likely stems, in part, due to movement of the cathode with respect to the nerve trunk and anode. During stimulation of the femoral nerve in the femoral triangle, the electrode can be shifted by the muscle contraction itself. In the present study, although we used a harness to provide pressure over the cathode, we did not assess if this pressure was constantly maintained during the fatigue protocol. On the other hand, stimulation over the muscle produces much more consistent torque between contractions (Baldwin et al., 2006; Bergquist et al., 2016;

Bergquist et al., 2012). Accordingly, in the present study SEQ produced contractions with the least variability. During HYBRID, variability was greater than SEQ but lower than NERVE which may be explained by the combination of nerve and muscle stimulation, whereby the high variability produced by nerve stimulation was counterbalanced by the consistency of torque during muscle stimulation.

Current and discomfort

During NMES, higher stimulation currents are related to more discomfort (Bergquist et al., 2012; Delitto et al., 1992; Wiest, Bergquist, Schimidt, Jones, & Collins, 2017). In the present study, a contraction of ~20 %MVC required ~40% and 50% less current during NERVE than HYBRID and SEQ, respectively. The lower current during NERVE may be because axons are bundled closely together right under relatively small stimulating electrodes. In contrast, when stimulation is applied over the muscle belly, larger electrodes and higher currents are needed because axons are distributed diffusely throughout the muscle (Alon et al., 1994). More current is thought to depolarize more receptors in the skin and throughout the muscle, such as A-delta fibers and some unmyelinated C-fibers (Yu, Chae, Walker, Hart, & Petroski, 2001). As predicted, NERVE produced less discomfort than SEQ and HYBRID. A significant correlation was found between current and discomfort, whereby increases in current were related to more discomfort. Likewise, previous investigations also found that less current was required when stimulation was delivered over the common peroneal nerve than over the tibialis anterior muscle belly to produce the same amount of torque, resulting in less discomfort during nerve stimulation in people with (Naaman et al., 2000) and without a SCI (Wiest, Bergquist, & Collins, 2017).

Discomfort during NMES has also been related to current density. When delivering NMES at a constant current, larger electrodes reduce current density and, consequently, discomfort (Alon et al., 1994). In the present study, different electrode sizes and currents were used to stimulate the nerve and the muscle, therefore, current density was also compared between NMES types. Although, during NERVE current density was significantly higher than HYBRID and SEQ, NERVE generated the least discomfort. Similarly, Martinsen et al. (2004) found that only current but not current density was directly related to the amount of discomfort produced over the hand area during electrical stimulation. The reason for this is that although larger electrodes produce less current density than smaller electrodes at a constant current, larger electrodes stimulate more nerve endings and induce a greater cutaneous sensation than smaller electrodes (Martinsen et al., 2004; Turi et al., 2014).

2.4.1 Clinical implications and limitations

NMES has been recommended as a way to prevent and/or reduce the secondary complications following a SCI (Chih-Wei et al., 2011; Deley et al., 2015; Dyson-Hudson & Nash, 2009). Of the three, we suggest that SEQ may be the best approach for imminent translation to clinical practice. SEQ has been shown to effectively reduce fatigability in upper and lower limbs muscles, including the quadriceps muscles which are often stimulated in NMES-based programs (Ibitoye et al., 2016; Maffiuletti, 2010; Martin et al., 2012). In the present study, all three NMES types showed promise for reducing contraction fatigability compared to conventional NMES. In particular, SEQ may be the best approach as it has been shown to be effective in reducing fatigability in upper and lower limbs muscles, including the quadriceps muscles which are often stimulated in NMES-based programs (Bergquist et al., 2016; Downey et al., 2015; Malesevic et al., 2010; Maneski et al., 2013; Popovic & Malesevic, 2009; Sayenko et al., 2015). SEQ is easily applied through surface electrodes over the muscle belly: hence, SEQ could be easily applied over all the main muscle groups. Accordingly, SEQ would be easily translated into NMES-based exercise therapy. In addition, SEQ produces the most consistent contractions which would be especially relevant when performing functional activities such as grasping. Furthermore, by rotating the anode and cathode among the four electrodes, charge build-up under the electrodes is reduced, minimizing tissue damage similar to the biphasic waveform used in clinical settings.

Despite the advantages of SEQ, a number of issues need to be addressed before its' implementation into clinical practice. For one thing, whether SEQ will effectively reduce fatigability of large contractions, when current intensity and MU overlap is high (Wiest, Bergquist, Schimidt, et al., 2017), is not known. When SEQ is delivered to produce large contractions, it is likely that its effectiveness on reducing fatigability may decrease (Bergquist et al., 2016). High stimulation intensities also increase discomfort, which may limit people with intact or heightened sensation from using

SEQ. Finally, to deliver SEQ in the current study, custom-made equipment was required. To facilitate the transition of SEQ into a clinical setting, more portable and user-friendly equipment will be needed.

NERVE also reduces fatigability and discomfort compared to conventional NMES (Lou et al, unpublished). In this study NERVE required less current than SEQ and HYBRID and produced contractions with the least discomfort. Thus, NERVE may be advantageous for use in people with intact sensation or heightened sensitivity because it can produce strong contractions with less discomfort (Hamid & Hayek, 2008). However, in our experience, finding the optimal location for the cathode in the femoral triangle is difficult, and maintaining stability during the contractions required pressure to be applied over the cathode. Hence, in our opinion, at this time stimulation over the femoral nerve is challenging and time-consuming and is not practical for clinical settings. Another issue with NERVE is the high variability in torque between contractions, which limits its applications for functional tasks. Finally, the effectiveness of NERVE on reducing fatigability may depend on the muscle stimulated. In the tibialis anterior muscle, stimulation over the nerve trunk did not reduce fatigability compared to conventional NMES (Lou et al., 2017), while in the plantar flexor muscles, stimulation over the nerve only reduced fatigability, in half of the participants, when contractions were produced by H-reflexes (Bergquist et al., 2014).

HYBRID involves stimulation over the nerve trunk, which as mentioned previously, has several practical issues yet to be resolved. In addition, HYBRID produced more discomfort than NERVE and more contraction variability than SEQ. Based on these findings, it is not justified to recommend the use of HYBRID over SEQ or NERVE.

Some aspects of the present experimental protocol may limit the extent to which the current experimental findings can be generalised to people with SCI participating in NMES-based exercise programs. For example, the current intensity required during NMES cycling in people with SCI is ~ 3 times higher than the current intensities used in the present study (88 ± 37 versus ~25±12 mA) (Popovic & Malesevic, 2009). In addition, presently fatigability was assessed during isometric contractions, however, fatigability of non-isometric contractions, which are observed during functional tasks, would be of more clinical relevance. Finally, the duty cycle of the fatigue protocol used in the present study (0.3 sec/07 sec off) corresponds to muscle activation of the quadriceps during walking in non-injured individuals (Pierrynowski & Morrison, 1985), which differs from the quadriceps activation during NMES-exercise in individuals with motor impairment (da Silva et al., 2016).

2.5 Conclusion

The level of fatigability that developed during fatigue protocols delivered using NERVE, SEQ and HYBRID was not different, and all three resulted in less fatigability than is typical of conventional NMES. SEQ produced contractions with the least variability, and NERVE resulted in the least discomfort. Of these three approaches, we suggest SEQ as the best candidate to be translated to clinical practice as it has consistently produced less fatigability than conventional NMES in people with SCI, produces less variability and is easy to apply and use. Although NERVE and HYBRID are also promising approaches for reducing fatigability there are several practical issues related to stimulating the femoral nerve that remain to be resolved before those approaches will be practical for use in NMES-based programs.

2.6 Figures

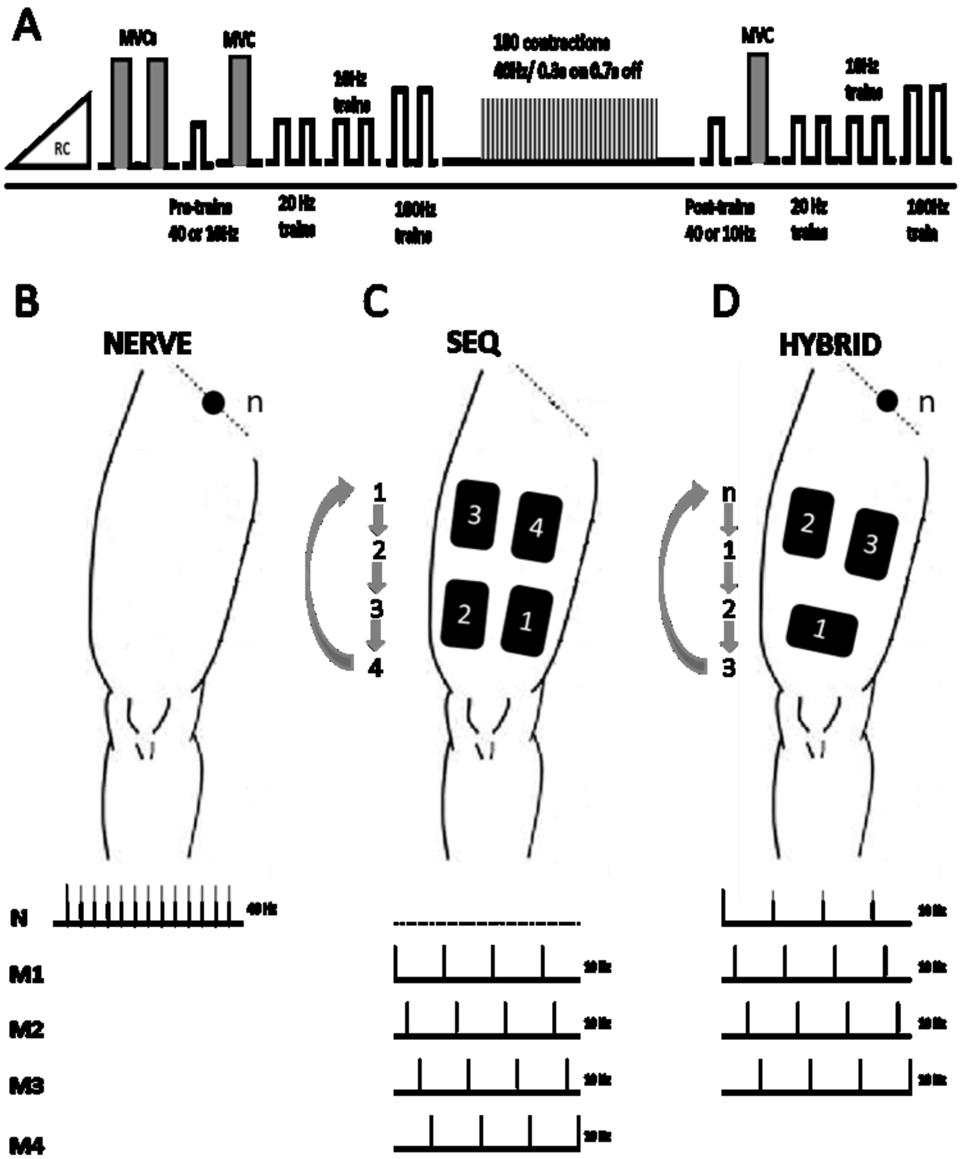

Figure 2-1. Schematic showing experimental procedures, electrode positioning and sequencing of stimulation pulses for each NMES type. Panel A shows the timing of the fatigue protocol and the pre- and post-measures. Panel B depicts NERVE, which is delivered at 40 Hz through one cathode over the femoral nerve trunk and an anode over the gluteal region (not shown). Panel C shows SEQ delivered at a net frequency of 40 Hz, with stimulus pulses rotated between four electrodes over the muscle belly. Panel D shows HYBRID which is delivered at a net frequency of 40 Hz, with stimulus pulses rotated between three electrodes over the muscle belly and one over the femoral nerve.

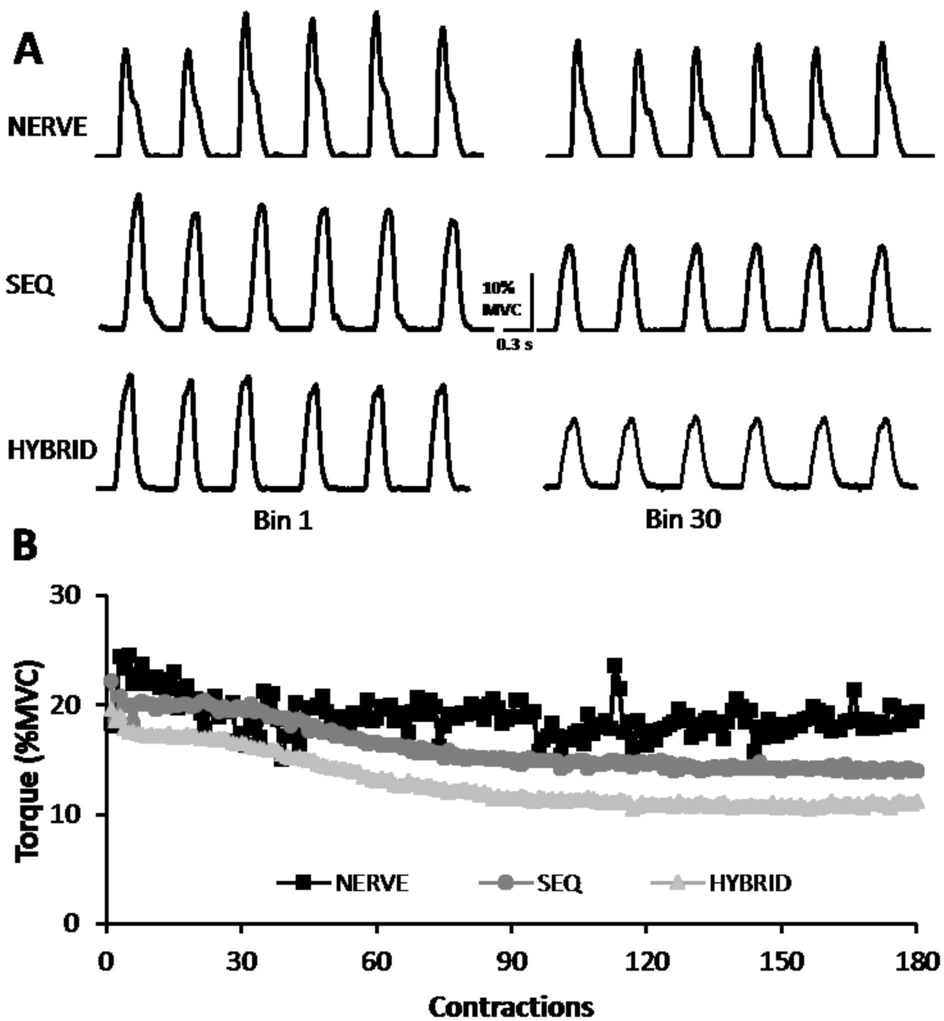

Figure 2-2. Data recorded from a single participant during fatigue protocols delivered using each type of NMES. Panel A shows torque recorded during the first 6 contractions (bin 1) and last 6 contractions (bin 30) of each fatigue protocol. Peak torque recorded during each contraction of the fatigue protocol for each NMES type is shown in panel B.

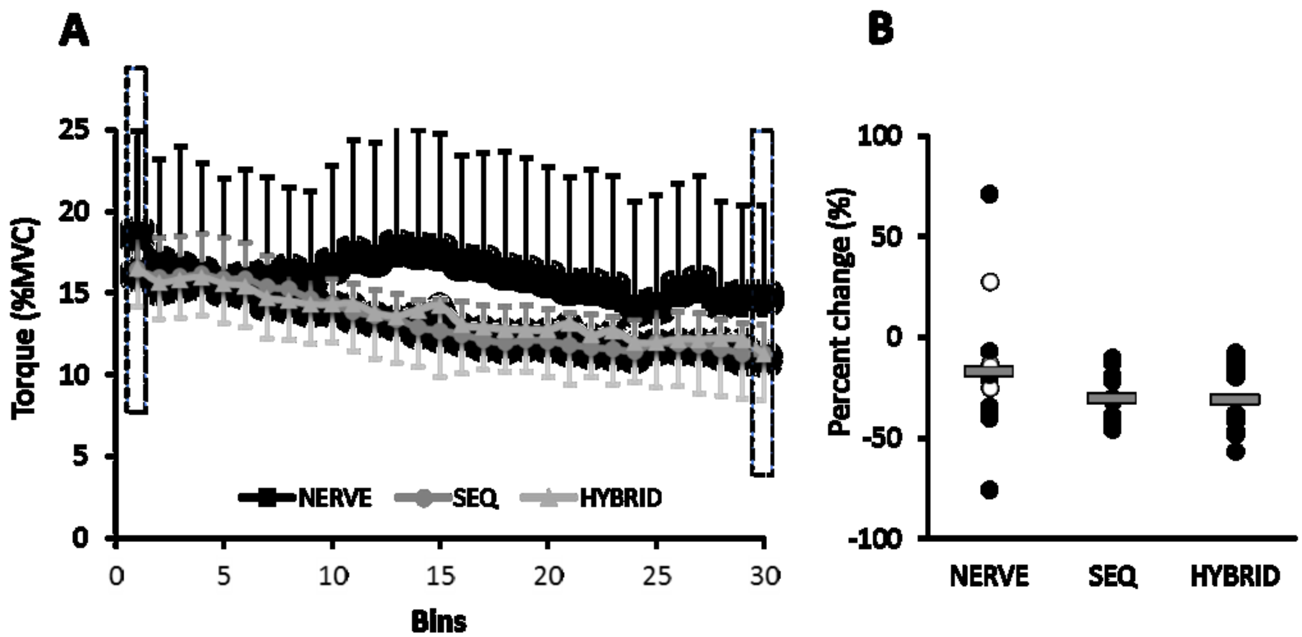

Figure 2-3. Peak torque recorded during the fatigue protocols averaged across the group of 14 participants. Panel A shows peak torque averaged into 30 bins across each fatigue protocol and averaged across the group. The dashed boxes show the peak torque produced at the beginning, and at the end of the fatigue protocol (bin 1 and bin 30) that were used for the statistical analyses. Panel B shows percent change in torque from bin 1 to bin 30 for each fatigue protocol. White circles in panel B show the data for participants in whom contractions were generated in part by H-reflexes during NERVE, black circles represent data for participants without H-reflexes. For SEQ and HYBRID participants were not divided into groups (n = 14). Asterisk (*) denotes $p < 0.05$.

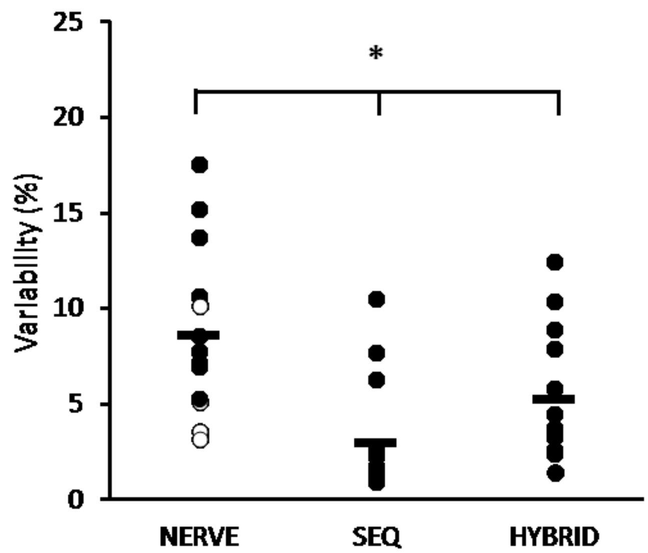

Figure 2-4. Variability in torque between consecutive contractions for each NMES type. White circles show data from participants in whom contractions were generated in part by H-reflexes during NERVE, black circles represent data for participants without H-reflexes. For SEQ and HYBRID participants were not divided into groups. Asterisk (*) denotes $p < 0.05$.

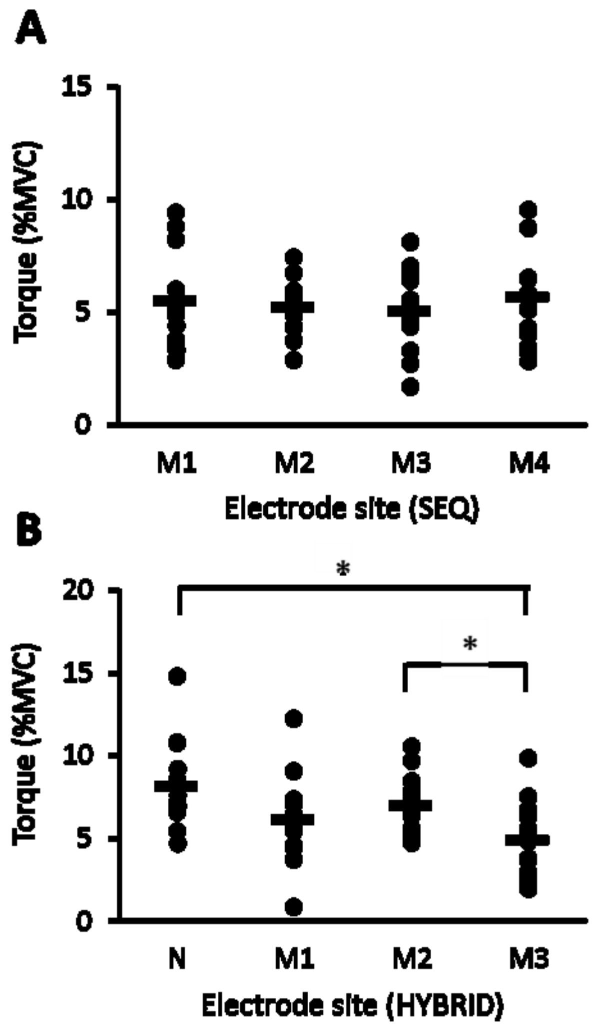

Figure 2-5. Peak torque produced before the fatigue protocols at each stimulation site during SEQ (Panel A) and HYBRID (Panel B). Asterisks (*) denote $p < 0.05$.

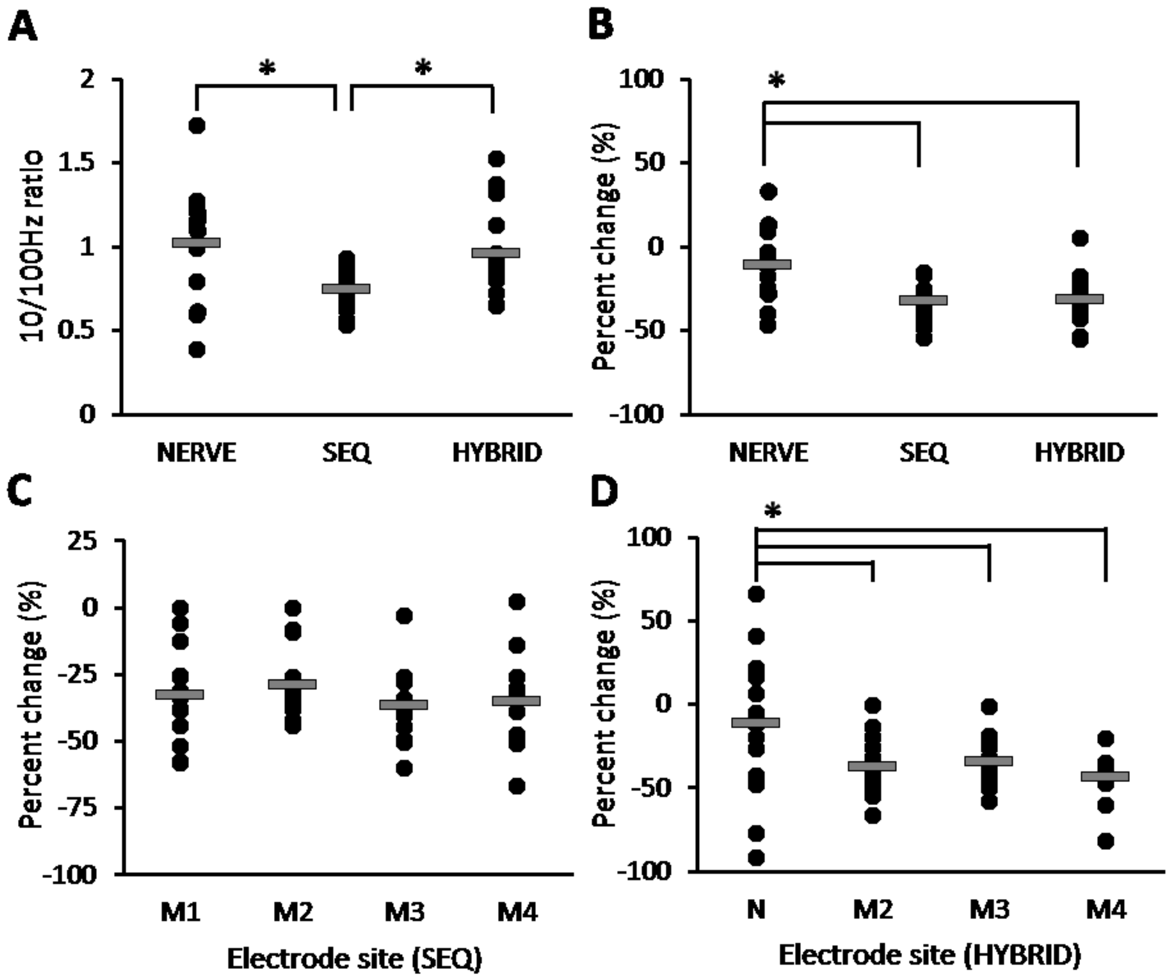

Figure 2-6. Measurements made before and after the fatigue protocols averaged across the group of 14 participants. Panel A shows the low frequency fatigue ratios for each NMES type. Panel B shows the percent change in torque between trains delivered immediately before and within one minute after each fatigue protocol. The percent change in torque between trains delivered to each stimulation site before and after the SEQ and HYBRID fatigue protocols are shown in Panels C and D, respectively. Asterisk (*) denotes $p < 0.05$.

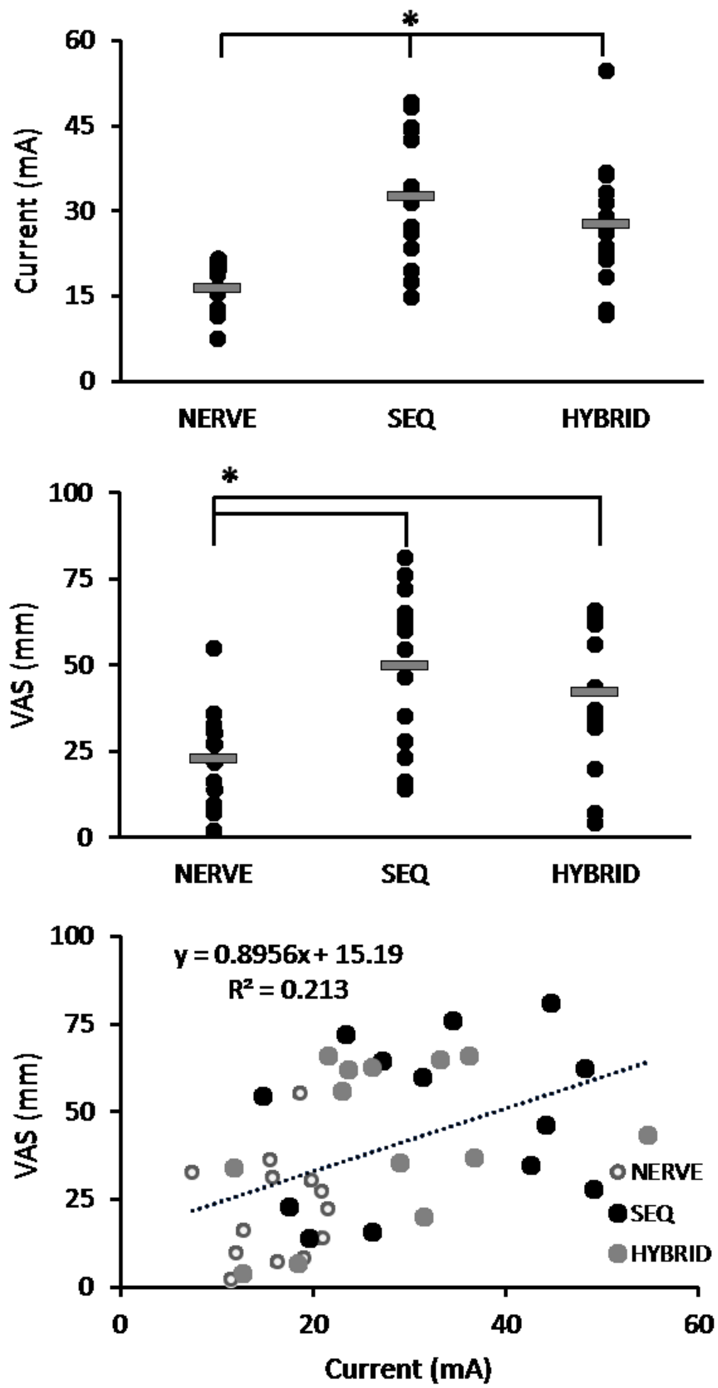

Figure 2-7. Current and discomfort recorded during the fatigue protocols. Panel A shows current delivered during each NMES type to produce ~20% MVC. Panel B shows discomfort assessed using Visual Analog Scale (VAS) during each NMES type. A linear regression between VAS scores and current (mA) is shown in panel C. Asterisk (*) denotes $p < 0.05$.

CHAPTER 3: GENERAL DISCUSSION

My thesis research was designed to compare contraction fatigability, variability, and discomfort between neuromuscular electrical stimulation applied over the femoral nerve (NERVE), neuromuscular electrical stimulation (NMES) rotated between four electrodes over the muscle belly (sequential NMES; SEQ), and hybrid neuromuscular electrical stimulation (HYBRID). Previous research has shown that NERVE and SEQ reduces contraction fatigability compared to conventional NMES (NMES delivered through two electrodes at ~ 40 Hz). Therefore, we hypothesized that HYBRID, a NMES approach combining NERVE and SEQ, would reduce contraction fatigability to a greater extent than NERVE or SEQ alone. We also hypothesized that NERVE would produce the most variability between contractions, as stimulation over the nerve has previously resulted in high variability in torque between contractions (Baldwin et al., 2006). In addition, we hypothesized that SEQ would result the most discomfort while NERVE would produce the least discomfort, due to in previous work stimulation over a nerve required less current and produces less discomfort than stimulation over a muscle (Bergquist, Clair, & Collins, 2011; Bergquist et al., 2012). Each experiment consisted of pre- and post-measures, and a fatigue protocol (180 contractions, at 40 Hz, ~20 % MVC). Contraction fatigability, variability, and discomfort were assessed during the fatigue protocol. No difference was found in fatigability between the three NMES types. SEQ produced contractions with the lowest variability in torque. NERVE required the lowest current consequently generating less discomfort than SEQ and HYBRID. Thus contrary to our hypothesis, the three NMES types resulted in the same amount of fatigability; however, based on previous studies that included conventional NMES, all three NMES types may have produced more fatigue-resistant contractions than typically develops during conventional NMES (Lou et al., unpublished). NERVE is a promising NMES approach but there are several issues related to the difficulty of accessing the stimulation site, movement of the cathode with respect to the nerve and anode, and high variability between contractions. HYBRID produced more variability than SEQ and more discomfort than NERVE, and the practical issues described for NERVE also apply to HYBRID. SEQ produced less variability in torque between consecutive contractions and can be easily applied. SEQ has also been shown to reduce fatigability compared to conventional NMES in people with and without a SCI in several muscles including the quadriceps which are the most frequently stimulated in clinical practice. Therefore, although all three NMES types are promising for reducing fatigability compared to conventional NMES, we suggest SEQ to be incorporated into clinical practice.

3.1 Implications

People with a SCI experience many other medical conditions associated to inactivity such as cardiovascular disease and muscular atrophy (Duffell et al., 2008; Dyson-Hudson & Nash, 2009; Kocina, 1997), which have been mainly attributed to sedentarity. NMES has been suggested as one solution to prevent and/or diminish these secondary complications. Typically, the use of NMES for fitness purposes consists of 30 to 45 minute-long sessions where NMES is delivered between 30 – 50

Hz to enable individuals to perform strengthening exercises (leg flexion/extension), row on a stationary rowing machine, or cycle a stationary bike - the most commonly performed in clinic - (Deley et al., 2015). However, due to the non-physiological way that conventional NMES activates MUs, rapid contraction fatigability develops. Thus people with a SCI are unable to exercise at sufficient intensities or durations to optimise the physiological benefits (Ibitoye et al., 2016; Maffiuletti, 2010; Martin et al., 2012). In the following section, the advantages, disadvantages and clinical implications of each alternative NMES approach studied in this thesis are discussed.

SEQ reduces contraction fatigability by dividing the frequency of stimulation among four electrodes positioned over the muscle belly. SEQ has been shown to reduce contraction fatigability compared to conventional NMES in both upper and lower limb muscles, including the quadriceps muscles which are most commonly stimulated in clinical practice (Bergquist et al., 2016; Downey et al., 2015; Ibitoye et al., 2016; Malesevic et al., 2010; Maneski et al., 2013; Popovic et al., 2009; Sayenko et al., 2015). For people with a SCI to improve cardiorespiratory fitness and muscle strength, exercise guidelines recommend at least 20 minutes of moderate to vigorous aerobic exercises twice per week and three sets of strengthening exercises for each major functioning muscle group, at a moderate to vigorous intensity, twice per week (Ginis et al., 2017). For people with a SCI, using SEQ to cycle or row may achieve exercise intensities at a moderate or even high level, and for longer periods of time than using conventional NMES. Compared to conventional NMES, SEQ also produce consistent torque between contractions which may be especially important for the performance of functional tasks such as grasping or walking (Bergquist et al., 2016). Another important advantage of SEQ is that is easily applied by using multiple electrodes over the muscle belly surface, facilitating its transition into clinical practice. In addition to NMES-exercise applications, SEQ may also be used to perform functional tasks that require fine motor-control of the muscles such as grasping. For example, Maneski et al. (2013) found that SEQ over the forearm could selectively activate the flexor digitorium superficial muscle, and produced less fatigability than conventional NMES. Hence, SEQ can be used to enhance the physiological benefits of FES exercise, restore fine motor control of a muscle or group of muscles, and can be easily translated into rehabilitation settings.

Besides these promising results of SEQ, there are some limitations of this technique that must be considered. The effectiveness of this approach in reducing contraction fatigability depends on the low frequency applied at each stimulation site and the corresponding low MU discharge rates (~ 10 Hz). In theory, if MUs are activated by more than one stimulation site (i.e. greater overlap in MUs recruited between electrode sites) this would result in MUs discharging at higher frequencies and consequently more contraction fatigability. Wiest et al. (2017) found that the amount of MU overlap between two stimulation sites increased as current intensity increased. If during SEQ, high stimulation intensities are delivered to produce strong contractions, there would be greater MU overlap and thus more fatigability. In the quadriceps, fast-fatigable MUs are mainly located at the surface of the muscle group while fatigue-resistant MUs are located deeper (Lexell et al., 1986). When stimulation is applied over the muscle belly, mainly those MUs closer to the electrodes would be recruited (Maffiuletti, 2010; Okuma et al., 2013). Thus, to activate the deeper fatigue-resistant fibers with SEQ, high current intensities are required. In addition to increasing MU overlap, high current intensities could be uncomfortable for people with intact or heightened sensation, limiting their participation in NMES-based programs. Another limitation of using SEQ is that complex equipment is required to distribute stimulation among the multiple electrodes. Although this equipment was effective for the purposes of our experiment, a more portable and user-friendly device would be best suited for a clinical setting. Finally, in order to translate SEQ into clinical practice, more clinically relevant research is needed to assess the effectiveness of SEQ on reducing contraction fatigability in a clinical population and during functional tasks such as cycling or rowing.

NERVE addresses another factor that contributes to contraction fatigability during NMES which is the random MU recruitment order, whereby fewer fatigue-resistant MUs are recruited than during voluntary contractions of similar amplitude (Bickel et al., 2011). In the quadriceps muscles, NERVE recruits MUs located throughout the muscle (Okuma et al., 2013; Rodriguez-Falces et al., 2013), including those fatigue-resistant MUs located in deep portions of the muscles (Knight & Kamen, 2005; Lexell et al., 1986), and MUs can be recruited synaptically through reflex pathways (Bergquist et al., 2012; Bergquist et al., 2014). Accordingly, NERVE reduced contraction fatigability compared to conventional NMES in the quadriceps muscles (Lou et al., unpublished). Hence, exercise using NERVE may prevent or diminish transformations from fatigue-resistant to fast-fatigable fiber types to a greater extent than conventional NMES in people with SCI (Deley et al., 2015; Mohr et al., 1997). Additionally, in this study NERVE required less current and produced less discomfort than SEQ and HYBRID. In previous work, less current was needed when stimulation was delivered over the nerve trunk compared to over the muscle belly in the quadriceps and in the ankle dorsiflexor muscles (Bergquist et al., 2012; Naaman et al., 2000). Therefore, NERVE may be mainly beneficial for people with intact or partial sensation, such as those with a stroke or with an incomplete SCI, to improve muscle strength, enhance cardiovascular conditioning and prevent muscle atrophy.

Although NERVE may have several advantages, there are several practical limitations of NERVE for NMES-based exercise programs. One limitation of NERVE over the femoral nerve trunk is accessibility to the stimulation location and the stability of the cathode. To stimulate the femoral nerve, the cathode is located over the femoral triangle, which is a complicated area for a clinician to access and may be uncomfortable for the patient. In addition, during NERVE there is greater risk of co-activation of other muscles, compromising the specificity of muscle activation and interfering with performance of a movement. Therefore, once the electrode is positioned, pressure needs to be applied to the cathode to effectively and consistently activate the axons innervating the target muscle and minimize movement during the contractions. In this study, we used a modified fall harness with custom-made accessories to produce constant pressure over the cathode. This set-up, however, could take more than 15 minutes to find the stimulation site and to apply adequate pressure over the cathode depending on the participants' morphological characteristics. Hence, the use of NERVE in common clinical settings where the time per patient is limited may not be practical. Another disadvantage of nerve stimulation is the high variability produced in torque between contractions (Baldwin et al., 2006; Bergquist et al., 2012) which can detract from the performance of functional activities such as walking, cycling, and rowing. Finally, the effectiveness of nerve stimulation to reduce fatigability will depend on the muscle stimulated and whether central pathways are activated, which can be variable between individuals (Wegrzyk et al., 2015). Stimulation over the nerve compared to conventional NMES reduced contraction fatigability in the quadriceps muscles (Lou et al., unpublished), but not in the tibialis anterior muscle (Lou et al., 2017). Furthermore, in the ankle plantar flexor muscles, nerve was effective in reducing fatigability only when H-reflexes were elicited in participants (Bergquist et al., 2014). Overall, NERVE could be beneficial for people with a SCI and other motor impairments, because it can more completely activate the muscle (i.e. superficial and deep) at lower currents and can recruit MUs through central pathways. However, more research needs to be conducted to resolve some of the previously mentioned issues before its implementation into clinical practice. Based on my experience, even if some of these issues were resolved such as electrode movement, only some people may use NERVE for general training because morphological characteristics such as lower abdominal fat could also interfere with the correct placement of the cathode in the femoral triangle.

HYBRID produced a similar amount of contraction fatigability as SEQ and NERVE, therefore it is expected that HYBRID may be as effective as NERVE and SEQ for reducing contraction fatigability compared to conventional NMES. However, HYBRID produced more discomfort than NERVE, generated contractions with more variability than SEQ, and has the same aforementioned practical difficulties with nerve stimulation limiting its applicability. Hence, there is no justification to prefer HYBRID over NERVE and SEQ. We suggest that a different HYBRID configuration than the one used in this thesis may result in less fatigability. One such configuration would incorporate four electrodes positioned over the muscle belly in addition to the electrode over the nerve trunk to increase the number of different MUs activated and reduce the stimulation frequency delivered to each stimulation site. Another configuration would involve a greater ratio of nerve stimulation per contraction to recruit more fatigue-resistant MUs. Although we encourage future research to conduct studies with a different HYBRID configuration, we suggest that some issues of nerve stimulation, such as movement of the cathode, needs to be addressed.

3.2 Limitations

One of the main limitations of this study was not including conventional NMES - the typical NMES type used in clinical practice. Our rationale for not including conventional NMES was that previous studies demonstrated NERVE and SEQ resulted in relatively less contraction fatigability in the quadriceps muscles (Bergquist et al., 2014; Downey et al., 2015; Nguyen et al., 2011; Popovic-Maneski, Malesevic, Savic, Keller, & Popovic, 2013; Popovic et al., 2009; Sayenko et al., 2015). In addition, it was predicted that HYBRID would reduce fatigability to an even greater extent because it combines NERVE and SEQ. Thus, we were expecting to find the best NMES approach among these already successful alternatives. Since conventional NMES was not included as a comparison we could not directly quantify the reduction of fatigability of NERVE, SEQ, and HYBRID in relation to conventional NMES, and were only able to compare our results with those reported in previous literature.

Another limitation of this study was that the custom-built stimulation distributor introduced noise into the EMG signal which made it impossible to measure H-reflexes during HYBRID and SEQ. Accordingly, it is uncertain whether central pathways contributed during HYBRID. Although H-reflexes were measured during NERVE, the sample size of participants who produced contractions in part through central pathways was smaller than people who did not; thus it was not adequate to conduct a statistical test. To be able to observe the effect of H-reflexes on contraction fatigability, future research should control this variable by including a larger number of people with H-reflexes during the fatiguing contractions.

This study included participants without neurological or musculoskeletal impairments. The main reason for this is because HYBRID is a newly developed approach of NMES which has not been tested before. Since SCI leads to several physiological adaptations such as increased muscle atrophy and prevalence of fast-fatigable muscle fibers (Gerrits et al., 2000), it is unknown whether the results obtained in this study would be replicated in people with a SCI, though it has been shown that SEQ reduces contraction fatigability in this population in the quadriceps muscles (Downey et al., 2015; Nguyen et al., 2011; Sayenko et al., 2015). In addition, fatigability was tested during isometric contractions, but in a rehabilitation setting NMES is usually applied during dynamic exercises such as cycling.

3.3 Future directions

Most of the investigations done to reduce contraction fatigability during electrically-evoked contractions are conducted during isometric contractions, but to validate those strategies for clinical practice, they should be tested during functional tasks (Ibitoye et al., 2016). Most studies also include short intervention periods with case studies (Nguyen et al., 2011) or cross-sectional designs (Popovic & Malesevic, 2009; Sayenko et al., 2014), which are designs generally used to study new approaches and when resources are limited. However, they are considered of low quality when determining the effectiveness of interventions due to the limited control of confounders. To accurately test the efficacy of sequential NMES on reducing contraction fatigability, higher quality studies need to be conducted including larger samples of people with SCI with longer intervention periods and functional tasks, ideally using a crossover as well as parallel designs.

The ability of SEQ to reduce contraction fatigability depends on the low frequency of stimulation (10 Hz) applied at each electrode site and the corresponding low MUs discharge rates. The MU discharge rates depend on the amount of overlap between stimulation sites, where, in theory, a more overlap would result in more contraction fatigability. Wiest et al. (2017) showed that higher stimulation intensities resulted in more overlap of MUs recruited between different stimulation sites (over the nerve and over the muscle) in the tibialis anterior muscle. Therefore, future research should investigate the amount of overlap between SEQ's four electrodes at different stimulation intensities, and the effect of stimulation amplitude in contraction fatigability. In addition, SEQ approaches in the lower limb muscles have been typically delivered through four electrodes (Maneski et al., 2013; Popovic & Malesevic, 2009; Sayenko et al., 2015), but possibly by distributing the frequency of stimulation between more than four electrodes, MU discharge rates would be reduced further, resulting in less contraction fatigability. Recently researchers have proposed that if stimulation parameters such as frequency or intensity could be modulated in each of those multiple electrodes independently, fatigability and the selectivity of the muscle contraction would be improved (Koutsou, Moreno, del Ama, Rocon, & Pons, 2016). SEQ with multiple electrodes in addition to its exercise applications could also be a good alternative for assisting with functional movements that require more muscle control such as walking and grasping (Malešević et al., 2012; Maneski et al., 2013). Hence, another future direction of SEQ could be the incorporation of multi-pad electrodes with independent control of stimulation parameters.

Higher MU discharge rates increase the metabolic demand on the muscle and hasten the onset of contraction fatigability (Vanderthommen et al., 2003). Interestingly, even though NERVE activated MUs at a higher stimulation frequency than SEQ and HYBRID (40 Hz vs ~10 Hz), there was no difference in contraction fatigability. This may be explained by a greater activation of fatigue-resistant MUs during NERVE than during SEQ and HYBRID. In the quadriceps muscles fatigue-resistant MUs are predominantly located in deeper portions of the muscle (Knight & Kamen, 2005; Lexell et al., 1986). NERVE, contrary to stimulation over the muscle belly where superficial MUs are mainly recruited, recruits MUs evenly throughout the muscle (Okuma et al., 2013). Therefore, those fatigue-resistant MUs located in deep portions of the quadriceps muscles can be activated. Accordingly, if NERVE was delivered at a lower frequency, between 20 – 30 Hz, it is possible that less contraction fatigability would be produced during NERVE than during SEQ and HYBRID. Thus, future work should assess contraction fatigability produced by NERVE delivered at lower frequencies than used in this study and SEQ.

Finally, there is only one known study that found a reduction in contraction fatigability of people with a SCI when contractions were produced in part with the contribution of central pathways (i.e. H-reflexes) (Bergquist et al., 2014). However, this study included a small sample size of eight participants, whereby only four participants presented central contributions (i.e. H-reflexes). In addition, H-reflexes have been found for some muscles such as triceps surae and quadriceps (Bergquist, Clair, Lagerquist, et al., 2011), and its amplitude is highly variable as H-reflexes can be affected by several factors (Misiaszek, 2003). Thus, the presence of H-reflexes depends on the muscle stimulated, the activity performed and the individual. Therefore, to determine the effect of H-reflexes on contraction fatigability, studies should measure H-reflexes during nerve stimulation in the quadriceps muscles with a larger sample size of people with SCI and performing functional tasks.

3.4 Summary

This thesis compared three types of NMES (NERVE, SEQ and HYBRID) for contraction fatigability, contraction variability and discomfort. In previous research NERVE and SEQ reduced contraction fatigability compared to conventional NMES, and therefore it was hypothesized that a new type of NMES that combines both (i.e. HYBRID) would reduce contraction fatigability to a greater extent. Our results showed that the three NMES types produced similar contraction fatigability, but SEQ produced contractions with the least variability and NERVE generated the least discomfort. Based on previous studies we suggest that these three NMES types produce less contraction fatigability than conventional NMES, however we recommend SEQ as the best way to deliver FES for NMES-rehabilitation programs due to its consistent contractions and ease of application. The next step for this work is to study SEQ in people with a SCI within functional tasks to produce more clinically relevant results.

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APPENDIX A. COMPARISON OF TWO TYPES OF NEUROMUSCULAR ELECTRICAL STIMULATION FOR THE REDUCTION OF CONTRACTION FATIGABILITY OF THE QUADRICEPS MUSCLES

Neuromuscular electrical stimulation (NMES) can produce contractions to increase functionality and reduce secondary complications for individuals with a spinal cord injury (Deley et al., 2015; Hamid & Hayek, 2008). However, the benefits of conventional NMES – NMES delivered through a single pair of electrodes over a muscle belly, are limited by rapid contraction fatigability and discomfort (Bickel et al., 2011; Ibitoye et al., 2016; Maffiuletti, 2010). Sequential NMES (SEQ) has been consistently shown to produce less contraction fatigability than conventional NMES (Downey, Bellman, Kawai, Gregory, & Dixon, 2015; Popovic & Malesevic, 2009; Sayenko, Nguyen, Hirabayashi, Popovic, & Masani, 2015). However, its effectiveness to reduce contraction fatigability depends on different MUs being recruited at each stimulation site, referred to as 0% overlap. Theoretically, with 0% overlap, SEQ delivered at a net stimulation frequency of 40 Hz would recruit unique populations MUs at 10 Hz; while at 100% overlap, each MU is recruited at 40Hz. The amount of overlap between different stimulation sites has been associated with stimulation intensity, whereby higher intensities produce greater MU overlap (Wiest, Bergquist, Schimidt, et al., 2017). However, it is unknown how the distribution of electrodes over the quadriceps muscle belly could affect the amount of overlap between stimulation sites, and consequently contraction fatigability during SEQ.

Researchers have applied SEQ in different ways in the quadriceps muscle. One of the most common ways is using five electrodes: four cathodes positioned over the proximal quadriceps muscle, and a “fixed” common anode located proximal to the knee (FIX SEQ) (Malesevic et al., 2010; Popovic & Malesevic, 2009).We developed a different way to deliver SEQ which involves four electrodes positioned over the quadriceps muscle belly without a fixed common anode. Instead, the anode and cathode rotate among the four electrodes in a "chasing" pattern (CHASE SEQ). We suggested that this rotation of anode and cathode through four electrodes located over the quadriceps muscles may recruit MUs with more selectivity, hence less MU overlap would be produced between stimulation sites than during FIX SEQ. The objective of this experiment was to compare CHASE and FIX SEQ on contraction fatigability in the quadriceps muscle. We hypothesized that less contraction fatigability would be produce during CHASE SEQ than during FIX SEQ.

METHODS

Eight participants (4 females and 4 males; 26.6±5.9 years) with no known neurological or musculoskeletal impairment were included for this study.

Experiment procedures

After providing informed written consent, participants participated in two experimental sessions, each lasting ~1 to 1.5 hours. One type of SEQ was delivered during each session, with sessions separated by at least 48 hrs. The order of the experiments was randomized for each participant. This study was approved by the Human Research Ethics Board at the University of Alberta. This experiment consisted of a fatigue protocol with electrical stimulation and maximal voluntary contractions (MVCs) before and after the fatigue protocol.

Position

Participants were seated in the chair of a Biodex dynamometer (System 3, Biodex Medical System, Shirley, New York). All procedures were performed on the right leg with the hip at ~120° and the knee at ~85°. The center of rotation of the knee was aligned with the axis of the dynamometer and the tibia was held in place by the arm of the dynamometer.

Maximum Voluntary Contractions

At the beginning of each session, participants performed two isometric knee extension MVCs, extending the knee against the arm of the dynamometer with their maximal force for 3 to5 s. If the peak torque produced during two consecutive MVCs was not within ~10% of each other, a third MVC was performed. MVCs were separated by ~1 minute. Participants received verbal encouragement to perform maximally and visual feedback of their torque on a computer monitor. The average torque of two contractions within 10% of each other was set as the MVC. These MVCs were used to normalize torque for each participant and set target levels for each of the fatigue protocols.

NMES

For FIX SEQ, adhesive gel electrodes (5.08 X 8.89 cm2; Richmar superstim) were placed over the quadriceps muscle belly. Five electrodes were placed over the thigh and four cathodes over the quadriceps, two cathodes over vastus lateralis, one cathode over rectus femoris, and one cathode over vastus medialis, targeting their respective motor points (Botter et al., 2011). The common anode was positioned on the distal portion of the thigh (Figure A-1A). For CHASE SEQ, four electrodes were placed over the quadriceps muscles in the same location as the cathodes during FIX (Figure A-1B). Stimulation during both FIX and CHASE SEQ was delivered by sending a pulse through each electrode in sequence one after another. Individually each electrode was stimulated at 10 Hz generating a net frequency of 40 Hz delivered to the entire muscle.

Stimulation Amplitudes

To set the stimulation intensity for each experiment, 0.3 s trains at 40 Hz stimulation frequency were delivered until the muscle generated a torque of ~15 % MVC. If the participant could not tolerate 12.5 %MVC, they were excluded from the study. Prior the fatigue protocol, testing trains were delivered to the quadriceps muscle (see below).

Fatigue protocol

One hundred and seventy contractions were evoked using trains delivered at 40 Hz. Each train lasted 0.3 s with 0.7 s rest between trains.

Data collection and analysis

Data were sampled at 5000 Hz using custom-written Labview software (National Instruments, Austin, TX) and stored on a computer for subsequent analysis using custom-written Matlab software (The Mathworks, Natick, MA). The MVC torque was quantified over a 0.3 s window centered on the peak during each MVC. The torque generated during the fatigue protocol was quantified as the average torque over a 0.3 s window and normalized to each participant's MVC. Contractions for the fatigue protocol were binned into five consecutives contractions (i.e. bin 1 = contraction 1 to contraction 5) and averaged, resulting in 34 averaged torque bins. Contraction fatigability was calculated as the percent change in torque from the first five contractions to the last five contractions. A paired T-test was used to determine the influence of $NMES_{type}$ on percent change in torque. To determine whether there was a significant decline in torque from the beginning (bin 1) to the end (bin 34) of the fatigue protocol a 2 (TIME) x 3 ($NMES_{type)}$ ANOVA test was conducted Statistical analysis was performed using Statistica 8.0 software (StatSoft, Tulsa, Oklahoma). Participant information such as demographic was summarized using descriptive statistics.

Results

Figure A-2 shows group data for torque across time (A), and percent change in torque (B). There was no significant difference in the relative change in torque between FIX and CHASE SEQ (-28.3±14 % and -29.2± 13% respectively) (t (7) = -

.348, p = 0.738) suggesting that both types of SEQ produced the same amount of contraction fatigability. To assess whether there was a significant decline in torque during each fatigue protocol a 2 ($NMES_{type}$) x 2 (TIME) rm ANOVA was run. There was a significant main effect of TIME ($F_{(1,7)}$ 35.64, p = 0.001), with ~5 %MVC decline in torque from bin 1 to bin 34. There was no main effect of $NMES_{type}$ ($F_{(1,7)}$ = 1.034, p = 0.343). No interaction was found between $NMES_{type}$ and TIME ($F_{(2,26)}$ = 0.628, p = 0.541). This indicates that there was a significant decline in torque during each fatigue protocol that did not differ between SEQ types (data not shown).

Discussion

We hypothesized that CHASE SEQ would result in less contraction fatigability than FIX SEQ because of less MU overlap between stimulation sites. Contrary to our hypothesis, there was no difference between FIX and CHASE SEQ on contraction fatigability. This would indicate that delivering SEQ with or without a common anode does not influence the discharge frequency of MUs. Although FIX and CHASE SEQ produced the same amount of contraction fatigability, we recommend CHASE SEQ as the best modality to deliver SEQ because the rotation of the cathode and anode between the four electrodes can reduce the build-up of charge under the stimulating electrodes. Accordingly, CHASE SEQ may prevent tissue damage during NMES-exercise applications such as cycling and rowing.

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