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A COMPARISON OF MULTIPATH AND CONVENTIONAL

NEUROMUSCULAR ELECTRICAL STIMULATION

by

Cody Brian Bremner

A Dissertation
Submitted to the Graduate School
and the School of Kinesiology
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

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ABSTRACT

A COMPARISON OF MULTIPATH AND CONVENTIONAL

NEUROMUSCULAR ELECTRICAL STIMULATION

by Cody Brian Bremner

December 2016

Neuromuscular electrical stimulation (NMES) is the use of an electrical current for the purpose of eliciting a muscular response, and these treatments are most often used clinically for the specific purpose of increasing quadriceps strength. It is commonly accepted that the effectiveness of NMES for this purpose is primarily determined by the NMES training intensity. However, spatially limited motor unit recruitment, fatigue and discomfort negatively impact NMES-induced torque, which subsequently reduces NMES training intensities. Due to the importance of NMES training intensity, a substantial amount of research has focused on strategies designed to increase NMES-induced torque production, as well as to reduce NMES-induced fatigue and discomfort. However, authors have indicated that additional strategies are needed, as many of the strategies supported by empirical evidence cannot be easily applied in clinical settings.

The Kneehab® XP (Theragen LLC, Leesburg, VA) is an electrical stimulator that incorporates a novel multipath current distribution strategy (m-NMES) marketed to address the primary factors limiting NMES training intensity, and as such it has gained a significant amount of attention in the literature. Relative to conventional NMES (c-NMES), authors have reported improved outcomes while using the novel m-NMES but due to a series of methodological limitations the influence of the multipath current distribution strategy on these outcomes remains unclear. Therefore, the purpose of this

project was to further investigate the influence that m-NMES has on NMES related outcomes.

A convenience sample of 21 participants completed two basic studies designed to compare the influence of m-NMES and c-NMES on maximum comfortable stimulus intensity and NMES-induced peak torque, as well as fatigue and discomfort related outcomes. The statistical analyses of each study did not reveal any significant differences across the two conditions deemed to be clinically relevant. Therefore, it does not appear that the novel multipath current distribution method influences the outcomes included during this project in a clinically meaningful manner. The large declines in NMES-induced torque that occurred, irrespective of the NMES condition, suggest the need for the development of additional strategies.

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I would like to recognize the following individuals for their support; without each of their individual contributions this project would not have been possible.

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Neurotech®/Theragen LLC. Thank you for providing a portion of the materials necessary for the completion of this project.

DEDICATION

This project is dedicated to my best friend and eternal companion, Stephanie. Thank you for putting your own aspirations on hold to support my educational goals. Words cannot describe my immense gratitude for your unwavering love and support.

I also want to recognize my parents: thank you for the prayers on my behalf and for the constant support throughout my life. Mom, thank you for showing me that education is worth the sacrifice, and for the many hours spent "facetiming" to discuss grammar and punctuation edits. Dad, thank you for teaching me to work hard, both manually and academically. To my two "extra" parents, Rick and Jennifer, thank you for always supporting me as one of your own. To my in-laws, Steve and Amy, thank you for embracing me with open arms.

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LIST OF ABBREVIATIONS

ACL	Anterior cruciate ligament
ANOVA	Analysis of Variance
cm	Centimeter
c-NMES	Conventional neuromuscular electrical stimulation
CNS	Central nervous system
CSA	Cross sectional area
CFT	Constant frequency train
EMG	Electromyography
ESCI	Exploratory Software for Confidence Intervals
GSR	Galvanic skin response
Hz	Hertz
H-reflex	Hoffman reflex
ICC	Intraclass correlation coefficient
KFAC	Kilohertz-frequency alternating current
mA	Milliamp
m-NMES	Multipath neuromuscular electrical stimulation
MVIC	Maximum voluntary isometric contraction
MRI	Magnetic Resonance Imaging
µsec	Microsecond
mm	Millimeter
ms	Millisecond
Nm	Newton-meter

Nm/kg	Newton-meter per kilogram
NMES	Neuromuscular electrical stimulation
NRS	Numerical rating scale
MVIC	Maximum voluntary isometric contraction
% MVIC	Percent maximum voluntary isometric contraction
PC	Low-frequency pulsed current
pps	Pulses per second
SDS	Simple descriptive scale
SEM	Standard error of measurement
SPSS	Statistical Package for Social Sciences
TENS	Transcutaneous electrical nerve stimulation
TTI	Torque-time integral
TKA	Total knee arthroplasty
T-TTI	Total torque-time integral
VAS	Visual analog scale
VFT	Variable frequency train
WPHF	Wide-pulse-high-frequency

CHAPTER I – INTRODUCTION

Statement of the Problem

Neuromuscular electrical stimulation (NMES) is the use of an electrical current for the specific purpose of eliciting a muscular contraction by stimulating peripheral motor nerves via electrodes fixed to the skin,[1-3] and it has been used as a therapeutic modality for many years. During the 1970's NMES gained popularity as a strengthening modality, but it was originally used in combination with ultrasound treatments for the purpose of allowing patients to experience a sensation and giving the impression that they were getting something from the treatment.[3] Today NMES treatments are common in orthopedic clinical settings as they can be used for muscle reeducation, preventing disuse atrophy, decreasing edema, decreasing muscle spasms, increasing range of motion and even as a strengthening adjunct for healthy individuals.[1,3-5] Despite this versatility, NMES is most often used for the specific purpose of enhancing quadriceps strength.[6]

Several studies have demonstrated that NMES treatments may lead to increased quadriceps strength in healthy[7,8] and injured[9-11] populations. Despite these positive results, higher levels of evidence (e.g., systematic reviews, meta-analyses[12]) have suggested that NMES treatments should not serve as a replacement for volitional strengthening; rather NMES is to be incorporated as an adjunct to traditional strengthening exercises.[5,6] Interestingly, based on their review of the literature Paillard[13] concluded that training programs that incorporate NMES and voluntary strengthening exercises, without performing them concurrently (e.g., superimposed contractions), resulted in greater muscular adaptations in healthy and injured populations than programs using either NMES or voluntary strengthening alone. Some authors have suggested that

NMES is a promising adjunct for clinicians attempting to enhance the quadriceps strength of healthy and injured individuals due to its different motor unit recruitment, which may alter the specific stresses placed on the muscle.[5,14] NMES-induced contractions may also elicit a greater cardiorespiratory demand than voluntary contractions of similar intensities.[15] Furthermore, NMES treatments may also serve as an alternative strengthening technique to break up the monotony of traditional strength training exercises.

NMES training intensity, which is most often defined as the ratio of NMES-induced torque to torque produced during a maximum voluntary isometric contraction (expressed as % MVIC),[5] is thought to be the primary determinant of the effectiveness of NMES treatments.[16,17] This belief is based on the established dose-response relationship, which indicates that NMES training intensity is positively related to strength gains.[7-10,18-20] Consequently, clinicians should maximize NMES training intensity to the extent possible,[17] but due to a series of limitations it is difficult to achieve and maintain a sufficient NMES training intensity.

Based on their review of the literature, Maffiuletti et al.[17,21] identified the primary limitations of NMES as: muscle fatigue leading to a premature decline in NMES-induced torque, spatially limited motor unit recruitment which constrains the amount of NMES-induced torque that can be achieved, and patient discomfort associated with the electrical stimulus and subsequent involuntary contraction. In agreement, others have also identified muscle fatigue,[22,23] spatially limited motor unit recruitment[24] and patient discomfort[5,23-27] as primary limitations of NMES. Although these factors differ from one another, each limits the NMES training intensity by restricting torque output during

NMES-induced contractions. Thus in an effort to increase NMES-induced torque, which subsequently enhances NMES training intensity, it is recommended that clinicians implement strategies with the potential to minimize fatigue and discomfort and maximize spatial recruitment.[23,24]

Strategies with the potential to minimize NMES-induced fatigue[28-35] and patient discomfort[36-41] associated with NMES treatments, as well as maximizing NMES-induced torque,[26,34,39,40,42-45] have been examined extensively. These strategies include but are not limited to: decreasing or increasing frequency, pulse duration or intensity of the electrical stimulus prior to beginning the treatment[26,31-33,46-55]; systematically altering the frequency, pulse duration or intensity over the course of the treatment[44,52,56-58]; using greater rest intervals between contractions[59-62]; use of variable frequency trains[28,30,55,63-67]; increasing electrode size[68-72]; altering electrode placement and/or orientation[39,41,69,70,73-75]; using different current waveforms[23,37,38,40,76]; implementing blunting strategies[77-81]; and altering joint position.[36,42,71,82,83] Unfortunately, some of these techniques supported by empirical evidence cannot be easily incorporated or are inaccessible within clinical settings,[24] and despite the extensive research in this area researchers have indicated that additional strategies are needed.[22]

The Kneehab® XP (Theragen LLC, Leesburg, VA) is a relatively new electrical stimulator approved by the United States Food and Drug Administration for marketing as a modality for muscle strengthening.[84] This device has been marketed to include a novel strategy with the potential to enhance NMES treatment efficacy, and as such it has gained a significant amount of attention in the literature.[21,27,84-90] Conventional NMES (c-NMES) devices transmit an electrical current from one electrode to another via a single fixed

path; but by using newly developed multipath™ technology, this novel device transmits an electrical current with altered pulse durations between four large electrodes integrated within a neoprene thigh garment via two separate channels.[21,27,84,88,91] Consequently, this device provides multipath current distribution, and thus it is referred to as multipath NMES (m-NMES).[21,27] According to the manufacturer, this m-NMES stimulator has the potential to enhance motor unit recruitment via improved patient comfort and spatial distribution of the stimulus leading to stronger NMES-induced contractions, while also minimizing muscle fatigue.[84,88,92]

By addressing the primary limiting factors of NMES training intensity, the novel m-NMES device has the potential to positively impact the efficacy of NMES as an adjunct for strengthening the quadriceps of healthy and injured individuals. Evidence-based practice requires that clinicians incorporate current best evidence addressing the efficacy of therapeutic interventions, along with their clinical expertise, when making clinical decisions.[93] Therefore, scientific examination of commercially available modalities, such as the novel m-NMES device, is needed to provide evidence that can be used by clinicians when making decisions with respect to therapeutic interventions (e.g., which NMES device to purchase or use as a strengthening adjunct).

Recent training studies incorporating the novel m-NMES device have demonstrated improved patient outcomes.[86-90] However, only one[87] of these training studies compared strength gains across patient groups receiving either c-NMES or m-NMES; thus creating an appropriate counterfactual framework by which the effectiveness of m-NMES could be compared to c-NMES (e.g., what would have happened if patients received c-NMES rather than m-NMES).[94] Relative to a group of patients receiving c-

NMES treatments and to a control group that did not receive any NMES, Feil et al.[87] observed greater quadriceps strength six weeks after an ACL repair within a group of patients receiving m-NMES treatments. Although these results appear to indicate that m-NMES is more effective than c-NMES, they should be interpreted with caution. The on:off ratio for the c-NMES condition was 10:20 during this study, whereas the m-NMES was 5:10. This difference ultimately resulted in an undesired systematic difference that may have confounded the results, since the different on:off ratios resulted in the m-NMES group performing approximately twice the number of repetitions as the c-NMES group over the course of each 20 minute treatment session. It is important to note that the duty cycle for both NMES groups was 1:2, which should have resulted in a similar total "on" time over the course of the 20 minute treatment.[87] To the best of our knowledge there are no published studies examining the impact of implementing the same amount of "on" time, while allowing the total number of contractions to differ between groups. Therefore, it is unclear whether the results observed by Feil et al.[87] are attributable solely to the use of m-NMES rather than c-NMES, because the extent to which the different on:off ratios may have confounded the results is unknown.

Feil et al.[87] also reported that the mechanisms by which m-NMES outperformed c-NMES during their study were unclear. Consequently, Maffiuletti et al.[21] and Morf et al.[27] completed two basic studies to determine if the results were attributable to the hypothesized benefits of the m-NMES device. While each study used a similar methodological approach, variations in the patient populations and results were observed. Maffiuletti et al.[21] used 10 healthy subjects, and did not observe significant differences with respect to fatigue related outcomes; which included the change between pre- and

post-test MVIC and doublet twitch torque, as well as the decline in NMES-induced torque across repetitions. In contrast Morf et al.[27] used 20 total knee arthroplasty (TKA) patients 6-12 months post-op, and reported significantly less fatigue under the m-NMES condition. However, the difference was only with respect to the change in pre- and post-test MVIC torque, whereas there was no difference when comparing the decline of NMES-induced torque across repetitions. Both studies reported significantly greater NMES-induced torque under the m-NMES condition while using a maximum tolerable intensity, and significantly greater patient comfort was observed under the m-NMES condition while using a variety of intensities (e.g., maximum tolerable, or 5% MVIC, 10% MVIC, 15% MVIC, 20% MVIC).[21,27]

Since the results of the fatigue related outcomes were non-significant, or inconsistent across these studies,[21,27] the proposed benefit of minimizing NMES-induced fatigue while using the m-NMES device does not appear to be supported by the current literature. The lack of support may be due to methodological limitations of the previous studies. For example, Morf et al.[27] identified the short treatment duration (20 contractions over a 5 minute period) and low training intensity (20% MVIC), as well as the performance of additional experimental trials prior to the fatiguing protocol, as possible limitations leading to their non-significant results. It is important to note that m-NMES appeared to outperform c-NMES with respect to patient comfort and NMES-induced torque during each of these basic studies. However, both sets of authors indicated that differences in the electrode configuration of the two NMES techniques may have contributed to the observed significant differences, and can be considered a limitation.[21,27]

Based on the previously discussed results and limitations of the two basic studies,[21,27] the mechanisms by which m-NMES outperformed c-NMES during the Feil et al.[87] study remain unclear. There are also parameters and outcomes associated with the m-NMES device yet to be investigated in the literature that warrant consideration. For example, studies[21,27,87] comparing m-NMES to c-NMES have predominantly implemented a 5:10 ratio, which is only one of seven options available on the m-NMES device. This is a potential limitation, since the other available ratios may have greater clinical relevance. For example, a 10:50 ratio has been previously recommended.[95] A meta-analysis[6] addressing the topic of NMES efficacy for quadriceps strengthening revealed that a 10:50 ratio was used most often by the included randomized controlled trials, and other authors have reported a similar observation.[86] In addition, one of the aforementioned clinical studies[86] using the m-NMES device utilized the 10:50 ratio option, and a 10:50 ratio is regularly implemented within clinical settings. Therefore, a comparison of m-NMES and c-NMES using a 10:50 ratio is warranted.

Due to the previously discussed methodological limitations of available studies comparing m-NMES and c-NMES,[21,27,87] and in order to advance the evidence-based decision-making process with respect to NMES treatments, further investigation of the novel m-NMES device is warranted. Therefore, the purpose of this project was to compare the effects of m-NMES and c-NMES on fatigue related outcomes and self-reported discomfort levels.

In order to appropriately examine NMES-induced fatigue, the initial contraction intensity should be standardized across conditions; as any systematic differences could significantly impact fatigue related outcomes and bias the results. Although this approach

is scientifically sound, it is also a limiting factor because a maximum comfortable stimulus intensity, which varies across individuals, should be utilized within clinical settings. In addition, standardizing the initial contraction intensity limits the clinical applicability of the results, as it does not allow for inferences regarding which NMES method allows for greater training intensities. Therefore, this project consisted of four test sessions that were subdivided into two manuscripts (Chapter IV, Chapter V) addressing similar sets of research questions and using a different methodological approach to standardize the stimulus intensity. Data collected during the first two test sessions were reported in Manuscript 1 (Chapter IV), whereas data collected during the last two test sessions were reported in Manuscript 2 (Chapter V).

Manuscript 1 Research Questions and Hypotheses

Manuscript 1 Research Questions

RQ_1 – While implementing a 10:50 on:off ratio and an initial training intensity of 30% MVIC, does the percent decline in MVIC torque differ between the c-NMES and m-NMES conditions?

RQ_2 – While implementing a 10:50 on:off ratio and an initial training intensity of 30% MVIC, does the percent decline in NMES-induced torque differ between the c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?

RQ_3 – While implementing a 10:50 on:off ratio and an initial training intensity of 30% MVIC, does the percent decline in torque-time integral (TTI) differ between c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?

RQ_4 – While implementing a 10:50 on:off ratio and an initial training intensity of 30% MVIC, does the total torque-time integral (T-TTI) differ between the c-NMES and m-NMES conditions?

RQ_5 – While implementing a 10:50 on:off ratio and a training intensity of 30% MVIC, does self-reported discomfort (mm) differ between the c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?

Manuscript 1 Research Hypotheses

H_1 – The percent decline in MVIC torque would be significantly greater after the c-NMES condition.

H_2 – The percent decline in NMES-induced torque would be significantly greater during the c-NMES condition and the percent decline in NMES-induced torque would be significantly greater over time. In addition, the rate of change would differ based upon NMES condition.

H_3 – The percent decline in TTI would be significantly greater during the c-NMES condition and the percent decline in TTI would be significantly greater over time. In addition, the rate of change would differ based upon NMES condition.

H_4 – The T-TTI would be significantly greater during the m-NMES condition.

H_5 – The self-reported discomfort levels would be significantly greater during the c-NMES condition and would significantly decrease over time. In addition, the rate of change would differ based upon NMES condition.

Manuscript 2 Research Questions and Hypotheses

Manuscript 2 Research Questions

RQ_1 – Does the maximum comfortable stimulus intensity (mA) differ between the c-NMES and m-NMES conditions?

RQ_2 – While using a maximum comfortable stimulus intensity, does the initial normalized NMES-induced torque (Nm/kg) significantly differ between the c-NMES and m-NMES conditions?

RQ_3 – While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does the percent decline in MVIC torque differ between the c-NMES and m-NMES?

RQ_4 – While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does the percent decline in NMES-induced torque differ between the c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?

RQ_5 – While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does the percent decline in TTI differ between c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?

RQ_6 – While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does the T-TTI differ between the c-NMES and m-NMES conditions?

RQ_7 – While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does self-reported discomfort differ between the c-NMES and

m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?

Manuscript 2 Research Hypotheses

H_1– The maximum comfortable stimulus intensity would be significantly greater during the m-NMES condition.

H_2– The initial normalized NMES-induced torque would be significantly greater during the m-NMES condition.

H_3 – The percent decline in MVIC torque would be significantly greater after the c-NMES condition.

H_4 – The percent decline in NMES-induced torque would be significantly greater during the c-NMES condition and the percent decline in NMES-induced torque would be significantly greater over time. In addition, the rate of change would differ based upon NMES condition.

H_5 – The percent decline in TTI would be significantly greater during the c-NMES condition and the percent decline in TTI would be significantly greater over time. In addition, the rate of change would differ based upon NMES condition.

H_6 – The T-TTI would be significantly greater during the m-NMES condition.

H_7 – The self-reported discomfort levels would be significantly greater during the c-NMES condition and would significantly decrease over time. In addition, the rate of change would differ based upon NMES condition.

Assumptions

1. Participants provided honest answers while completing the health and physical activity questionnaire.

2. The wash-out period between test sessions was sufficient and carry-over effects did not significantly impact the results.[96]

3. Participants provided maximum effort during MVICs, and recorded MVIC peak torque values represented true maximum values.

4. Participants completely relaxed during all NMES-induced contractions (e.g., participants did not voluntarily aid or inhibit NMES-induced torque production).

5. Participants provided honest answers while completing the visual analog scale (VAS), and self-reported discomfort levels reflected each participants' true level of discomfort.

6. Self-selected maximum comfortable stimulus intensities reflected each participant's true maximum comfortable stimulus intensity (e.g., the highest stimulus intensity that does not cause pain).[77]

Delimitations

1. The project duration was restricted to six sessions.

2. Individuals attending The University of Southern Mississippi and/or residing within the Hattiesburg community; who were male, recreationally active, healthy, age 18-35; with a body mass index (BMI) of $\leq$30 kg/m^2.

3. Individuals capable of tolerating a stimulus intensity resulting in a NMES training intensity of $\geq$30% MVIC by the end of two familiarization sessions.

4. Individuals capable of producing three consecutive MVICs within 10% of one another during each test session.

5. Each test session consisted of 18 NMES-induced contractions and the following NMES parameters were used: 70 Hz frequency, 400 μsec pulse duration and a 10:50 on:off ratio.

6. NMES was only applied to the quadriceps of the dominant leg, and the ensuing review of the literature focuses primarily on the use of NMES as a strengthening modality specific to the quadriceps. However, as previously mentioned this is the muscle group most often treated with NMES.[6]

Limitations

1. Participants were exposed to NMES treatments during only six sessions, which likely does not allow for training effects, so corresponding outcomes were not included (e.g., strength gains). Therefore, the results of this project do not provide direct evidence that m-NMES is, or is not, more effective than c-NMES with respect to rehabilitation and/or strength training in injured or healthy populations.

2. The generalizability of the results was limited by the small regional convenience sample of healthy males, that are recreationally active and 18-35 years old.

3. Skin impedance may change daily,[97] but no attempts were made to measure and subsequently standardize each participants' skin impedance across days. However, it is important to note that the anterior thigh of the test leg was shaved and cleaned each day in an effort to reduce skin impedance. Participants were

also be asked to arrive well hydrated each day, but no control over hydration status was attempted.

4. Participants were asked to refrain from strenuous activities for 12 hours prior to reporting each day, but continuous observation of participants to ensure compliance was not possible.

5. Fatigue related outcome measures included in this project are considered to be general assessments of fatigue, and as such the results did not allow for distinction between central and peripheral fatigue mechanisms.[98]

6. A direct measure of nerve accommodation, which partially contributes to NMES-induced torque declines,[99,100] was not included in this project. Therefore, the ability to differentiate between the contributions of muscular fatigue and nerve accommodation to declines observed in NMES-induced torque was limited.

7. NMES-induced torque production is attributable to central and peripheral pathways,[101,102] however, measures capable of differentiating the contribution of each pathway to overall torque production were not included. Therefore, the results were not sufficient to determine which NMES method (c-NMES, m-NMES), if any, enhances central recruitment pathways.

8. The c-NMES electrodes did not replicate the size of the m-NMES electrodes exactly (427 cm^2 vs. 360 cm^2), which limited the ability to attribute the observed difference in maximum comfortable stimulus intensity across conditions to current distribution method (e.g., multipath or single path).

9. Due to a lack of availability, it was not possible to blind the primary investigator tasked with measuring the outcomes to treatment condition. Thus, an experimenter expectancy threat remained possible.[94]

10. Due to a lack of equipment, it was not possible for participants to complete each of the six sessions at the same time. Therefore, a history threat remained possible.[94]

Definitions

The following are definitions of terms used in this project that may be less known to the reader, but that are not specifically defined elsewhere within the document. Thus, they have been provided to facilitate the reader's comprehension.

Asynchronous- The normal pattern of motor unit recruitment, by which the activation of motor units is temporally spaced. The recruitment of a given motor unit is temporally spaced from the recruitment of a previously recruited motor unit but their forces are summed.[103]

Doublet torque- The peak torque produced during a contraction occurring in response to a doublet electrical train, which consists of two electrical pulses separated by a brief inter-pulse interval (e.g., 100 ms).[104] A change in the doublet torque following a fatiguing protocol is indicative of peripheral fatigue.[105]

Galvanic skin resistance (GSR) - A method by which changes in skin resistance are monitored. It is used to objectively assess pain levels as it is considered to measure the response of the sympathetic nervous system to a painful stimulus such as NMES.[40]

Hoffman reflex (H-reflex)- An electromyography (EMG) technique that can be used to assess motor neuron excitability levels,[106] thus it is considered to be a measure of

central fatigue.[105] The H-reflex is an electrically induced twitch response observed in an EMG signal that is equivalent to the stretch reflex, as it occurs due to the depolarization of Ia afferent axons in response to an electrical stimulus. [107,108] These large axons (Ia afferent) are responsible for transmitting sensory information with respect to changes in muscle length from muscle spindles to the spinal cord.[109] In an EMG signal, the H-reflex appears after an M-wave.[107,108]

Low:High Frequency Ratio- The ratio of peak torques produced in response to an electrical testing train consisting of a low frequency relative to an electrical train consisting of a high frequency (e.g., 10 Hz:100 Hz, 20 Hz:60 Hz).[46,52,104] Low:High frequency ratios are used to assess low-frequency fatigue.[46,52,104] Low frequency fatigue is characterized as a long lasting decrease in force production, with the decrease occurring at a greater rate when electrically inducing a contraction using a low-frequency stimuli rather than using a high-frequency stimuli.[110,111] Low-frequency fatigue occurs as a result of a variety of activities, and low-frequency stimulation is not the only possible cause.[111]

M-wave- An EMG technique used to assess a muscle response because a change in the M-wave amplitude is considered to indicate a change in the number of motor units activated.[112] An M-wave occurs due to the direct stimulation and subsequent depolarization of efferent nerve fibers in response to a brief electrical stimulus,[107,108] thus it is considered to be a measure of peripheral fatigue as it excludes the central nervous system (CNS). In an EMG signal, the M-wave is observed following the stimulus artifact.[108]

Non-selective- The random pattern of motor unit recruitment occurring during NMES treatments, since the muscle fiber types recruited by the NMES stimulus are not recruited in any type of order or pattern (e.g., fast to slow or slow to fast).[14,113]

Spatially fixed- The altered pattern of motor unit recruitment occurring during NMES treatments.[14,113] The number of motor units recruited by a given NMES stimulus is fixed, thus when the muscle becomes fatigued additional fibers are not recruited to prevent the drop in force production.[14]

T2 Signal- A form of signal generated during magnetic resonance imaging (MRI), and it is determined by the manner in which the protons of the tissues being imaged respond (e.g., relaxation times) to the radiofrequency pulses emitted by the MRI machine.[114] The intensity of the T2 signal ranges from bright to dark, and is determined by the type of tissue imaged (e.g., solid masses and fluid are bright, fat is dark).[114] Increases in T2 signal intensity following NMES have been used to examine the pattern of motor unit recruitment and to differentiate the muscular areas activated during the treatment from the areas that were not activated.[26,50,51]

Temporally synchronous- The altered pattern of motor unit recruitment occurring during NMES treatments.[14,113] The motor units recruited by the NMES stimulus are recruited at the same time, or in a temporally synchronous manner, which has been suggested as causing a greater metabolic demand and subsequently resulting in greater fatigue.[51]

Torque-time integral (TTI)- The area under the torque-time curve. With respect to isometric contractions, the TTI is considered to represent the amount of isometric work done during a given contraction.[47,115,116] As such, changes in the TTI over course of a

series of NMES-induced contractions have been used as a measure of fatigue during previous NMES studies.[23,29,47,115,116]

Twitch torque- The peak torque produced during a single twitch contraction, which occurs in response to a single electrical pulse.[104] A change in the twitch torque following a fatiguing protocol is indicative of peripheral fatigue.[105]

CHAPTER II – REVIEW OF RELATED LITERATURE

Overview

As mentioned previously, Maffiuletti et al.[17,21] identified the primary limitations of neuromuscular electrical stimulation (NMES) treatments as: muscle fatigue leading to a premature decline in NMES-induced torque, spatially limited motor unit recruitment which constrains the amount of NMES-induced torque that can be achieved, and patient discomfort associated with the electrical stimulus and subsequent involuntary contraction. These factors are considered to be limitations because they ultimately influence NMES-induced force output and the subsequent NMES training intensity used during treatments, which is considered to be the primary determinant of strength gains associated with this treatment modality.[7-10,16-20] What follows is a review of the literature related to the limiting factors of NMES, with a focus on the primary strategies that have been developed to address these factors. The purpose of this review was to provide background information to enhance the reader's understanding of the current state of the literature and establish the need for this project.

Muscle Fatigue

Fatigue Overview

Various definitions of fatigue exist in the literature,[110] which has led to some confusion with respect to the interpretation of fatigue related results.[117] Regardless of the definition used, there are some common characteristics associated with fatigue. Specifically, fatigue involves a reversible decline in one or multiple systems of the body.[118] For the purposes of this project muscle fatigue is operationally defined as an exercise-induced decrease in a muscle's ability to produce force.[98,110] NMES-induced

contractions were used to represent "exercise", while the quadriceps represented the "muscle" of interest. It is important to note that using involuntary electrically evoked contractions to represent exercise during a fatigue related study is an acceptable technique.[110]

Based on their extensive review of the literature, Enoka and Stuart[117] concluded that rather than the result of a single universal underlying factor, declines in force attributable to fatigue are the result of many underlying mechanisms; which include central drive failure, neuromuscular propagation failure, metabolic substrates and excitation-contraction coupling failure. In addition, they reported that the extent to which each underlying mechanism contributes to the overall decline in muscle force appears to be task dependent.[117] Fatigue can be subdivided into two main categories based on the underlying mechanisms responsible for the force declines, which are central and peripheral fatigue.[98] Peripheral muscle fatigue refers to the decrease in muscle force production attributable to changes occurring at or distal to the neuromuscular junction; whereas central fatigue refers to the decrease in muscle force attributable to a decreased ability to voluntarily activate the muscle.[98]

NMES-induced Fatigue

NMES-induced contractions appear to result in similar amounts of fatigue irrespective of the fiber type composition (e.g., more slow twitch than fast twitch, or fast-twitch than slow twitch) of the muscle being stimulated.[63] Despite this consistency across muscle groups, NMES-induced contractions have been shown to result in greater muscle fatigue relative to voluntary contractions.[15,51,62,119,120] The altered motor unit recruitment present during NMES-induced contractions relative to voluntary contractions

(addressed in the motor unit recruitment section), is believed to be the primary contributing factor to the greater fatigue observed during NMES treatments.[119,120]

Although it is commonly accepted that NMES-induced contractions lead to greater fatigue relative to voluntary contractions, the relative contribution of central and peripheral fatigue mechanisms responsible for NMES-induced fatigue is not as well understood. With respect to NMES-induced contractions of the quadriceps, Zory et al.[121] concluded that the decrease of approximately 20% in MVIC torque they observed following 30 NMES-induced contractions was primarily attributable to peripheral fatigue. This conclusion was based on their observation that voluntary activation levels did not change, while M-wave amplitude significantly decreased following the NMES-induced contractions.[121] In a more recent study, Fouré et al.[104] also attributed the roughly 30% decline in quadriceps MVIC torque they observed immediately following 40 NMES-induced contractions solely to peripheral fatigue; which was based on significant decreases in post-test doublet and twitch torque, as well as declines in the low:high frequency ratio (10:100 Hz) and rate of force development, while voluntary activation was unaltered.

It appears that NMES-induced fatigue of the quadriceps is primarily due to peripheral fatigue,[104,121] but this may not hold true across different muscle groups. For example, Boerio et al.[122] concluded that peripheral, as well as central mechanisms, contributed to NMES-induced muscle fatigue while stimulating the triceps surae, even though they used a similar treatment protocol to that of Zory et al.[121] (e.g., 6.25 second:20 second on:off ratio and a maximum tolerable intensity). This conclusion was based on their observation that voluntary activation deficits and a reduction in electromyography

(EMG) signal, which represented neural drive from supraspinal centers to the muscle and neuromuscular propagation failure, occurred along with the declines in MVIC torque following the NMES treatment.[122] Although Fouré et al.[104] attributed NMES-induced fatigue solely to peripheral factors immediately following the NMES treatment, this did not hold over the course of a 4 day recovery period during which MVIC deficits persisted. During the recovery period they observed significant declines in voluntary activation in addition to peripheral factors that were related to the remaining MVIC deficits. Consequently, they concluded that prolonged NMES-induced fatigue of the quadriceps may be attributable to both central and peripheral mechanisms.

Motor Unit Recruitment

Voluntary Motor Unit Recruitment

A motor unit is defined as a motor neuron's cell body (soma), dendrites, axon, axonal branches and the muscle fibers innervated by the axonal branches.[103,123,124] The axon of a single motor neuron is grouped together with other axons to form a peripheral spinal nerve, with each axon of a spinal nerve innervating tens to thousands of fibers of the same fiber type.[103,124,125] Although there are a variety of muscle fiber types, researchers generally use three main groupings based on the fiber's metabolic characteristics and contraction speed; the fiber types are: Type I (slow twitch, slow oxidative), Type IIa (fast twitch, fast oxidative glycolytic) and Type IIb (fast twitch, fast glycolytic).[124] The muscle fibers of a single motor unit are dispersed throughout the muscle rather than being grouped closely together, and all fibers belonging to a motor unit are recruited when the motor neuron fires.[103]

Based on their seminal study using the gastrocnemius muscles of cats, Burke and colleagues[126] are credited with developing the classic motor unit typology system.[125] Their classification of motor units was based on the response of motor units to stimulus trains eliciting unfused tetani (twitches), as well as each motor unit's sensitivity to fatigue. The unfused tetanic response of each motor unit was examined for the presence of a "sag" property defined as a rapid increase in tension followed by a decline in the tension that eventually plateaued. Each motor unit was also repeatedly stimulated to produce a series of contractions. Sensitivity to fatigue was determined using a fatigue index, which compared the peak tension produced during the initial tetanic contraction relative to that produced after 2 minutes (120th contraction). Motor units were then classified based on the presence or absence of the sag property and the corresponding fatigue index scores. The classifications created by Burke and his colleges were: fast twitch fatigue sensitive (FF), fast twitch fatigue resistant (FR) or slow twitch fatigue resistant (S). According to Enoka,[125] Burke's classical motor unit typology is not applicable to human motor units. Therefore, human motor units are often classified based on their recruitment threshold.[123,125]

It is commonly accepted that during voluntary contractions motor unit recruitment order is based on motor unit size, with the recruitment of smaller motor units innervating fatigue resistant slow twitch fibers occurring first, while progressively larger motor units innervating fatigable fast twitch fibers are recruited as force production requirements increase.[124,125,127] This pattern of recruitment is referred to as the Henneman principle, as it is based on the work of Dr. Elwood Henneman.[128,129] Interestingly, the Henneman principle is considered to be applicable to all types of voluntary contractions as well as

reflexes.[125] In addition to the size principle, motor unit recruitment during voluntary contractions is believed to be asynchronous.[103]

NMES Motor Unit Recruitment

Voluntary and NMES-induced contractions are similar in that the axon/axonal branches of a motor neuron ultimately elicit a muscular response, irrespective of the method used. Despite this similarity, motor unit recruitment in response to NMES is significantly different from that of voluntary contractions. The initial difference is that the axon/axonal branches of a motor neuron depolarize in response to an electrical signal generated by an external stimulator rather than to one generated by the central nervous system (CNS). Furthermore, the action potential generated in response to NMES is conducted in both directions along the axon, due to the lack of normal hyperrepolarization occurring behind the signal that is present during signals generated by the CNS.[130] When the signal is transmitted away from the neuromuscular junction, or contrary to the physiological direction, it is referred to as antidromic conduction; whereas when the signal is transmitted in the physiological direction it is referred to as orthodromic transmission.[130]

In addition to bidirectional conduction along axons, the order of motor unit recruitment differs significantly during NMES-induced contractions relative to the voluntary recruitment order previously discussed. The traditional theory related to NMES motor unit recruitment proposed that the recruitment order was reversed, preferentially activating large fast twitch fatigable units followed by the smaller slow twitch less fatigable units.[3,113,131] According to Gregory and Bickel[14] this theory was based on the observations that NMES-induced contractions result in greater amounts of

fatigue relative to voluntary contractions[15,51,62,119,120] and because larger motor units are believed to have a decreased resistance to current.[131]

A seminal study was performed by Adams et al.[51] to test this theory. These authors employed magnetic resonance imaging (MRI) to map the pattern of motor unit recruitment following voluntary and NMES-induced isometric contractions of the quadriceps using changes in the intensity of the T2 signal. They identified the location of the activated muscle fibers and determined percent of activated cross sectional area (% CSA) by examining changes in the T2 signal following the contractions. Substantial inter-individual differences in the location of activated fibers following NMES-induced contractions of 25% MVIC, 50% MVIC and 75% MVIC were observed. Furthermore, activated fibers were located in deep and superficial areas, regardless of the contraction intensity. According to Bickel et al.,[113] this particular observation is in contrast to another commonly held view that NMES primarily stimulates superficial nerves and is incapable of recruiting deep muscle fibers. Adams et al. also observed a significant linear relationship between NMES-induced torque and the % CSA activated (R^2= 0.74). This relationship lead the authors to hypothesize that NMES does not preferentially activate the larger fast twitch motor units, rather the linear relationship suggests that the location of the motor neuron branches is the primary determinant of which muscle fibers are recruited during NMES treatments. This hypothesis is in agreement with the observations of Knaflitz et al. [127] that NMES recruitment order is not reversed but is dependent upon the location of the motor axons relative to the stimulating electrodes, with the fibers innervated by the superficial axons being recruited first.

Adams et al.[51] reported that the T2 signal following voluntary contractions of the same duration and intensity changed very little relative to the NMES-induced contractions. They suggested that the differences in the T2 signal changes after NMES-induced and voluntary contractions provides support to the theory that voluntary contractions recruit motor units in an asynchronous manner in an effort to limit metabolic demand and fatigue. Whereas NMES motor unit recruitment occurs in a synchronous manner with the same fibers recruited repeatedly, which results in a greater metabolic demand and the subsequent greater levels of fatigue.[51] McNeil et al.[120] also recognized synchronous motor unit recruitment as the mechanism responsible for the greater metabolic demand and subsequent declines in MVIC (fatigue) they observed following NMES-induced contractions, relative to voluntary contractions consisting of the same torque-time integral (TTI).

It appears that the traditional theory that NMES motor unit recruitment occurs in a reverse order can no longer be supported. According to Bergquist et al.[101] the current thought among researchers is that the axons' diameter, as well as their distance relative to the electrodes, determines which axons are depolarized; which is based on the understanding that a nerve is more easily stimulated the larger it is, as well as the more superficial it is.[3] Consequently, the current theory regarding NMES-induced motor unit recruitment is that it occurs in a non-selective manner, in addition to being temporally synchronous and spatially fixed.[14,113] The latter two points suggest that the same motor units are repeatedly recruited at the same time throughout the NMES treatment.[4,50]

This novel theory of motor unit recruitment during NMES has been examined in the literature. Jubeau et al.[132] compared the time to peak torque following a

superimposed supramaximal stimulus during a series of voluntary and NMES-induced quadriceps contractions. The time to peak torque was significantly shorter under the voluntary condition, regardless of the contraction intensity (e.g., 20% MVIC, 40% MVIC, 60% MVIC). In addition, time to peak torque did not differ during NMES-induced contractions, regardless of the contraction intensity, whereas it significantly decreased as the contraction intensity increased under the voluntary condition. The authors concluded that fast and slow twitch motor units are recruited during sub-maximal NMES-induced contractions, as evidenced by the consistently slower time to peak torque observed under the NMES condition. Furthermore, due to the lack of variation across contraction intensities during the NMES-induced contractions, the authors concluded that the NMES stimulus does not recruit motor units in any systematic way related to muscle fiber type.

Central and Peripheral Pathways

When considering motor unit recruitment in response to NMES, it is also important to discuss two distinct pathways identified in the literature as contributing to the overall NMES-induced force production. Based on their review of the literature, Collins et al.[102,133] and Bergquist et al.[101] suggested that NMES motor unit recruitment occurs via peripheral and central pathways or mechanisms. These authors defined the previously discussed recruitment pathway, in which motor axons are depolarized in response to the NMES stimulus, as the peripheral pathway. In addition to the well-known peripheral pathway, these authors proposed the existence of an additional central pathway. This less familiar pathway is the process by which a sensory volley, caused by the concurrent depolarization of sensory (afferent) axons during NMES, results in the

synaptic recruitment of motor neurons within the spinal cord. Motor unit recruitment due to the sensory volley is hypothesized to occur via the Hoffman reflex (H-reflex), and/or due to activation of motor neurons within the spinal cord due to the generation of persistent inward currents in spinal neurons caused by the continuous flow of high-frequency afferent stimuli.[102,133-135] The latter pathway is believed to have the potential to produce asynchronous recruitment,[135] but while some muscles may be capable of asynchronous recruitment via central pathways the quadriceps may not be capable of producing this response.[136,137]

There is some evidence to support the presence of a central recruitment pathway during NMES treatments in the literature. Collins et al.[134] observed that relative to the force produced after placing a nerve block proximal to the site of stimulation, plantar flexion force generated via NMES over the triceps surae was much greater without the presence of a nerve block. A follow-up study performed by the same authors also demonstrated a similar pattern while stimulating the triceps surae or tibialis anterior with respect to the presence or absence of a nerve block.[133] Furthermore, during both of these studies[133,134] NMES-induced force was produced while using stimulus intensities below the motor threshold, and NMES-induced contractions persisted after the cessation of the electrical stimulus. These authors concluded that such observations suggest the presence of a central pathway for motor unit recruitment during electrically induced contractions. Lagerquist et al.,[135] also compared NMES-induced torque production of the triceps surae with and without a tibial nerve block. In agreement with the observations of Collins et al.[133,134], under the nerve block condition significant decreases in torque output were observed over the course of a 30 second stimulus; whereas significant increases occurred

without the presence of a nerve block. As with the previous studies, these authors concluded that their results demonstrate that the sensory volley produced during NMES treatments results in the synaptic recruitment of motor neurons within the spinal cord.

As mentioned previously motor unit recruitment is believed to be synchronous during NMES treatments.[14,113] However, it has been suggested that recruitment via the central pathway has the potential to recruit motor units in a similar order to voluntary contractions and in an asynchronous pattern,[101,134,135] thus it has been hypothesized that enhancing motor unit recruitment via central pathways has the potential to limit NMES-induced fatigue.[29,134,135] Based on their review of the literature, Bergquist et al.[101] suggested that to maximize central contributions during NMES motor unit recruitment the stimulus parameters should include long pulse durations, high frequencies and low stimulus intensities. Despite this recommendation, a recent study[29] demonstrated that both conventional NMES and a wide-pulse-high-frequency (WPHF) approach resulted in central pathway motor unit recruitment. In addition, as evidenced by a significantly greater decline in the TTI under the WPHF condition, this method resulted in greater amounts of fatigue. These results lead the authors to conclude that using WPHF to enhance central recruitment in an effort to reduce fatigue is questionable.

The location of NMES application, over the nerve trunk rather than over the muscle belly, has also been proposed as having the potential to influence the relative contribution of peripheral and central pathways to motor unit recruitment. With respect to the quadriceps, Bergquist et al.[136] examined this hypothesis while applying NMES over the femoral nerve trunk located within the femoral triangle or via electrodes over the quadriceps muscles, with a similar NMES training intensity being maintained across

conditions. Under each condition the peak to peak amplitudes of M-waves and H-reflexes, as well as the root mean square of asynchronous activity, were measured via surface EMG recordings. The M-wave amplitude was significantly greater while stimulating over the muscle belly, whereas H-reflexes were significantly greater while stimulating the nerve trunk. Due to the fact that H-reflex amplitudes are considered a measure of central recruitment while M-wave amplitudes are representative of peripheral recruitment, the authors concluded that when NMES is applied over the femoral nerve trunk the motor unit recruitment and subsequent contraction occurs predominantly via the central pathway. Asynchronous activity was also considered a measure of central recruitment, but no such activity was observed under either condition, so the authors concluded that enhancement of central recruitment during stimulation of the femoral nerve trunk was attributable to the H-reflex. Similar results have been observed when applying NMES to the belly of the triceps surae and tibial nerve trunk, with the exception that asynchronous activity partially contributed to the NMES-induced force production while stimulating over the muscle belly.[137] Despite the apparent involvement of a central pathway under this condition, overall NMES-induced torque was still primarily attributable to peripheral recruitment.[137]

It is important to note that the NMES training intensity was relatively low (e.g., 10-20% MVIC) during these studies.[136,137] Lower intensities were most likely selected to prevent elimination of central pathway contributions via large peripheral pathway contributions, as antidromic transmission of action potentials along motor axons prevents the central pathway mechanisms from activating motor units previously activated via peripheral pathways.[101,102,138] This technique presents a variety of problems that make it

problematic to implement clinically with respect to NMES of the quadriceps. Specifically, the femoral nerve trunk is difficult to access, the electrodes have a tendency to move and the quadriceps contractions produced via this method are inconsistent.[101,136]

Strategies to Reduce NMES-induced Fatigue

Overview

As mentioned previously, NMES-induced contractions have been shown to result in greater muscle fatigue relative to voluntary contractions, which is attributed to the altered motor unit recruitment pattern during NMES treatments.[15,51,62,119,120] Muscle fatigue negatively influences NMES treatment efficacy, as it contributes to the decline in NMES-induced torque that is frequently observed over the course of a treatment. It has been suggested that strategies with the potential to minimize NMES-induced fatigue are of significance,[31,47] and NMES-induced fatigue is considered to be the greatest concern with respect to the limitations of this treatment modality.[33] Consequently, strategies with the potential to limit fatigue have received a significant amount of interest in the literature.

Strategies with the potential to limit NMES-induced fatigue often involve manipulation of the parameters readily controlled on NMES devices, with the most widely studied parameter being the stimulus frequency.[47] Prior to addressing these strategies it is important to discuss the different types of muscular responses that can occur in response to NMES, based on the parameters selected by the clinician or researcher. A response resulting in a short muscular contraction followed by complete relaxation is referred to as a twitch contraction, while a sustained muscular contraction occurring in response to the stimulus is referred to as a tetanic contraction or tetany.[3]

When one twitch is superimposed on another twitch before total relaxation can occur it is referred to as summation,[4] but this response is not desired during NMES treatments. A high stimulus intensity but low frequency (e.g., <15 Hz or pps) stimulus is used to elicit a twitch contraction, whereas a high stimulus intensity and high frequency (e.g., >40 Hz or pps) stimulus is used to elicit tetanic contractions. Summation results when the stimulus consists of frequencies between those required to achieve a tetanic or twitch response (e.g., 15-25 Hz or pps).[1,4] The pulse duration recommended for twitch or tetany is the same (e.g., 300-500 μsec).[1] Tetanic contractions are of primary concern for this project, as these are used clinically during NMES treatments for the purpose of reducing strength loss and atrophy or for increasing strength.

Frequencies and Pulse Durations

Kesar et al.[46] examined the influence of stimulus frequency, as well as the pulse duration, on NMES-induced fatigue. They used three separate fatiguing protocols with an initial training intensity of 20% MVIC. The first protocol combined a low frequency (11.5 Hz) and long pulse duration (600 μsec), the second protocol combined a medium frequency (30 Hz trains) and medium pulse duration (150 μsec) and the final protocol combined high frequency (60 Hz) with a medium pulse duration (131 μsec). The low frequency and long pulse duration protocol resulted in the lowest percent decline in the NMES-induced torque, as well as the smallest amount of low frequency muscle fatigue as evidenced by a greater low:high frequency ratio (20 Hz:60 Hz). The authors hypothesized that fatigue was minimized during the low frequency condition as a result of lowering the metabolic demand by reducing the number of pulses generating action potentials, as well as lowering the levels of intracellular calcium. Based on the results

from this study, the authors concluded that performance of NMES-induced isometric contractions is maximized by using a long pulse duration and low frequency stimulus, as this combination has the potential to minimize fatigue.

Gregory et al.[33] also compared the influence of pulse duration and frequency on NMES-induced fatigue, but they maintained a similar total charge (frequency x pulse duration) during each protocol. The stimulus intensity was set to elicit contractions torques of 50% MVIC. Although the initial NMES-induced torque was similar across protocols, when using the same total charge (e.g., stimulation with a high frequency low pulse duration or high pulse duration low frequency were similar) the decline in NMES-induced torque over the course of 60 1 second contractions was greater during higher frequency conditions. However, it should be noted that 1 second contractions are not clinically practical for strengthening purposes. Similar to the conclusions of Kesar et al.[46], Gregory et al. concluded that frequency should be minimized in an effort to limit NMES-induced fatigue.

Gorgey et al.[32] examined the relative importance of pulse duration and frequency on NMES-induced fatigue, but they also included the stimulus intensity as a variable of interest. They measured percent decline in NMES-induced torque, which represented fatigue, during four different protocols. Three of the four protocols maintained similar parameters to a standardized protocol as well as to one another, with the exception of either using a low frequency, short pulse duration or low stimulus intensity. A significant percent decline during the 11^{th}, 21^{st} and 30^{th} contractions was observed, irrespective of the protocol used. Interestingly, relative to the standardized protocol, manipulating the stimulus intensity and pulse duration did not significantly influence NMES-induced

fatigue; but relative to the other three protocols, decreasing the frequency from 100 Hz to 25 Hz significantly reduced the percent decline in NMES-induced torque. It is important to note that in contrast to the studies of Keser et al.[46] and Gregory et al.[33], the initial NMES-induced torque was not held constant across protocols in this study. Greater initial torque was observed while using the standardized protocol, followed by the low frequency, low stimulus intensity and short pulse duration procedures. Despite these initial variations, due to the differences in percent decline across the protocols the torque produced during the low frequency condition was greater than all the other protocols by the 11th contraction (1 minute into the protocol) and this continued throughout the remainder of the treatment. Based on these results it may be acceptable to utilize a lower frequency protocol despite the fact that this may result in a smaller initial torque relative to a higher frequency protocol, allowing for greater torque production over the course of a NMES treatment. Also of interest is the fact that under the shorter pulse duration condition Gorgey et al. observed less torque per active cross sectional area (CSA; Nm/cm^2) relative to the other protocols, but despite this difference the observed NMES-induced fatigue was not reduced when compared to the standardized protocol using the same frequency and stimulus intensity.

In a later study Bickel et al.[31] also examined the influence of frequency, pulse duration and stimulation intensity on NMES-induced fatigue, but in contrast to Gorgey et al.,[32] the initial NMES-induced torque was standardized across protocols, using 25% MVIC as the benchmark. Despite this difference in methodology, they observed similar results, with a significantly lower decline in NMES-induced torque (roughly 25% drop)

occurring during the low frequency protocol; whereas the low pulse duration and low stimulus intensity protocols had roughly a 50% drop.

Despite consistent results across a variety of studies demonstrating reduced levels of fatigue when decreasing the frequency,[31-33,46] there are inconsistencies reported in the literature. Matsunaga et al.[48] observed greater fatigue under a low frequency condition relative to a high frequency condition (20 Hz vs. 100 Hz). Although it is unknown why their results were in direct contrast to the other studies, differences may have occurred because Matsunaga and colleagues measured NMES-induced fatigue over the course of a 60 minute treatment, as well as using a longer on:off ratio (4:56) and larger beginning training intensity (60% MVIC). Despite possible differences in methodology, the observations of Matsunaga et al. suggest inconclusive evidence with respect to the influence of stimulation frequency and muscle fatigue.[46]

Furthermore, Russ et al.[116] reported no differences in fatigue and metabolic cost between a high and low frequency protocol while maintaining similar NMES-induced force. It is important to note that these authors utilized a frequency of 80 Hz to represent low frequency, but this is often considered to be a high frequency. However, for the purposes of this study 80 Hz was considered to be low because the higher frequency was 100 Hz. A lack of differences in fatigue and metabolic changes have also been observed in a study using high and low frequencies of 100 Hz and 20 Hz, respectively.[47] Although this observation is in agreement with the results of Russ et al., it is important to note that this study used rats rather than human subjects.

Gorgey et al.[32] suggested that the non-significant observations of Russ et al. [116] occurred because the two frequencies that they utilized are within the plateau portion of

the force-frequency curve. Russ et al. also acknowledged this as a limitation of their study, and indicated that NMES-induced fatigue may still be positively influenced by lower frequencies. However, they postulated that because their study uniquely maintained force across the two protocols, NMES-induced fatigue is influenced more so by the resultant force production than by the stimulus frequency. This argument appears to be supported by the results of an additional study performed by the same primary author. While not matching initial force values, Russ and colleagues[49] observed greater percent declines in NMES-induced torque with higher frequencies (40 & 80 Hz) relative to lower frequencies (20 Hz). These results are in agreement with other studies demonstrating a decrease in fatigue while using lower frequencies [31-33,46], but Russ et al.[49] reported that the initial peak torque was a better predictor of fatigue than the number of pulses of the stimulus or the initial force-time integral (measure of isometric work). This relationship provides some support to their original argument that NMES-induced force rather than frequency influences fatigue.[116] However, because Bickel et al.[31] observed significantly less fatigue while utilizing a low frequency protocol, despite standardizing the initial NMES-induced torque across conditions, there appears to be a lack of consistent support to the conclusions of Russ et al.

Although many of these studies demonstrated that frequency influences NMES-induced fatigue, most of the fatiguing protocols were not clinically relevant as they consisted of short contraction durations (e.g., 300 ms, 1 second, 3 seconds) rarely used on:off ratios (e.g. 300 ms:700 ms, 1 second:1 second, 3 seconds:3 seconds, 4 seconds:56 seconds) and/or a large number of contractions (e.g., 30, 60, 150). It is likely that these settings were chosen in an effort to magnify NMES-induced fatigue, but their

observations can only be extrapolated to clinical protocols used during NMES treatments. Consequently, future studies should verify that these results hold while implementing clinically relevant protocols (e.g. 10 second contraction, 10:50 on:off ratio, 20 contractions).

Based on the results of studies previously discussed,[29,31-33,46,49] it appears that higher frequencies increase NMES-induced fatigue. Therefore, it has been recommended that to minimize fatigue, while maximizing NMES-induced torque, clinicians should lower the frequency while increasing the pulse duration and stimulus intensity.[32] Despite this recommendation, there is a lack of consistency in the literature because some studies report no difference or greater fatigue with lower frequencies. [47,48,116] Due to these results, some have maintained that lowering the frequency while increasing the pulse duration is not a sufficient strategy for minimizing fatigue.[47] In addition, further research is also needed to better understand the costs associated with longer or shorter pulse durations, as it has been reported that the influence of this parameter on NMES-induced muscle fatigue is less understood than other parameters (e.g., frequency, stimulus intensity).[32]

Systematically Altering Frequencies

To this point, the studies reviewed primarily examined the impact of using a high or low frequency on NMES-induced fatigue. Systematically altering the stimulus frequency over the course of the treatment is another strategy that has been examined to reduce fatigue. Downey et al.[57] reported that relative to a high (40 Hz) or low (20 Hz) constant frequency protocol, systematically altering the frequency throughout the treatment significantly improved the amount of time in which dynamic NMES-induced

quadriceps contractions were adequately performed. This observation held true regardless of whether the frequency was systematically altered from lower to higher or higher to lower values. Consequently, the authors indicated that altering the frequency from high to low frequencies, or vise-versa, may be a viable strategy to enhance NMES efficacy. These authors also observed a greater amount of time in which contractions were adequately performed while using lower frequencies (e.g., 20-30 Hz) relative to higher frequencies (e.g., 30-40 Hz), and this observation held true irrespective of whether the frequency was altered from low to high or high to low. Based on this observation, that lower frequencies (20-30 Hz) appeared to enhance performance irrespective of the initial frequency or fatigued state, the authors recommended using lower frequencies to delay the onset of NMES-induced fatigue; which is in agreement with some of the studies previously discussed.[33,46]

Kebaetse et al.[58] also examined the influence of systematically altering the frequency on fatigue during dynamic NMES-induced contractions. Similar to Downey et al.[57] they observed less fatigue when altering the stimulus frequency from a low to high, as evidenced by a greater number of contractions performed under this condition. However, in contrast to Downey et al. they observed a significant increase in fatigue when altering the stimulus frequency from high to low. Thus, these authors suggested that lowering the stimulus frequency may not be an effective strategy for minimizing NMES-induced fatigue; whereas increasing the stimulus frequency over the course of the treatment may be an effective strategy. It is difficult to determine why differences in these two studies occurred, but it may be due to the fact that the higher frequency

protocol used by Kebaetse et al. consisted of a series of doublets rather than equally spaced pulses.

Systematically altering the frequency during a treatment consisting of isometric contractions has also been shown to influence fatigue. Binder-Macleod et al.[56] compared the rate and amount of decline in NMES-induced quadriceps torque under two conditions using the same maximum tolerable intensity. During the frequency modulation protocol they systematically decreased the initial frequency of 60 pps by 5 pps throughout the treatment, with the final 10 contractions using a frequency of 30 pps. The constant frequency protocol maintained a frequency of 60 pps throughout the treatment. The rate of decline was significantly less by the 4th contraction and by the 6th contraction the NMES-induced torque was significantly greater under the condition in which the frequency was systematically decreased. Therefore, systematically reducing the frequency during repetitive NMES-induced isometric contractions may also minimize fatigue. It is important to note that unlike systematically increasing the stimulus intensity, in order to alter the frequency most NMES units require the clinician to interrupt the selected protocol in order to implement this strategy; thus this strategy may lack clinical applicability.

Positive results, with respect to decreases in NMES-induced force, have also been observed while systematically increasing the frequency from 20 Hz to 40 Hz.[139] However, these results were not consistent across different stimulus intensities. Kesar et al.[52] performed a study that systematically increased the frequency while also comparing the influence of modulating the frequency to modulating pulse duration. Under the modulated frequency condition they observed increases in NMES-induced peak torque

and the TTI over the course of the fatiguing protocol, but modulating the pulse duration did not prevent a decline in NMES-induced peak torque. Despite not preventing a decline in peak torque, modulating the pulse duration resulted in a smaller decline relative to maintaining constant parameters. Although modulating frequencies and pulse duration minimized or prevented a decline in peak torque and TTI during the fatiguing protocol, smaller declines in the pre- and post-test peak torque elicited by 60 Hz testing trains and the low:high frequency ratio (20 Hz:60 Hz) were observed following the constant parameter condition; which suggests that significantly greater fatigue occurred following the modulated conditions. The authors concluded that the superior post-test fatigue associated with the constant parameter condition occurred due to a smaller metabolic demand on the muscle, because the parameter modulation conditions resulted in greater NMES-induced torque over the course of the fatiguing protocol.

The observations made by Kesar et al.[52] that NMES-induced torque over the course of the fatiguing protocol was enhanced by modulating frequency, while coming at the expense of greater post-test fatigue, highlights an important concept. As suggested by Holcomb,[140] strategies that maintain NMES-induced torque over the course of a treatment are most important. Thus, strategies that result in greater NMES-induced torque over the course of the treatment, like the one implemented by Kesar et al., may be preferred over less fatiguing protocols.

There is a lack of consistency among the results of studies examining the influence of modulating frequency on NMES-induced fatigue. For example, one study[58] demonstrated that increasing the frequency from low to high minimized fatigue but systematically decreasing the frequency did not, while another study[57] showed similar

improvements while increasing or decreasing the frequency. Furthermore, one study[56] demonstrated that systematically decreasing the frequency minimized the decline in NMES-induced torque, while another study[52] demonstrated that increasing the frequency increased torque production rather than simply minimizing the decline. Due to the lack of consistency across these studies it is difficult to determine the optimal method of modulating the frequency to eliminate or minimize NMES-induced fatigue. It may be that the impact of frequency modulation is patient dependent, since inter-individual differences have been shown to occur with respect to frequency modulation.[56] Although the optimal method of frequency modulation is unknown, this method does appear to have promise with respect to reducing or eliminating the decline in NMES-induced torque frequently occurring during treatments. Therefore, future studies should build upon the results of the previous studies in an effort to provide clinicians with an evidence-based recommendation with respect to frequency modulation.

On:Off Ratio or Duty Cycle

The rest intervals during NMES treatments are determined by the selected on:off ratio or duty cycle, which is defined as the ratio of the time in which current is flowing relative to when it is not flowing.[1,60] Typical on:off ratios within the clinical setting range from 10-15 seconds on with an off time of 8-50 seconds.[56] The impact of duty cycle has also been examined to determine its influence on NMES-induced fatigue. Packman-Braun[60] observed that participants with hemiparesis were able to maintain NMES-induced wrist extensor torque above 50% of the initial value for a longer period of time while using a 1:5 duty cycle (5:25 on:off ratio), relative to a 1:3 (5:15 on:off ratio) or 1:1 (5:5 on:off ratio) duty cycle.

Interestingly, Packman-Braun[60] suggested that her results may be less applicable to larger muscles such as the quadriceps, but later studies examining the influence of duty cycle while treating the quadriceps in healthy individuals do not support this hypothesis. Cox et al.[59] compared rest intervals of 35, 50 and 65 seconds during a series of 10 NMES-induced contractions. They observed significant reductions in NMES-induced torque during the 35 second condition, however, no difference was observed between the 50 second and 65 second conditions. This led these authors to hypothesize that longer rest intervals may allow for enhanced metabolic recovery. Rankin and colleagues[61] compared fatigue during and after 10 NMES-induced quadriceps contractions at 30% MVIC using duty cycles of 1:1, 1:5 and 1:12. They observed significant declines in the NMES-induced torque throughout the treatment under each of the three conditions, but the decline was progressively greater as rest time decreased. These authors also examined recovery for 60 minutes following the treatment, as well as after 24 hours. Only high frequency fatigue following the 10 second rest condition returned to baseline within 24 hours post treatment. The authors hypothesized that this occurred because less work was performed under the 10 second rest condition, as evidenced by the greater decline in torque. In a more recent study, Holcomb et al.[62] compared the percent decline in NMES-induce torque of the quadriceps over the course of 5 repetitions while using a 30 second or 120 second rest period. The shorter rest lead to a 29.6% decline, whereas the decline during the longer rest period was only 8.2%.

Although longer rest intervals resulted in less NMES-induced fatigue, in each of the studies reviewed NMES-induced torque was lower during the final contraction relative to the initial contraction of the treatment. Based on the results of these studies it

appears that progressively increasing rest intervals does reduce NMES-induced fatigue, but rest periods greater than 120 seconds may be required to eliminate declines in force production. However, longer rest intervals between contractions result in longer treatment times, thus it is not practical to expect clinicians to use rest periods greater than 120 seconds. Therefore, solely increasing rest intervals may not adequately reduce NMES-induced fatigue, so other strategies should be used in conjunction with longer rest periods.

Stimulus Intensity

The influence of the stimulus intensity on NMES-induced fatigue has also been examined. In their seminal study examining NMES motor unit recruitment with MRI, Adams et al.[51] altered the stimulus intensity to achieve quadriceps torque outputs of 25% MVIC, 50% MVIC and 75% MVIC. The authors observed declines in the NMES-induced torque following 5 sets of 10 contractions at each intensity. The average percent decline in torque was significantly greater following the 25% MVIC condition relative to the 75% MVIC condition, which seems to suggest an inverse relationship between NMES-induced fatigue and stimulus intensities. Slade et al.[55] observed a lack of significant differences over the course of 180 constant frequency trains (CFTs) while using a stimulus intensity eliciting 25% MVIC and 50% MVIC, with percent declines of 60% and 65% respectively. Consequently, they concluded that their results do not support the existence of an inverse relationship.

In contrast to these two studies, Binder-Mcleaod et al.[53] reported that the rate and amount of NMES-induced fatigue significantly increased with greater stimulation intensities (20% MVIC vs. 50% MVIC), irrespective of the stimulation frequency used.

Godfrey et al.[54] also demonstrated greater fatigue during higher stimulation intensities, as evidenced by greater declines in NMES-induced force and force-time integrals of the thenar muscles following stimulation of the median nerve using a supramaximal intensity relative to a submaximal intensity. This observation lead the authors to suggest that submaximal stimulation intensities may positively influence muscle fatigue induced by electrical stimulation. Due to the inconsistencies in the reviewed studies, it is unknown which stimulus intensity minimizes fatigue.[46] Furthermore, it appears that the stimulus intensity has less of an influence on NMES-induced fatigue than the frequency of the stimulus.[31,32]

Variable Frequency Trains

The use of variable frequency trains (VFTs) in place of CFTs is another strategy that has also been hypothesized as having the potential to reduce NMES-induced fatigue. A train is defined as the pattern of the electrical pulses while the stimulator is on.[140] Constant frequency trains generally consist of moderate frequency pulses (e.g., 20-40 Hz) with consistently spaced interpulse intervals; whereas VFTs implement two or three pulses with short interpulse intervals resulting in higher frequencies (e.g., 80-100 Hz), prior to a series of pulses with longer interpulse intervals resulting in lowering of the frequency.[30,65,66,140,141] Variable frequency trains are hypothesized to be advantageous due to the "catchlike" property of skeletal muscle; which refers to the observation that a high frequency stimulus is required to reach an initial maximum force, but lower frequencies are capable of holding the contraction.[65,142] Binder-Macleod et al.[65] were the first authors to demonstrate the catchlike property in whole human skeletal muscle, when

they observed greater peak quadriceps torque wile implementing a VFT that incorporated a 20 pps stimulus after an initial high frequency stimulus relative to a CFT of 20 pps.

The ability of VFTs to reduce NMES-induced fatigue relative to CFTs has been examined in the literature, with inconsistent results. Deley et al.[30] reported less NMES induced fatigue, represented by the decline in MVIC and NMES-induced torque, while using a VFT protocol compared to a protocol of CFTs. Bigland-Ritchie et al.[64] compared the decline in NMES-induced force and force-time integrals while stimulating the thenar muscles with VFTs and CFTs. These authors chose to standardize the initial force-time integral rather than match initial torque or the number of pulses, and they also concluded that VFTs may attenuate the rate of NMES-induced fatigue. In contrast to Bigland Ritchie and colleagues, Thomas et al.[67] concluded that VFTs and CFTs produce similar amounts of fatigue despite also performing their study using the thenar muscles. Furthermore, they observed similar results regardless of the health status (healthy vs. spinal cord injury) of the participants. It is unknown why the results of these two studies differ, but Thomas and colleagues hypothesized that it may have been due to their small sample size of healthy controls.

Similar to Thomas et al.,[67] Papaiordanidou et al.[28] observed comparable levels of NMES-induced fatigue, as evidenced by equivalent decreases in MVIC torque, following CFTs and VFTs protocols, but as concluded by the authors their results do not indicate that NMES-induced fatigue is reduced via VFTs. They also reported no differences across the protocols with respect to the mechanisms responsible for the observed fatigue. Binder-Macleod and colleagues have also performed many similar studies in which CFTs and VFTs were compared. In a 2001 study,[66] they did not observe differences in fatigue,

as evidenced by similar declines in NMES-induced torque for both VFT and CFT fatiguing trains. In contrast, during other studies[115,143] they observed greater fatigue during repetitive stimulation with VFTs relative to CFTs.

Based on the inconsistent results among the studies comparing CFTs and VFTs, the extent to which VFTs reduce NMES-induced fatigue is currently unknown. However, based on their review of the literature, Papaiordanidou et al.[28] recently suggested that the fatigue induced by CFTs and VFTs appears to be similar. It is important to note that only one[30] of the studies reviewed utilized a stimulus train of sufficient duration to allow for a prolonged contraction time during the fatiguing protocol, but this study used a 1:1 duty cycle which ultimately limits the clinical applicability of this study. Future studies should examine the influence of VFTs on NMES-induced fatigue while utilizing clinically relevant treatment parameters. Specifically, an appropriate on:off ratio and treatment time should be utilized (e.g., 10:50 for 15-20 minutes). Although VFTs do not appear to be advantageous with respect to NMES-induced fatigue, they may be advantageous with respect to increasing NMES-induced torque when the muscle has been fatigued (discussed in detail in a later section).[141] Others have suggested that when the goal is to obtain high levels of NMES evoked torque VFTs may be a better choice.[28]

Accommodation

Many of the previously discussed studies[30-33,46,49,51,54,55,59,61,62,64,66] utilized the decline in NMES-induced torque throughout the stimulation protocol as a measure of muscle fatigue, but caution should be exercised as these declines may be attributable to a combination of fatigue and accommodation of the motor nerve .[99,100] Alon and Smith[144]

defined accommodation as the transient process by which the threshold required to excite the nerve increases in response to the electrical stimulus. Accommodation has been suggested as contributing factor to declines in NMES-induced force output because an increased nerve threshold has the potential to result in a diminished number of recruited motor units.[28,77,99,100]

Although evidence demonstrating that accommodation contributes to the decline in NMES-induced force production has been considered to be mostly anecdotal,[145] recent research has provided some evidence supporting the premise that accommodation contributes to the declines in NMES-induced force output. Papaiordanidou et al.[100] compared declines in NMES-induced plantar flexion torque across protocols consisting of high and low frequencies, as well as long and short pulse durations. Although each of the protocols resulted in significant declines throughout the treatment, significantly greater declines in NMES-induced torque occurred under both of the higher frequency protocols. Despite this difference, no other differences in the other fatigue measures (e.g. post-test MVIC and twitch torque loss) were observed between protocols. Furthermore, the decreases in post-test twitch torque were positively related to the declines in NMES-induced torque occurring during the low frequency protocols, whereas no such relationship was observed with respect to the high frequency protocols. The authors concluded that the lack of a consistency between results, as well as greater percent declines in NMES-induced torque relative to declines in MVIC torque, suggest that the greater declines observed under the high frequency conditions are not solely attributable to greater muscle fatigue. They subsequently hypothesized that the differences occurred

because the higher frequency stimulus led to an increased threshold of excitability in the stimulated axonal branches, or accommodation.

Matkowski et al.[99] performed an additional study to examine whether accommodation contributes to declines in NMES-induced force production by indirectly assessing changes in the proportion of active motor units during NMES-induced contractions of the quadriceps. While applying NMES at an initial training intensity of 20% MVIC, these authors also applied a supramaximal stimulus to the femoral nerve during and after each of the 15 NMES-induced contractions that produced superimposed and post-tetanic twitches. To determine the proportion of active motor units during NMES the superimposed and post-tetanic twitches were placed in a muscle activation formula similar to that used for voluntary activation. The decline in NMES-induced torque throughout the protocol and the change in post-test MVIC were also assessed. Similar to Papaiordanidou et al.,[100] Matkowski et al.[99] observed greater percent declines in NMES-induced torque than in post-test MVIC, with NMES-induce torque decreasing roughly 60% while MVIC only declined roughly 20%. Relative to their initial values, the post-tetanic twitch torque significantly decreased while the superimposed twitch torque significantly increased over the course of the protocol. This resulted in a roughly 60% decrease in the proportion of active motor units during NMES. The authors concluded that these results are indicative of a reduction in the number of motor units activated by NMES, which they attributed to accommodation. Subsequently, they indicated that accommodation was the mechanism primarily responsible for the greater reduction in NMES-induce torque relative to MVIC.

In an earlier study, Randolph et al.[145] observed NMES-induced torque declines across 10 repetitions while also observing concurrent declines in discomfort; which can be attributed to accommodation in sensory nerves, but they also hypothesized that accommodation affected motor nerves therefore contributing to their observed declines in NMES-induced torque. Further evidence that accommodation contributes to the declines in NMES-induced torque is provided in a study performed by Holcomb et al.,[44] in which they demonstrated that increasing the stimulus amplitude roughly 5% every other contraction eliminated declines in NMES-induced torque across 10 repetitions without increasing patient discomfort. Interestingly, increases in the excitation threshold have also been observed following MVICs, with greater increases occurring following contractions of longer duration. [146] Accommodation may also occur in afferent nerves, but it appears to be greater for motor axons relative to sensory axons.[146,147]

Although the previously discussed studies provide evidence that accommodation is a contributing factor to the decline in NMES-induced force, the relative contribution of accommodation to the declines in NMES-induced force output remains unknown.[145] Furthermore, as evidenced by the suggestion that further research is needed to better understand the effects of the fluctuations in the threshold required for excitation of motor axons during NMES-induced contractions,[100] it can be concluded that a lack of comprehensive understanding exists with respect to accommodation. Accommodation has been attributed to hyperpolarization of the nerve axons, which alters the membrane potential to a more negative state, and some have hypothesized that the primary mechanism causing hyperpolarization is the sodium potassium pump.[28,146,147] Despite the need for more research, it is understood that if accommodation occurs the nerve cannot

be excited without increasing the intensity of the electrical stimulus.[148] Although the term "fatigue" and "accommodation" are often used interchangeably, it is important to acknowledge the difference between accommodation and fatigue with respect to the mechanisms responsible for the declines in NMES-induced force output, as others have done.[44,145,149]

Strategies to Enhance Patient Comfort

Overview

Similar to fatigue and accommodation, NMES-induced torque and the subsequent NMES training intensity are restricted by patient comfort. The stimulus intensity used during NMES treatments is directly related to the amount of NMES-induced torque production, as well as the amount of muscle CSA activated.[17,50,51] In order to elicit NMES training intensities sufficient for strength gains, stimulus intensities must be adequate. However, it is difficult to achieve or maintain sufficient intensities because they are often uncomfortable. NMES-induced discomfort is most often considered to be the result of the concurrent depolarization of thinner type III and IV sensory fibers, which are responsible for delivering pain information to the CNS,[109] and this subsequently results in the perception of discomfort.[150] It is also important to note that NMES-induced discomfort has been shown to only decrease roughly 50% following a sensory nerve block.[151] Furthermore, Alon and Smith[144] observed that 50% of participants reported that muscle cramping was the sensation that limited their willingness to tolerate greater stimulus intensities, whereas only 18% of their participants identified pins and needles as the limiting sensation. Others have reported that the muscle pulling or tearing sensation experienced during NMES treatments limits higher stimulation intensities more so than

the uncomfortable sensation related to the stimulus being applied.[78] Thus, NMES-induced discomfort appears to be attributable to muscular (e.g., cramping) mechanisms as well as sensory mechanisms.

Regardless of which factors primarily contribute to NMES-induced discomfort, the fact remains that discomfort may lead to patient resistance toward turning the stimulus intensity up to a level sufficient for producing adequate NMES training intensities, or may even lead to patient non-compliance.[39,97] Consequently, the patient discomfort experienced during NMES treatments has been identified as a primary limitation of this modality.[16] A variety of strategies have been examined in an effort to improve patient comfort, but despite these efforts additional strategies have been solicited.[16]

Electrode Size

Electrode size influences the current density, which is defined as the quantity of current per unit area (e.g., mV/cm^2), and the perception of NMES is amplified as the current density increases.[152] Thus, increasing electrode size to reduce current density has received considerable attention as a possible strategy by which clinicians can reduce patient discomfort during NMES. Alon et al.[68] examined the influence of electrode size on patient comfort while stimulating the gastrocnemius with electrode sizes consisting of 2.25 cm^2, 9 cm^2, 20.25 cm^2 and 40.3 cm^2. The stimulus intensity was increased and the level at which each participant reported reaching their pain threshold and pain tolerance was recorded under each condition. The NMES-induced torque at each level was also recorded. Participants were able to tolerate greater stimulus intensities, which lead to greater torque production, under the larger electrode conditions; but similar levels of

torque were observed between the two largest, as well as the two smallest electrodes at the pain threshold. As a result, the authors recommended the 20.25 cm^2 electrode to enhance comfort during NMES of the gastrocnemius. In contrast to the results of Alon et al., Lyons et al.[70] reported greater comfort while stimulating the gastrocnemius with smaller round electrodes relative to larger round electrodes, as evidenced by the participants' tolerance of greater stimulus intensities under the small electrode condition. Lyons et al. suggested that their contrasting results may have occurred due to variations in the stimulus parameters used in each study.

Although the results of these two studies appear to be in direct contrast, with one[70] supporting the use of small electrodes and the other[68] supporting large electrodes, the small (19.63 cm^2) and large electrodes (38.48 cm^2) used by Lyons et al. [70] were comparable in size to the two largest electrodes used by Alon et al.[68] Thus, it seems that larger electrodes do not always enhance patient comfort, but when taken together, the results of these two studies suggest that an optimum electrode size may exist; which appears to be approximately 20 cm^2 when stimulating the gastrocnemius. In agreement, Alon et al.[68] reported that unreasonably large electrodes do not appear to enhance excitation, and they suggested that the ideal electrode size for maximizing comfort and force production is primarily dependent upon muscle size.

Forrester et al.[69] also examined the influence of electrode size on patient comfort. In addition, they examined the influence of electrode shape while stimulating the quadriceps, tibialis anterior and biceps brachii to determine the extent to which these effects hold true across muscle groups. The electrode sizes tested were 5.08 cm^2, 4.39 cm^2 and 3.66 cm^2, and the shapes consisted of round, square and square with a serrated

leading edge. Under each condition the muscle was stimulated with an intensity required to achieve 10% MVIC. As evidenced by similar visual analog scale (VAS) scores across each of the conditions, the authors concluded that differences in electrode shape and size did not influence patient comfort. Furthermore, Naaman et al.[41] also reported no significant differences with respect to discomfort while stimulating the tibialis anterior with different shapes and sizes of electrodes.

These results[41,69] directly contrast the studies previously discussed that reported differences across electrode sizes, although it is important to note that the significant results were inconsistent across studies.[68,70] It is unknown why these inconsistencies occurred. Forrester et al.[69] suggested that an inability to elicit meaningful differences may be attributable to small differences in the size of their electrodes. This proposition seems to be supported by the observations of Alon et al.[68] that increasing the electrode size 6.75 cm^2 (2.25 cm^2 to 9 cm^2) did not significantly alter the pain threshold, while a larger increase of 11.25 cm^2 (9 cm^2 to 20.25 cm^2) did. However, further increasing the electrode size by 20.05 cm^2 (20.25 cm^2 to 40.3 cm^2) did not significantly alter comfort, which seems to suggest a limit to the positive influence of increasing electrode size to improve comfort. The inconsistencies may have also occurred due to the differences in the measure used to assess comfort. Forrester et al. measured patient comfort with a VAS at a standardized training intensity. Participants in the study by Naaman et al.[41] provided a numeric rating grade between 0 and 10 and the intensity was altered across conditions to elicit the same level of contraction, whereas the other studies[68,70] assessed the level of stimulus intensity tolerated under each condition.

With the exception of the study by Forrester et al.[69], the previously discussed studies did not examine the influence of electrode size on patient comfort while stimulating the quadriceps. In a study[71] reporting no difference between the quadriceps twitch torque produced while maintaining a constant stimulus intensity under a small (20 cm^2) and large (36 cm^2) electrode condition, a number of participants anecdotally reported greater comfort with the larger electrodes. Although Alon[150] did not directly measure patient discomfort while using electrodes measuring 9 cm^2, 36 cm^2 and 81 cm^2, he did measure the amount of quadriceps torque produced while using stimulus intensities just below the pain threshold (non-painful contraction) as well as when the pain threshold (painful contraction) was reached. Participants were able to tolerate greater stimulus intensities under both of the conditions using the 81 cm^2 electrodes (9 cm X 9 cm or 5 cm X 16.2 cm), resulting in significantly greater amounts of non-painful and painful NMES-induced torque production. Consequently, Alon concluded that using larger electrodes can elicit greater quadriceps contraction torque without discomfort. Patterson and Lockwood[72] compared the influence of electrode size on discomfort using five different electrode sizes ranging from 20-60 cm^2, with the size increasing 10 cm^2 under each condition. In contrast to Alon, these authors assessed self-reported discomfort under each condition while stimulating the quadriceps to elicit a training intensity of 25% MVIC. Despite the fact that there were no significant differences in the stimulus intensity required to achieve 25% MVIC, greater discomfort was observed under the 20 cm^2 and 30 cm^2 conditions; whereas no such differences were observed with respect to the three larger electrode sizes.

As with applying NMES to the gastrocnemius, the results of Patterson and Lockwood[72] also seem to suggest a limit to the positive influence of increasing electrode size for the purpose of improving comfort while stimulating the quadriceps. However, the optimal size for applying NMES to the quadriceps remains unknown because the results of Alon[150] suggested greater comfort while using electrodes much larger than 30 cm^2, whereas the results of Patterson and Lockwood did not demonstrate significant improvements when using 60 cm^2 relative to 40 cm^2.

It is commonly accepted among clinicians that patient comfort is improved as electrode size is increased because larger electrodes result in lower current densities, but based on the previously discussed studies[41,68-70,72,150] there appears to be a lack of consensus in the literature to support this belief. It has been suggested that the optimal electrode size is dependent upon the size of the muscle being treated,[68] thus further studies are warranted to determine the optimal electrode size for each of the various muscle groups frequently treated with NMES.

Electrode Placement

The influence of electrode placement has also been examined as a potential strategy for improving patient comfort. It is commonly accepted that a motor point is the location by which the motor nerve is most hypersensitive to stimulation.[69] A motor point is defined electro-physiologically as the portion of skin at which the least amount of stimulus intensity is required to produce a visible twitch contraction, or anatomically as the point at which the motor nerve branches and enters the muscle belly.[68,69,153,154] Although these two definitions are often used interchangeably, Gobbo et al.[24] recommended that the electrophysiological and anatomical definition be differentiated,

with the later representing motor entry points rather than motor points. Furthermore, they suggested that two of the major NMES limitations, restricted spatial recruitment and patient discomfort, can be effectively reduced by placing electrodes over the motor points; which was based on the hypothesis that applying NMES over a motor point results in depolarization of primarily motor axons, whereas applying the stimulus away from these points results in greater concurrent sensory axon depolarization and necessitates higher stimulus intensities to reach and recruit motor axons.

Motor points can be identified by using published atlases illustrating their location,[152] or manually by using a pencil electrode. The pencil electrode method requires that the frequency be set low, to elicit a twitch (e.g., 2 pps), while the skin is scanned using the lowest stimulus intensity capable of producing a detectable twitch response.[24,39] A recent study has demonstrated that relative to using an atlas to identify motor points of the tibialis anterior and vastus lateralis, the pencil electrode method results in greater patient comfort, torque production and metabolic stress.[39] However, it is important to note that during this study different stimulus intensities were used under each condition, which may have confounded the results.

During their previously discussed study, Forrester et al.[69] also examined the impact of electrode placement. The authors observed that the stimulus intensity required to elicit 10% MVIC and the reported discomfort levels were significantly lower when placing the electrodes over the motor points rather than placing electrodes away from the motor points. Exceptions to these findings were that similar stimulation intensities and comfort levels were observed when the electrodes were placed 2 cm proximal or medial to the motor point of the tibialis anterior. Furthermore, they observed that moving the

electrodes closer together tended to increase the discomfort more so than moving the electrodes further apart. The authors hypothesized that this trend was attributable to greater activation of afferent nerve fibers due to the superficial flow and increased current density that occurs when electrodes are in close proximity.[155]

Different electrode placement strategies appear to influence patient comfort,[39,41,69,70] with greater comfort occurring when the electrodes are placed over motor points.[39,69] It is important to note that with respect to the tibialis anterior, greater comfort has not always been reported when placing the electrodes over the motor point.[41,69] However, with respect to NMES of the quadriceps, the electrodes should be placed over the motor points due to the poor accessibility of the femoral nerve trunk.[101] Other authors have also recommended placing electrodes over the motor points.[24] As evidenced by their observation of inter-individual differences with respect to motor point locations, Botter et al.[153] suggested that the usefulness of identifying motor points via the atlas method is limited. Thus, in agreement with these authors, the pencil electrode method was deemed more appropriate for motor point identification for this project. With respect to the influence of electrode placement on patient comfort, it is also important to note that using a crossed or parallel electrode arrangement does not appear to influence perceived discomfort levels while stimulating the quadriceps.[73]

Current Parameters

It has been suggested that the optimal NMES waveform is one that elicits the least amount of discomfort while producing the desired NMES training intensity.[156] Thus, a variety of current parameters have also been examined to determine the extent to which they can improve patient comfort during NMES treatments. During their previously

discussed study, Naaman et al.[41] reported greater levels of NMES-induced discomfort when increasing the stimulus frequency and altering the stimulus intensity to maintain the same level of contraction across protocols. Although this was significant among healthy and neurologically impaired participants, the increased discomfort was significantly greater in the neurologically impaired group. Furthermore, they observed that as the pulse duration increased greater amounts of discomfort were reported in the neurologically impaired group, but no such difference was observed within the healthy group.

Bennie et al.[40] compared patient comfort during a series of 4 minute NMES-induced isometric quadriceps contractions using four different waveforms. The conditions tested were Russian, Interferential, biphasic sine and biphasic square waveforms, and the stimulus intensity was altered throughout the course of each contraction to maintain a training intensity of 10% MVIC. The interferential waveform was incapable of producing contractions of 10% MVIC, thus this condition was not included in the analysis. The amount of stimulus intensity required to maintain a contraction of 10% MVIC over the course of 4 minutes was significantly less during the sine waveform condition. Despite this difference, there were no significant differences across the three conditions with respect to verbal rating scale scores, which represented subjective discomfort levels. These authors also assessed objective discomfort levels by measuring changes in galvanic skin resistance (GSR), which they suggested represents the response of the sympathetic nervous system to painful stimuli. During the Russian condition the changes in GSR over the first minute of stimulation were significantly greater than during either of the other two conditions, and changes in GSR were

significantly greater under the sine waveform relative to the square waveform condition. Due to their observation that participants consistently rated their discomfort lower under the sine waveform condition, and as evidenced by the lower levels of the required stimulus intensity to maintain 10% MVIC, the authors concluded that the sine waveform was most comfortable. Caution should be exercised with respect to this conclusion because no significant differences were observed in subjective discomfort levels and GSR changes were significantly lower during the square waveform condition. However, the authors hypothesized that their lack of significant differences with respect to self-reported discomfort was attributable to their small sample size.[40] It should be noted that the extent to which these results are clinically applicable is unclear, as it is unlikely that clinicians will implement NMES-induced contractions lasting longer than 10-20 seconds.

Low-frequency pulsed currents (PCs) consist of biphasic or monophasic pulses delivered at a range of frequencies between 1-200 Hz, whereas kilohertz-frequency alternating currents (KFACs) consist of biphasic waveforms delivered at a range of frequencies between 1000-10,000 Hz.[23,37,157] Consequently, the biphasic waveforms included in the aforementioned study[40] can be categorized as PCs while the Russian and Interferential waveforms can be categorized as KFACs. It is important to note that KFACs may also be referred to as burst-modulated alternating currents because the alternating current is often delivered in the form of bursts followed by inter-burst intervals,[157] but for the purpose of consistency they were only referred to as KFACs. The proposed theoretical advantage associated with KFACs is that higher frequencies lead to reduced skin impedance, ultimately allowing greater amounts of current to be delivered to motor nerves.[2] Russian and Aussie currents are two commonly used forms of KFACs

within the clinical setting.[37] The standard Russian current is defined as having a carrier frequency of 2,500 Hz delivered at a burst frequency of 50bps and a burst duty cycle of 50% (e.g., 10 ms on :10 ms off)[95]; whereas the standard Aussie current is defined as having a lower carrier frequency of 1,000 Hz and 20% duty cycle, but is also delivered at a burst frequency of 50bps.[37]

Vaz et al.[38] compared self-reported discomfort and the amount of stimulus intensity required to achieve NMES-induced quadriceps contractions of 10% MVIC under a PC and Russian condition. They reported significantly lower discomfort, as well as lower stimulation intensity requirements under the PC condition. In contrast, Laufer et al.[23] observed similar levels of self-reported discomfort while stimulating the wrist extensors at a maximum tolerable intensity under standard Russian (e.g., 50bps and 50% burst duty cycle) and PC (50 pps) conditions. These authors also included variations of the standard Russian current (e.g., 20bps and/or 20% burst duration) in their comparison, and they reported that the variation consisting of 20bps and a 20% burst duration resulted in greater levels of discomfort relative to the standard Russian and PC conditions.

A more recent study[37] compared the impact of two PCs to two KFACs with respect to patient comfort during NMES of the quadriceps. Russian and Aussie waveforms were used to represent KFACs, while the PCs differed in pulse duration only. The pulse durations selected were 200 µsec or 500 µsec, which mimic the pulse durations of Russian and Aussie currents, respectively. Under each of the conditions the stimulus intensity was increased until the participant reported reaching their maximum tolerable discomfort level, at which point NMES-induced torque and self-reported discomfort scores using a VAS were recorded. The participants received each of the NMES

conditions with the quadriceps in a relaxed state, and when superimposed during an MVIC. There were no significant differences in self-reported discomfort across NMES conditions regardless of whether the stimulus was superimposed or applied to the relaxed muscle. This observation is limited because the stimulus intensity was not held constant across conditions but rather the intensity was increased until the participant reported reaching their maximum tolerable discomfort under each condition. Consequently, it would be expected that similar levels of discomfort were experienced because participants reported reaching the same self-reported discomfort level prior to beginning each condition. Likely, a more appropriate approach for comparing NMES-induced discomfort levels is to hold either the stimulus intensity or contraction intensity constant across conditions, which has been done in other studies.[36,38,40,41] It is important to note that similar NMES-induced torque was observed across each of the conditions tested, except when applying Russian to the muscle in a relaxed state[37]; thus it may be speculated that similar results could have been observed had the researchers standardized the contraction intensity across conditions.

A 2015 meta-analysis,[76] which is considered to provide the highest level of evidence,[12] compared the influence of KFACs and PCs on self-reported patient discomfort. The KFACs included in this study were limited to Aussie and Russian waveforms. According to the authors the grouped data obtained from studies meeting the inclusion criteria, which included only one of the previously discussed studies,[37] revealed that patient comfort was slightly enhanced when using PCs. However, there were no statistically significant differences revealed during the meta-analysis. Consequently, the authors concluded that based on the current literature both the KFAC and PC approach

induce similar levels of discomfort, thus they recommended that clinicians can choose either of these options.

Blunting Strategies

Blunting strategies, which interrupt the perception of discomfort by the CNS or distract patients during treatments, may also be used to diminish or "blunt" the perception of NMES-induced discomfort.[81] One such blunting strategy that has been examined in the literature is the addition of sensory level transcutaneous electrical nerve stimulation (TENS) during NMES treatments. Laufer et al.[78] compared self-reported discomfort levels during NMES prior to and immediately following a 20 minute TENS treatment. However, self-reported comfort levels were not significantly improved via this technique. It is important to note that their results may have been confounded by allowing participants to alter the stimulus intensity from their initial maximum tolerable stimulus intensity prior to the post-treatment NMES protocol. Consequently, it is plausible that the significant increase they observed in the stimulus intensity from pre- and post-test masked potential changes in comfort. Although they did not directly measure discomfort, Holcomb et al.[77] examined whether applying TENS and NMES simultaneously would result in greater NMES-induced peak torque of the quadriceps. They postulated that if this approach effectively improved comfort, greater stimulus intensities could be comfortably tolerated, which would result in greater NMES-induced torque production. However, no significant differences were observed in NMES-induced peak torque while using a maximum comfortable stimulus. Based on the results of these studies, it does not appear that TENS decreases NMES-induced discomfort.

Another blunting strategy that has been examined is the application of cryotherapy prior to or during NMES treatments. Miller et al.[79] applied a 2 minute ice massage over the area of skin identified as the vastus medialis and rectus femoris motor

points prior to applying NMES to the area. Similar to Holcomb et al.,[77] these authors did not directly measure discomfort but inferred that greater levels of NMES-induced torque were indicative of greater comfort. As evidenced by greater NMES-induced torque under the ice massage condition, they concluded that this strategy decreased NMES-induced discomfort and they attributed these results to the analgesic effect of cryotherapy. This study provides some evidence to support the use of cryotherapy prior to NMES treatments, but due to the methods of this study the duration of the improved comfort following a 2 minute ice massage is unknown since only three contractions were performed under each condition. It is unlikely that the analgesic effects of a 2 minute ice massage would last the duration of a 15-20 minute NMES treatment.

Van Lunen et al.[80] examined the strategy of applying a longer duration cryotherapy treatment in an effort to improve comfort by assessing the maximum tolerable stimulus intensity (mV) every 4 minutes over the course of a 20 minute ice bag treatment. They observed a significant increase in the maximum tolerable stimulus intensity from baseline over time in both the treatment and control groups, but they also observed significant between group differences in favor of the cryotherapy treatment condition at the 12, 16 and 20 minute time points. Despite these between group differences there were no significant differences in the NMES-induced torque output, with torque significantly increasing over time within each group. The authors subsequently concluded that this strategy has the potential to improve tolerance levels, but that it does not ultimately result in improved torque production. Based on their systematic review, which included the two studies[79,80] previously discussed, Nolan et al.[81] also concluded that cryotherapy prior to or during NMES treatments has the potential to

improving patient comfort, but this improved comfort may not lead to greater NMES-induced torque output. Improving patient comfort is important as it may allow for greater stimulus intensities which theoretically result in greater NMES-induced force production,[17,50,51] however, if cryotherapy reduces discomfort without leading to greater amounts of force production it may not be a clinically useful strategy. Consequently, Nolan et al. indicated that further research is warranted to determine if this blunting strategy decreases or increases NMES-induced torque production.

Additional Comfort Strategies

There are additional strategies that also warrant discussion. For example, while stimulating the quadriceps with a standardized stimulus intensity, self-reported comfort has been shown to significantly improve while applying NMES with the knee flexed to 60° relative to a more extended position.[36] Acclimation or habituation, which is the process by which patients become accustomed to the NMES stimulus and are able to subsequently tolerate greater amounts of current also warrants attention. Alon and Smith[144] reported that healthy participants were able to tolerate significantly greater stimulus intensities over the course of a single session consisting of 10 NMES-induced quadriceps contractions, as well as between sessions over the course of 6 days. Consequently, they concluded that patient tolerance may improve between and within NMES treatment sessions in part due to acclimation. Others have also reported an improved tolerance over the course of a series of initial NMES sessions.[97,158]

Patient Characteristics Influencing NMES Tolerance and Comfort Levels

It is also important to note that some unalterable patient characteristics may have the potential to influence NMES-induced discomfort levels and thus limit the amount of

stimulus intensity that can be tolerated. One such patient characteristic is an individual's personality, which has been shown to influence the NMES-induced discomfort experienced.[25] Another patient characteristic that may influence NMES-induced discomfort is gender, however, there appears to be a lack of consensus in the literature regarding this topic. Some authors have reported that males tolerate greater stimulus intensities during NMES,[73,97,144,145] while others have reported no such gender differences.[75,80,154] Gender differences with respect to the amount of stimulus intensity required to achieve excitatory thresholds (e.g., sensory, motor) have also been reported.[159,160]

The impact of a possible gender difference, with respect to tolerance levels, on the subsequent NMES training intensities is also unclear. Despite failing to reach statistically significant gender differences with respect to the amount of stimulus intensity tolerated, Alon et al.[154] observed that female participants produced significantly lower NMES training intensities while stimulating the triceps surae at maximum comfortable and maximum tolerable intensities. Alon and Smith[144] observed that males tolerated significantly greater stimulus intensities, which also resulted in significantly greater NMES training intensities within the male cohort, while stimulating the quadriceps at a maximum tolerable intensity. In contrast, Laufer et al.[97] reported that male total knee patients tolerated significantly greater stimulus intensities while treating the quadriceps, but this failed to result in a significant gender difference with respect to NMES-training intensity. Bergman et al. [73] and Randolph et al.[145] also observed similar results within a cohort of healthy individuals, while Kramer[82] reported no gender difference in NMES-training intensities while stimulating the quadriceps at a variety of knee angles and using

a maximum tolerable intensity. Based on the results of these studies, the influence of gender differences with respect to the amount of stimulus intensity tolerated on NMES training intensities is unclear.

In addition to tolerance levels, it is important to discuss a possible gender difference with respect to self-reported discomfort levels. Maffiuletti et al.[159] did not observe any statistically significant gender differences in self-reported discomfort during NMES-induced quadriceps contractions while using a stimulus intensity required to produce 10% MVIC. However, they did observe significantly greater discomfort levels reported by females while using a stimulus intensity required to reach motor threshold. They postulated that the discomfort levels were not different across genders during the supramotor condition due to large amounts of inter-individual variability. And they observed that a larger number of females were unable to achieve the supramotor level; thus they concluded that females perceived the noxious stimulus associated with NMES to be more uncomfortable.

During a follow-up study Maffiuletti et al.[160] did not observe any gender differences with respect to self-reported discomfort levels recorded immediately after the highest excitatory level tolerated by each participant (e.g., sensory, first motor or second motor). Furthermore, Bergman et al.[73] also observed similar levels of self-reported discomfort across genders. It is important to note that these results are limited because during the Bergman et al. study each participant increased the stimulus intensity to their individual maximum tolerable level, which should have resulted in similar levels of self-reported discomfort across participants; whereas during the Maffiuletti et al. study not all of the participants rated discomfort following the same excitatory threshold. Based on

the results of these studies,[73,159,160] the influence of gender on self-reported NMES-induced discomfort appears to be unclear.

It is also important to gain an understanding of how subcutaneous tissue thickness differences may impact the amount of stimulus intensity tolerated, as well as self-reported discomfort levels. Miller et al.[156] observed that female participants with the greatest amount of subcutaneous tissue thickness tolerated significantly greater stimulus intensities while using a maximum comfortable stimulus. However, as with previously discussed studies,[73,97] these authors reported that the significant difference in the maximum comfortable stimulus intensity tolerated by the participants with greater amounts of subcutaneous tissue did not result in significantly greater NMES training intensities. They postulated that the thicker subcutaneous tissue acted as a poor conductor, which prevented the current from reaching the underlying quadriceps muscle bellies. The participants with greater amounts of subcutaneous tissue were subsequently able to tolerate greater stimulus intensities without eliciting strong muscle contractions, which may have limited the amount of muscular discomfort previously discussed,[78,144,151] thus allowing for greater stimulus intensities to be tolerated.

Medeiros et al.[161] stimulated the quadriceps in a cohort of healthy women while using a maximum tolerable stimulus intensity, and the women were separated into two groups representing thick and thin subcutaneous tissue amounts. As with Miller et al.[156] they observed that greater stimulus intensities were tolerated by those with greater amounts of subcutaneous tissue. However, in contrast to Miller et al., Medeiros et al. observed significantly lower NMES training intensities within the thick subcutaneous tissue group despite the greater stimulus intensities used by this group; which is also in

direct contrast to studies reporting no difference between groups using significantly different stimulus intensities.[73,97]

With respect to self-reported discomfort levels and subcutaneous tissue thickness, Medeiros et al.[161] reported no significant difference between the thick and thin subcutaneous tissue groups; which should have been expected because a maximum tolerable intensity was used by each participant during their study. During their previously discussed study examining gender effects, Maffiuletti et al.[160] also categorized participants as obese or non-obese based on body mass index (BMI) scores. In agreement with Medeiros et al., they did not observe any significant differences in self-reported discomfort levels of participants categorized as obese or non-obese while measuring discomfort scores immediately after the highest excitatory threshold tolerated by each participant. They also observed a strong positive relationship between BMI and stimulus intensity required to reach motor thresholds. Interestingly, BMI was a better predictor of the intensity required to achieve motor threshold than the subcutaneous tissue thickness measured at the site of stimulation, with BMI explaining 56%-61% of the differences in the stimulus intensity required to achieve motor thresholds and subcutaneous tissue thickness only explaining 26%-50%.

It is important that clinicians consider patient body type while providing NMES treatments, since obesity may influence the amount of stimulus intensity required to achieve motor thresholds.[160] As with gender, the influence of subcutaneous tissue thickness and/or obesity on patient comfort is unclear due to methodological limitations of the aforementioned studies,[160,161] but it does appear that individuals with greater amounts of subcutaneous tissue tolerate greater stimulus intensities.[156,161] However,

Maffiuletti et al.[160] concluded that obese participants demonstrated a lower tolerance to NMES, as evidenced by the fact that a larger number of obese participants were unable to reach the motor thresholds during their study.

In addition to body type and gender, menstrual cycle phases may also impact NMES-induced discomfort levels. Teepker et al.[162] compared sensory and pain thresholds while applying an electrical stimulus to the wrist extensors on days 1, 4, 14 and 22 of a 28 day menstrual cycle. Although there were no significant differences with respect to the sensory threshold, the participants demonstrated significantly greater pain tolerance levels on days 14 and 22 relative to day 1. Consequently, NMES may be more comfortable, or greater stimulus intensities may be tolerated, when treating females during the later phases of their 28 day menstrual cycle. Furthermore, these results suggest that researchers should collect data between days 4 and 22 of a 28 day cycle during studies in which NMES-induced discomfort is assessed in females over time; which has been done previously.[37]

Strategies to Maximize NMES-induced Force Production

Overview

To this point the literature reviewed has primarily focused on potential strategies for improving NMES-induced fatigue and/or nerve accommodation, as well as discomfort, because improving these factors may ultimately impact the amount of force produced during NMES-induced contractions; thus, improving the overall effectiveness of this treatment modality by enhancing NMES training intensities. However, strategies having the potential to directly improve NMES-induced force production also warrant discussion.

Current Parameters

It is commonly accepted that increasing the stimulus intensity results in a linear increase in NMES-induced force output.[17,50,51] Similarly, it is commonly accepted that increasing the stimulation frequency results in greater NMES-induced force production, but only to a certain extent because the established force-frequency relationship curve demonstrates a sigmoidal relationship between frequency and NMES-induced force output; with a plateau in substantial force increases occurring at roughly 60 Hz.[33,52,53]

In contrast to stimulus intensity and frequency, the impact of pulse duration on NMES-induced force output is not as well understood. Based on their review of the literature, Gorgey et al.[26] reported that the influence of pulse duration on NMES-induced force production is underappreciated, and that knowledge regarding the optimal pulse duration is limited. These authors consequently performed a study in which the quadriceps of participants were stimulated using a standardized stimulus intensity, which was set to elicit a contraction of 45% MVIC. The NMES conditions consisted of a long and a short pulse duration condition (250 μsec or 450 μsec). Relative to the shorter pulse condition (250 μsec), the authors observed significantly greater NMES-induced peak torque while using the longer pulse stimulus (450 μsec) during the first NMES-induced contraction. Consequently, the authors concluded that longer pulse durations enhance NMES-induced torque production. It is important to note that the stimulus frequency was not standardized across the two conditions, with a lower frequency used during the shorter pulse duration condition. Thus, as acknowledged by the authors, this study was limited because different frequencies may have confounded the results. However, the authors contended that inconsistent frequencies were not a sufficient explanation for the

20% difference in NMES-induced torque they observed between conditions because the two frequencies they used are located within plateau portion of the force-frequency curve (60 vs. 100 Hz).

Despite this limitation, additional evidence also suggests that increasing the pulse duration results in greater NMES-induced force output. In an earlier study, Gorgey et al.[50] compared the influence of frequency, pulse duration and stimulus intensity on NMES-induced torque production of the quadriceps while using four different NMES protocols. Three of the protocols differed from a reference protocol by either lowering the stimulus intensity, frequency or pulse duration. These authors reported that increasing the pulse duration from 150 μsec to 450 μsec, while maintaining a constant frequency and stimulus intensity, resulted in significantly greater NMES-induced torque production. Although decreasing the frequency and stimulus intensity also resulted in a significant decrease in NMES-induce torque, the authors concluded that pulse duration appears to influence NMES-induced torque production more than the other parameters; as evidenced by a 55% decrease while using a shorter pulse duration whereas decreases of 18% and 34% occurred while using a lower frequency and stimulus intensity, respectively.

Increasing the pulse duration, frequency or stimulus intensity results in greater NMES-induced force output, [17,26,33,50-53] but it is important to note the mechanism responsible for the subsequent increases appears to differ across these parameters.[50] Greater stimulus intensities have been shown to result in a linear increase in the amount of muscular area activated by the stimulus,[50,51] thus the mechanism responsible for greater torque production while treating the quadriceps with higher stimulus intensities

has been attributed to a greater amount of muscle activation.[50] In contrast, the amount of muscular area activated by high and low frequencies appears to be similar, thus the mechanism responsible for the improved torque while treating the quadriceps using higher frequencies has been attributed to enhanced twitch summation.[50] Similar to higher stimulus intensities, Gorgey et al.[50] observed that using a longer pulse duration while treating the quadriceps resulted in a greater amount of muscle CSA activated by the stimulus, but the subsequent increases in torque and in the amount of muscle activated were not proportional. This observation lead the authors to suggest that the increased amount of muscle activated did not adequately explain the subsequent increase in torque observed while using a longer pulse duration. Furthermore, during their later study Gorgey et al.[26] reported that despite a significant difference in torque production, the volume of activated muscle CSA (cm^3) was not significantly different between the long and short pulse duration conditions. Consequently, both sets of authors postulated that the greater force output associated with the longer pulse duration conditions may be attributable to greater fast-twitch fiber recruitment.[26,50]

Although increasing the stimulus intensity, frequency or pulse duration may result in greater NMES-induced force output, there are limitations associated with these approaches that must be acknowledged. Clinicians are often unable to simply increase the stimulus intensity until a desired force output is achieved because, as has been previously discussed, high stimulation intensities are often uncomfortable. Simply increasing the stimulus intensity is also limited because stimulators used within clinical settings have a limited stimulus intensity output capacity; so even if an individual is able to tolerate high stimulus intensities the machine would ultimately reach its output

capacity. Increasing the frequency is a limited approach because high frequencies may result in greater amounts of muscle fatigue, which has been discussed previously. Greater fatigue limits the increase in force production that can occur while increasing the frequency, thus some authors have suggested that simply increasing the frequency to increase torque is also a limited approach.[26] If the hypothesis that longer pulse durations result in greater activation of fast-twitch fibers holds true,[26,50] then using longer pulse durations to increase NMES-induced force output would likely result in greater amounts of fatigue. Thus, similar to increasing the frequency, the usefulness of this approach to increase NMES-induced force output may be questionable; but it is important to note that the influence of pulse duration on fatigue is currently unclear.[32]

The influence of KFAC and PCs waveforms on NMES-induced force output has also been examined. During their previously discussed study comparing the influence of KFAC and PC on NMES-induced discomfort, Dantas et al.[37] also observed similar amounts of NMES-induced torque across each condition, with the exception of significantly lower torque evoked during the Russian condition with the quadriceps in a relaxed state. Consequently, they concluded that for the purposes of maximizing NMES-induced quadriceps torque PCs or Aussie currents are advantageous relative to Russian current.[37] The previously discussed meta-analysis[76] reported that NMES-induced torque was slightly greater when using PC waveforms, but as with comfort there were no statistically significant differences. Consequently, the authors concluded that based on the current literature both KFACs and PCs approaches induce similar levels of torque, which provides further support to their previously mentioned recommendation that clinicians can choose either PCs or KFACs. In agreement with this conclusion, Laufer et al.[23]

observed similar NMES training intensities, while using a maximum tolerable stimulus intensity, irrespective of whether the waveform used was PC or any of three Russian variations.

Variable Frequency Trains

During their study comparing CFTs and VFTs, Papaiordanidou et al.[28] observed greater NMES-induced torque during the initial 50 contractions of the VFT condition. Consequently, they concluded that when the goal is to obtain high levels of NMES-induced torque VFTs may be a better choice than CFTs.[28] However, based on their review of over 50 articles, Binder-Macleod and Kesar[141] concluded that VFTs may be advantageous with respect to increasing NMES-induced torque only when the muscle has been fatigued.

Some of the previously discussed studies examining the influence of VFTs and CFTs on NMES outcomes also examined torque output after the muscle had been fatigued. Binder-Macleod et al.[65] reported that after the muscle had been fatigued, the VFT stimulus induced greater average torque relative to three different CFT protocols. Slade et al.[55] observed that prior to a fatiguing protocol VFTs produced significantly a smaller TTI relative to CFTs, whereas following the fatiguing protocol VFTs elicited a significantly greater TTI; which occurred due to greater peak torque and reduced slowing of the rate of rise while using VFTs. Bickel et al.[63] compared the torque produced with a VFT to that of a CFT with the muscle in a fatigued state. They observed a greater TTI, as a result of greater NMES-induced torque and faster rates of contraction, under the VFT condition.

Based on their review of the literature, Binder-Macleod and Kesar[141] reported that an increase in sarcoplasmic calcium concentration and an increase in the series elastic component stiffness are the two primary mechanisms hypothesized as the mechanism by which an increase in force occurs while using VFTs. Although VFTs appear promising for increasing NMES-induce torque when the muscle has been fatigued, this is currently a limited strategy because most stimulators used within clinical settings do not include VFTs as an option.

Electrode Placement

The strategies involving electrodes previously discussed primarily focus on improving comfort in an effort to indirectly enhance NMES-induced force production, but electrode placement strategies may also have a direct impact. Brooks et al.[75] examined whether placing electrodes in a parallel or perpendicular fashion relative to the muscle fibers maximized NMES-induced torque production while stimulating the quadriceps. They observed that despite maintaining a similar stimulus intensity across conditions, NMES-induced torque was significantly greater when the electrodes were placed parallel to the muscle fibers. This observation held true across genders, and the 17% difference in NMES-induced torque resulted in NMES training intensities of 44% MVIC and 27% MVIC during the parallel and perpendicular conditions, respectively. The authors postulated that greater torque occurred during the parallel condition because this method facilitates excitation-contraction coupling processes. They further postulated that because the inter-electrode distance was smaller there was a greater current density under the parallel condition, which may have increased the number of motor units activated by the stimulus. Although current density is a term commonly used to describe

the amount of current per unit area of the electrodes used,[152] it is important to note that these authors appeared to use the term to describe the amount of current per unit area of the thigh between the electrodes.

This later hypothesis is questionable because in order to elicit a motor response the current density must be sufficient at the motor axons to cause depolarization.[4] When electrodes are placed within close proximity a higher current density occurs within superficial tissues; whereas greater spacing among electrodes allows for higher current densities in deeper tissues, which is where the muscle and motor axons are located.[4] Consequently, wider spacing between electrodes is considered to be advantageous while attempting to stimulate deeper structures (e.g., motor axons), as this allows for greater dispersion of current.[155] It is important to note that during a preliminary study Petrofsky et al.[163] observed that a greater percentage of the stimulation current reached the deeper quadriceps muscle tissue while using a smaller inter-electrode distance, but they did not assess the subsequent NMES-induced torque output. During a more recent study, Viera et al.[74] observed that NMES-induced torque was significantly enhanced by increasing the distance between three pairs of electrodes placed over the muscle bellies of the three superficial quadriceps. Furthermore, they reported that the increase in torque corresponding with an increase in stimulus intensity plateaued at relatively low stimulus intensities under the smaller inter-electrode distance conditions, while no such plateau was observed under the larger inter-electrode distance conditions. These authors subsequently concluded that the inter-electrode distance used while stimulating the quadriceps significantly impacts the amount of NMES-induced torque, with greater inter-electrode distances being advantageous. It is important to note that Viera et al. allowed

the stimulus intensity to vary, as a maximum tolerable intensity was identified prior to each condition. However, the stimulus intensity used under three of the four conditions tested was not significantly different, with significantly smaller stimulus intensities used only during the smallest inter-electrode distance condition.

Bergman et al.[73] examined the influence of crossing or maintaining electrode pairs parallel to one another while stimulating the quadriceps via two independent channels. The authors reported that the NMES training intensity was significantly greater under the crossed electrode condition, despite the lack of a significant difference with respect to the maximum tolerable stimulus intensity used under each condition. The authors postulated that the crossed pattern outperformed the parallel pattern because the greater distance between electrodes may have allowed for a greater amount of muscle to be recruited, which is in agreement with the aforementioned results of Viera et al.[74] but contrary to the hypothesis of Brooks et al.[75] Bergman et al. also postulated that the flow of current under the crossed condition better matched the pennate muscle fiber arrangement of the quadriceps. This hypothesis also appears to partially contradict the results of Brooks et al., but it is important to note that the crossed or parallel pattern used by Bergman et al. referred to the channel arrangement of the two electrode pairs and not the orientation of the electrodes. During both conditions of the Bergman et al. study the electrodes were oriented in a similar fashion to the electrode orientation used during the parallel condition in the Brooks et al. study. Therefore, it is difficult to directly compare and contrast these two studies.

Bergman et al.[73] also provided a third hypothesis to explain their results, suggesting that the crossed electrode arrangement may have enhanced motor unit

recruitment by allowing greater current flow through motor points of the quadriceps, specifically the rectus femoris motor point. There appears to be some support in the literature for this hypothesis because stimulating over manually identified motor points has been shown to enhance NMES-induced torque of the quadriceps[39]; which subsequently lead Gobbo et al.[24] to conclude that placing electrodes over manually identified motor points may maximize spatial recruitment and the subsequent NMES-induced force production.

When discussing the influence of electrode placement on NMES-induced force production, it is important to note that while stimulating the quadriceps many of the previously discussed studies used a two electrode arrangement,[26,50,69,75,150,159] while others used an arrangement of four[36,42,44,73,145] or even three[21,27] electrodes. To the best of our knowledge there have yet to be any studies comparing which approach, if any, is best. Consequently, further research is warranted to determine which combination of electrode number, size, orientation and placement maximizes NMES-induced force output and patient comfort while stimulating the quadriceps.

Joint Position

With regards to maximizing NMES-induced torque while stimulating the quadriceps it is also important to briefly discuss the influence of the knee joint angle used during NMES treatments. During volitional contractions of the quadriceps it has been well established that the greatest peak torque occurs with the knee in roughly 60°-70° of knee flexion,[164-166] which is most likely due to the optimal overlap of thick and thin filaments associated with the well-known length-tension relationship principle. With respect to involuntary contractions, two older studies[82,83] investigated NMES-induced

torque production at a variety of knee joint angles. Each of these studies reported that greater NMES induced torque occurred with the knee positioned in the middle portion of the available range of motion, but each had a substantial limitation because the authors did not standardize the stimulus intensity across the tested knee positions; which likely biased the results because knee joint angle have subsequently been shown to influence NMES-induced discomfort levels.[36] McNeal and Baker[71] also examined the influence of knee joint angle on NMES-induced peak torque, but they standardized the stimulus intensity across conditions. As with the earlier studies, they observed greater torque with the knee positioned in the mid-range of motion, however, the clinical relevance of their study is questionable because they only assessed the torque produced during twitch rather than tetanic contractions.

Due to the limitations of these studies,[71,82,83] Bremner et al.[42] recently performed a study comparing NMES-induced peak torque with the knee positioned at 60° and 15°. These authors used a clinically relevant NMES protocol while maintaining a constant stimulus intensity, and they observed significantly greater NMES-induced peak torque with the knee at 60°. Consequently, Bremner et al. concluded that the knee joint angle during NMES treatments impacts peak torque in the same manner in which voluntary contractions are affected, thus the knee should be positioned within the mid-range of motion in order to maximize NMES-induced peak torque.

Multipath NMES

Multipath NMES (m-NMES) is a novel NMES treatment approach identified in the literature as having multiple pathways, or multiple electrode pairings, by which current is distributed via two separate channels; whereas during conventional NMES (c-

NMES) the current is distributed within each channel between a single pair of electrodes, or along a single pathway.[21,27,84,88] Furthermore, during m-NMES the specific pathways by which current is distributed are dynamically changed by opening and closing pathways for preset time periods within each pulse of current delivered with the first channel.[21,84,88] It has been suggested that m-NMES, which is commercially available as the Kneehab® XP (Theragen LLC, Leesburg, VA),[27] is advantageous because it provides an asynchronous stimulus and improves spatial distribution through dynamically changing the pathways by which current is distributed and by dynamically altering the pulse duration.[24,87]

The Kneehab® XP consists of a portable stimulation unit connected to a neoprene garment with four self-adhesive reusable gel electrodes integrated into the garment.[27,86] The four electrodes are referred to as electrodes A, B, C and D, and they differ in size; with their sizes being 10 cm X 20 cm, 3 cm X 18 cm, 10 cm X 7.5 cm and 7 cm X 14 cm, respectively.[21,84,87] When the garment is appropriately placed over the quadriceps, electrode A is located superficial to the proximal rectus femoris and is oriented perpendicular to its muscle fibers; electrodes B and C are located superficial to the proximal and distal vastus lateralis, with electrode C and the distal portion of electrode B oriented perpendicular to its muscle fibers and the proximal portion of electrode B oriented parallel to its muscle fibers; electrode D is located superficial to the distal vastus medialis and is oriented somewhat parallel to its muscle fibers.

The Kneehab® XP produces a symmetric bi-phasic current that is delivered through two distinct channels, but as previously mentioned, within each channel the current is distributed between multiple electrode pairings or pathways and the pathways

of the first channel are dynamically altered within each electrical pulse.[21,27,84,88] With respect to the pathways by which current of the first channel (medial channel) is distributed, the A electrode serves as one pole and the other three electrodes serve as the opposite pole; whereas the D electrode serves as one pole and the other three electrodes serve as the opposite pole with respect to the pathways of the second channel (lateral channel).[21,27,87] The pulse duration of the electrical pulses delivered through the medial channel is 400 μsec, while the pulse duration of the pulses delivered through the lateral channel is only 100 μsec.[21,88] The pathways by which the current is distributed within the medial channel are created by the A and C as well as the A and D electrode pairings during the first 300 μsec of each pulse; while the pathway by which the current is distributed changes during the final 100 μsec with the current only being distributed via the A and B electrode pairing.[21] In contrast, the pathways by which the current is distributed in the lateral channel do not change within each pulse. The pathways by which the current is distributed within the lateral channel are created between the D electrode and each of the other three electrodes (Figure 1).[21] It is also important to note that the Kneehab® XP has six NMES programs that determine the frequency of the individual electrical pulses delivered by each channel, as well as the on:off ratio used throughout a given treatment.[167] Thus, it appears that the frequency and on:off ratio of each channel are the same, whereas the pulse durations differ.

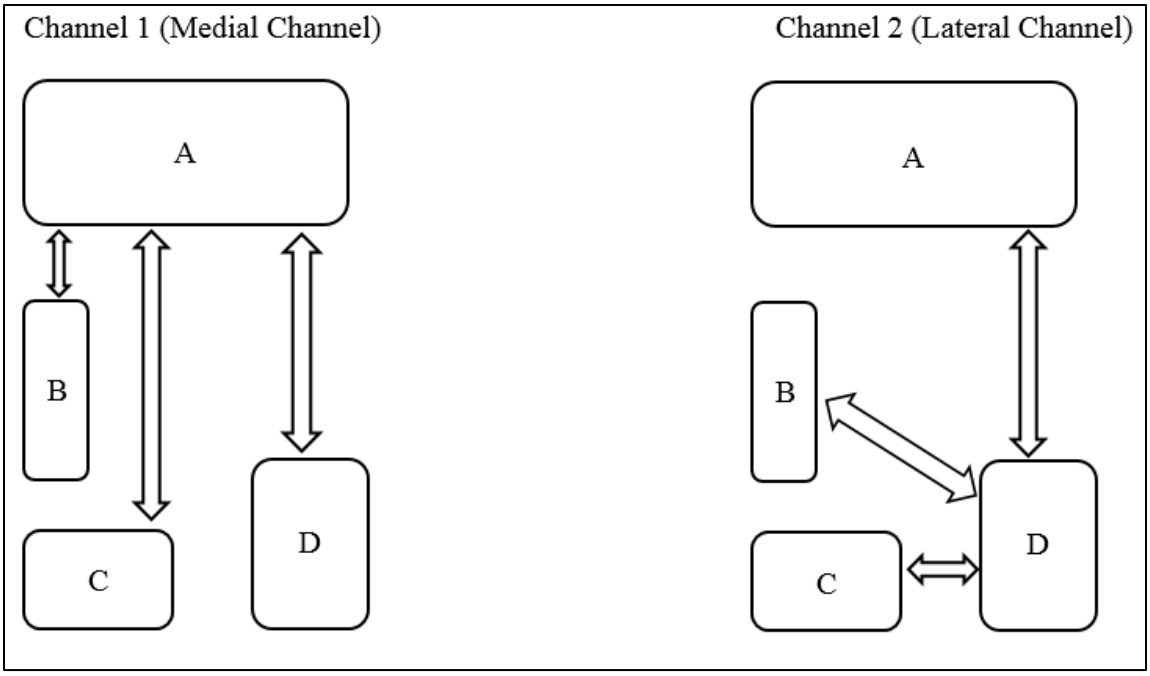


Figure 1. Multipath Current Pathways.

Note. Figure based on information provided by Paessler et al.[84]

In order to achieve the dynamic pulse duration and dynamic pathways, Crowe and Minogue[91] developed a control circuit with a series of output terminals that connected to each of the four electrodes. The control circuit is capable of subdividing each electrical pulse into a series of time periods, or slots. Through a series of switches it is determined whether a given electrode serves as a cathode, anode or is inactive for each time slot of a given electrical pulse.[91] Consequently, each 400 µsec pulse delivered via the medial pathway can be thought of as being subdivided into four 100 µsec time slots. During the first three time slots of each pulse the switches are manipulated to allow the current to flow between the A, C and D electrodes, with A serving as one pole and C and D serving as the opposite. However, during the last 100 µsec time period the switches are manipulated to only allow current to flow between the A and B electrodes (Table 1; Figure 1). It is unnecessary to subdivide the 100 µsec pulse delivered via the lateral

channel into a series of time slots, because current flows between the same pathways without manipulating the pulse (Table 2; Figure 1).[21]

Table 1

Channel 1 Current Pathway Distribution During Each Pulse

Electrode	Time 1 (0-100 µsec)	Time 2 (101-200 µsec)	Time 3 (201-300 µsec)	Time 4 (301-400 µsec)
A	Active	Active	Active	Active
B	Not Active	Not Active	Not Active	Active
C	Active	Active	Active	Not Active
D	Active	Active	Active	Not Active

Note. The A electrode serves as one pole while the other electrodes serve as the other pole.

Table 2

Channel 2 Current Pathway Distributed During Each Pulse

Electrode	Time 1 (0-100 µsec)	Time 2 (101-200 µsec)	Time 3 (201-300 µsec)	Time 4 (301-400 µsec)
A	N/A	N/A	N/A	Active
B	N/A	N/A	N/A	Active
C	N/A	N/A	N/A	Active
D	N/A	N/A	N/A	Active

Note. The D electrode serves as one pole while the other electrodes serve as the other pole. N/A indicates not applicable.

The theory of this device is grounded upon the principles of temporal and spatial summation.[91] Temporal summation has been defined as the process by which subthreshold stimuli from the same source are summed over time, and spatial summation has been defined as the process by which subthreshold stimuli from various sources are summed over space.[3] Consequently, the developers[91] suggested that the device utilizes temporal summation when a given electrode is active over consecutive time slots, whereas spatial summation is caused when two or more electrodes are active at the same

time. As previously discussed, with respect to the medial channel the current is distributed between the A and C as well as the A and D electrodes during the first three time slots, whereas during the final time slot the current is distributed only between the A and B pathway. This pattern ultimately allows temporal summation during the first 300 µsec of each pulse because current is delivered over a series of consecutive time slots, but current density is lowered under electrode C and D (by splitting the overall current between two electrodes) without altering current density under electrode A. However, greater current density occurs under the B electrode, relative to what was experienced under electrodes C and D, during the last 100 µsec of each pulse because the current is only running between the A and B electrodes. Through altering the current density as well as the amount of time an electrode is active, the developers indicated that both forms of summation occur by using a pattern such as the medial channel of the Kneehab® XP device discussed in this example. [91]

Visual Analog Scale Validity Evidence

A VAS, which consists of a 100 mm horizontal line with vertical anchors and verbal descriptors at each end, has been frequently utilized in the literature as a measure of self-reported discomfort during NMES treatments.[21,23,27,29,36-38,69,72,160,161] In general, the verbal descriptors placed at each end of a VAS are "no pain" and "worst imaginable pain"[168]; however, it is important to note that during NMES studies the descriptors are often altered from "pain" to "discomfort" (e.g., "no discomfort", "worst possible discomfort").[21,27,29,36,37,159,161]

The most frequent form of validity evidence with respect to the VAS is construct validity, particularly in the form of convergent validity. The observed correlations

between VAS scores and scores on various scales used to assess the same latent variable (e.g., discomfort/pain) range from 0.30 to 0.95.[169-172] Downie et al.[171] examined convergent validity by comparing VAS scores to scores on a simple descriptive scale (SDS) and numerical rating scale (NRS). Scores on the VAS had a correlation of 0.78 with scores on the SDS, whereas VAS scores had a correlation of 0.91 with the NRS scores. These authors concluded that their results provide convergent validity evidence, since the scales appear to measure the same underlying construct.

Good et al.[172] also used the NRS to examine convergent validity, however, they also examined discriminant validity. The correlation between five sets of VAS and NRS scores taken over the course of two days ranged from 0.85-0.92, which provides evidence of convergent validity because the two measures of the same construct were highly correlated. In order to assess discriminant validity the authors used an alternative NRS scale that was designed to measure a different construct ("distress"). The correlations between the VAS designed to measure pain intensity and the alternative NRS scale were much lower, ranging from 0.43-0.78 across the five measurements. The authors concluded that these observations provide discriminant validity evidence, since the two measures of different constructs were not as highly correlated as the two measures of the same construct.

Some authors have indicated that due to the absence of a gold standard, criterion validity evidence has not been established with respect to the VAS.[169,170,173] In addition, there does not appear to be any reference evaluating the content validity of VAS scores in the literature.[169] Although these two forms of validity evidence are lacking, there appears to be adequate construct validity evidence (discriminant and convergent) to support the

use of the VAS; since the VAS is highly correlated with similar measures of the same construct (e.g., discomfort/pain) and not as highly correlated with measures of different latent variables.[171,172]

Conclusion

As evidenced throughout this literature review, there is a lack of consensus with respect to the most appropriate methods to limit NMES-induced fatigue and patient discomfort in an effort to indirectly maximize NMES-induced force production, as well as with respect to directly maximizing NMES-induced force production. Some have suggested that the primary determinant of NMES-induced quadriceps torque production may be uncontrollable intrinsic factors rather than controllable extrinsic factors,[174] which may explain this lack of consensus. However, a recent meta-analysis concluded that in order to clarify the ideal NMES treatment methods, further well-designed studies are needed.[76]

Based on the current state of the literature, additional strategies such as the multipath method that was studied in this project, are needed. Furthermore, Gobbo et al.[24] suggested that some of the advances in evidence based strategies for improving NMES are not being used by clinicians because they are difficult to integrate within clinical settings. This project was also needed because the m-NMES device used in this study provides a potential strategy that can easily be implemented by clinicians as well as patients.

CHAPTER III - METHODOLOGY

Study Design

Two single-blind cross-over studies with sequence order counterbalanced were performed in a university setting (Figure 2 & 3). A design allowing for within-participant comparisons was selected in an effort to eliminate the possibility of inter-participant differences resulting in confounding factors. For example, subcutaneous tissue thickness differences[156,161] and personality factors[25] have been shown to impact neuromuscular electrical stimulation (NMES) related outcomes similar to those included in this project. This project was subdivided in the form of two separate manuscripts (Chapter IV and Chapter V). However, in an effort to facilitate the reader's comprehension the methodology is presented in a unified format throughout this chapter (see Appendix A for detailed diagrams of the study design and methods specific to each manuscript).

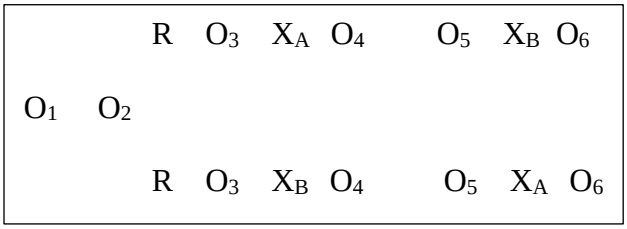


Figure 2. Manuscript 1 Cross-Over Study Design

Note. "O" = observation, "X_A" = m-NMES treatment condition while standardizing the stimulus intensity at 30% MVIC, "X_B" = c-NMES treatment condition while standardizing the stimulus intensity at 30% MVIC (served as the control condition for manuscript 1), "R" = participants assigned to condition sequence using a probabilistic technique that ensured an equal number of participants in each sequence. O_1, O_2 = familiarization session observations, O_3, O_5 = pre-test observations, O_4, O_6 = post-test observations. It is important to note that due to rolling participant enrollment observations did not occur at the same time for all participants. The notation used in this figure is based on the notation used by Shadish et al.[94].

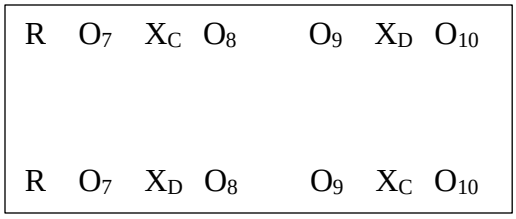


Figure 3. Manuscript 2 Cross-Over Study Design

Note. "O" = observation, "X_C" = m-NMES treatment condition while standardizing the stimulus intensity using a maximum comfortable stimulus intensity, "X_D" = c-NMES treatment condition while standardizing the stimulus intensity using a maximum comfortable stimulus intensity (served as the control condition for manuscript 2), "R" = participants assigned to condition sequence using a probabilistic technique that ensured an equal number of participants in each sequence. O_7, O_9 = pre-test observations, O_8, O_{10} = post-test observations. It is important to note that due to rolling participant enrollment observations did not occur at the same time for all participants. The notation used in this figure is based on the notation used by Shadish et al.[94]

Due to the incorporation of probabilistic counterbalancing of test session order, as well as the deliberate introduction of two NMES interventions prior to measuring their effect on fatigue and discomfort related outcomes, the design selected for this project was categorized as an experimental design.[94] An experimental design was selected as this approach facilitated the primary investigator's ability to make causal inferences.[94]

Design Strengths and Weaknesses

According to Shadish et al.,[94] in order to infer a causal relationship the following three conditions must be satisfied:

1. The cause must precede the effect.
2. The cause and effect must be related.
3. All other alternative explanations must be unlikely.

The methodology implemented throughout this project ensured that the proposed cause (e.g., NMES treatment condition, time) preceded any observed effects on fatigue and discomfort related outcomes. Furthermore, the use of a probabilistic method to counterbalance test session order reduced the likelihood of alternative explanations (e.g.,

order effect, selection bias), which are often referred to as threats to internal validity, thus allowing the NMES treatment condition to be the primary remaining systematic difference.[94] It is important to note that for the purposes of this project the conventional NMES (c-NMES) treatment condition served as the control condition. Therefore, the c-NMES condition provided an appropriate counterfactual framework necessary for establishing an effect (e.g., provided information related to what would have happened had participants not received multipath NMES [m-NMES] treatments).[94]

Threats to internal validity are considered to be any plausible causes of an observed effect other than the independent variable(s)[94]; which were defined as NMES treatment condition and time for the purposes of this project. Despite the efforts to facilitate causal inferences by implementing an experimental design, it is important to acknowledge that some threats to internal validity remained plausible. Due to the use of a rolling enrollment strategy, a history threat remained possible because an event impacting the outcome(s) could have occurred concurrently with the participation of one individual that did not occur for a previously enrolled individual or an individual enrolled at a later date.[94] A rolling enrollment strategy was utilized because the laboratory equipment only allowed the measurement of one participant at a time.

In addition, due to a lack of availability of other investigators, it was not possible to blind the primary investigator tasked with measuring the outcomes to the NMES treatment condition. Consequently, the threat of expectancy also remained possible[94]; but it is important to note that the primary investigator made efforts to standardize feedback and participants were blinded to treatment condition to reduce the plausibility of this threat. Due to the large number of sessions (6 total sessions) and contractions within

each session, testing and experimental mortality were also possible threats.[94] However, as discussed later, the probabilistic assignment of test session sequence was delayed until the third and fifth sessions in an effort to reduce the plausibility of experimental mortality[94]; which appears to have been an effective strategy since each participant assigned a test session sequence completed all six sessions of the study.

As with threats to internal validity, threats to external validity remained possible; which may have limited the generalizability of the results of this project.[94] By using a healthy male population, rather than including females and/or injured participants, the primary plausible threat limiting the external validity or generalizability of this project is the interaction of the causal relationship(s) with units; which is defined by Shadish et al.[94] as occurring when an effect observed in a particular population does not hold true in other populations.

Power Analysis and Sample

A Priori Power Analysis

In an effort to strengthen the statistical conclusion validity of this project by reducing the plausibility of the threat of low statistical power, *a priori* power analyses were performed to determine the necessary sample size to maintain adequate power.[94] The *a priori* power analyses were performed using G*Power software (version 3.1.9.2),[175] and a power analysis for each of the planned statistical analyses was completed (e.g., dependent *t*-test, two-way repeated measures ANOVA). The dependent *t*-test power analysis indicated a minimum requirement of 17 participants to successfully complete all sessions in order to maintain statistical power above the commonly accepted

0.80 threshold (Table 3)[94]; whereas a minimum of 12 participants was required with respect to the two-way repeated measures ANVOA power analysis (Table 4).

Table 3

A Priori Power Analysis Inputs for the Dependent t-tests

Parameter	Selected Input
Effect size (Cohen's *d*)	0.65
Alpha Level (α)	0.05
Power (1 - β)	0.80
Tail(s)	One

Table 4

A Priori Power Analysis Inputs for the Two-Way Repeated Measures ANOVAs

Parameter	Selected Input
Effect size (Cohen's *f)*	0.325
Alpha level (α)	0.05
Power (1 - β)	0.80
Number of groups	2
Number of measurements	17
Correlation among repeated measures	0.5
Nonsphericity correction (ε)	0.4

To determine the appropriate effect size for the *a priori* power analyses, the results of two similar studies comparing c-NMES and m-NMES were examined.[21,27] With respect to fatigue related outcomes, only Morf et al.[27] observed a statistically significant difference between the c-NMES and m-NMES conditions. However, only one of the three fatigue related outcomes measured by these authors reached statistical significance (e.g., significantly greater percent decline in MVIC after c-NMES relative to m-NMES). This statistical difference had a corresponding small to medium effect size (Cohen's *d* = 0.38), but the mean difference between the two NMES conditions was only 3%; which

suggests that a small to medium effect size corresponding with this outcome variable lacks clinical meaningfulness. Consequently, a medium to large effect size was selected for the a priori power analyses because any statistically significant differences with small to medium effect sizes would likely lack clinical relevance. The specific medium to large effect size values used during the power analyses (Table 3 & 4) were obtained by calculating half the distance between the medium and large effect size values defined by Cohen.[176] It is also important to note that other authors have selected a large effect size (e.g., Cohen's $f = 0.68$) for an *a priori* power analysis during a NMES study examining similar dependent variables,[37] which further supports the selection of a medium to large effect size as appropriate for this project. The alpha level (α) and power ($1 - \beta$) values were selected based on commonly accepted values.[94] In addition, single-tailed tests were selected due to the directional research hypotheses (see Chapter I), which were based on the results of similar studies.[21,27]

Sample

A convenience sampling technique was used to obtain the sample for this project, with participants being recruited from the university and community population via flyers, posted campus announcements and recruitment emails. In order to facilitate participant recruitment, participants were incentivized by being entered into a lottery for a chance to win one of four $50 gift cards to a local electronics store. Due to the results of the previously discussed power analyses and the possibility of experimental mortality, a rolling enrollment strategy was used with the original objective of collecting data until 17 participants successfully completed all six sessions.

Although a sample of 17 participants that successfully completed all six sessions was originally obtained, it was determined that some of the participants' data needed to be excluded from the statistical analyses due to a unique limitation of the c-NMES unit used throughout this project. Specifically, the c-NMES device has an obscure setting that may automatically reduce the stimulus output when the unit senses a change in impedance over the course of the NMES-induced contractions.[177] As would be expected, it was observed that the percent decline in MVIC, in NMES-induced torque and in torque-time integral (TTI), as well as the total torque-time integral (T-TTI) and self-reported discomfort levels, were influenced when this automatic step-down occurred, ultimately resulting in an undesired systematic bias in c-NMES test sessions during which the stimulus output was automatically reduced by the unit. Of the original 17 participants, the c-NMES device automatically reduced the stimulus output during six of the participants' test sessions corresponding to the study addressed in manuscript 1 (Chapter IV); whereas it occurred during seven of the participants' test sessions corresponding to the study addressed in manuscript 2 (Chapter V). Consequently, the data of these participants was excluded from the appropriate statistical analyses.

In an effort to improve the final sample size the rolling enrollment strategy was continued, and an additional four participants successfully completed all six test sessions. As with the original group of participants, the c-NMES device automatically reduced the stimulus output throughout the c-NMES condition test sessions of some of the additional participants. Specifically, the c-NMES device automatically reduced the stimulus output during one of the participant's test sessions corresponding to the study addressed in

manuscript 1 (Chapter IV); whereas it occurred during three of the participants' test sessions corresponding to the study addressed in manuscript 2 (Chapter V).

The final sample included a total of 21 participants that successfully completed all six test sessions; but due to the automatic reduction in the stimulus output by the c-NMES device, only data from 14 participants were used during the statistical analyses for manuscript 1. Since the automatic reduction of the c-NMES stimulus output did not influence the initial maximum comfortable stimulus intensity and initial normalized NMES-induced torque, the data of all 21 participants for these variables were used during the corresponding statistical analyses in manuscript 2. However, with respect to the other statistical analyses for manuscript 2, only data from the 11 participants that did not experience an automatic decrease in the stimulus output during the c-NMES condition were used.

The desired sample size of 17 participants indicated by the *a priori* power analyses was not achieved during all statistical comparisons; thus a type II error may have occurred due to low statistical power, which reduces the strength of the statistical conclusion validity.[94] However, medium to large effect sizes were selected for the power analyses because small to medium effect sizes would not likely have been clinically meaningful with respect to the variables included in this project. As discussed in future chapters, the effect sizes observed corresponding to the non-significant findings were predominantly small; which suggests that even if the desired sample size of 17 participants was obtained, significant findings would not have been meaningful. Therefore, the sample size was considered adequate to answer the research questions,

since it allowed the primary investigator to identify clinically meaningful differences and it is similar in size to a previous publication addressing similar research questions.[21]

Instrumentation

Torque Recording

A Quickset 4 Biodex dynamometer (Biodex Medical Systems Inc., Shirley, New York) was used to measure and record isometric knee extension torque during all voluntary and NMES-induced contractions, at a sampling rate of 100 Hz (e.g., one sample taken every 10 ms). Participants were asked to remove their shoe from the dominant foot prior to being seated on the dynamometer. The set-up followed standard procedures suggested by the manufacturer. During all voluntary and NMES-induced contractions participants were seated in the dynamometer chair with the seat back tilt at 85° and the dominant leg secured within a lever arm fixed at 60°. This position was chosen for testing as this approximates the joint position at which maximum quadriceps torque production occurs.[164,166] In addition, this joint position has been used in previous NMES studies,[26,50] and has been recommended as the position at which NMES treatments should occur.[42] The axis of rotation of the dynamometer was aligned to the anatomical axis of the test knee, and the lower leg was secured in the fixed lever arm via an ankle strap placed 2-3 cm above the lateral malleolus.[27] The dynamometer was calibrated to the manufacturer's specifications prior to beginning the project to ensure reliable measurements. In addition, stabilization straps were used to prevent undesired movement of the upper body and participants were asked to cross their arms over their chest while performing all voluntary and NMES-induced contractions (Figure 4).[27,178]

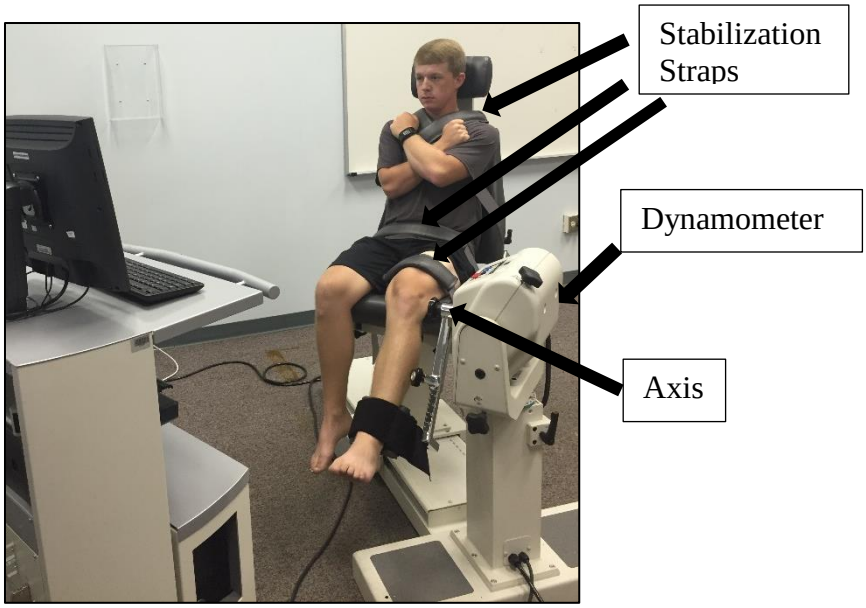


Figure 4. Isokinetic Dynamometer Set-up

Note. Participant performing a voluntary isometric contraction with the lever arm fixed at 60^0.

Conventional Neuromuscular Electrical Stimulation

All c-NMES treatments were applied using a Sonicator® Plus 940 stimulator (Mettler Electronics® Corp., Anaheim, CA). To maintain consistency across the two NMES conditions, the c-NMES parameters were as similar as possible to the parameters used with the Kneehab® XP program 6 (Table 5). Although the sample size was negatively impacted by an unforeseen feature of the device selected for the c-NMES condition, it is important to note that this stimulator was selected because it allowed the primary investigator to more closely match the settings of the m-NMES device; thus reducing the plausibility of confounding factors and facilitating causal inferences.[94] For example, other c-NMES units available in the laboratory did not allow the use of a biphasic waveform while simultaneously setting two channels to co-contract mode. Using other available c-NMES units would have resulted in different basic current types across the two NMES conditions (e.g., Russian vs. biphasic), ultimately leading to additional

systematic differences and limiting the ability to attribute any observed differences to the multipath current distribution method.

Table 5

Parameters of Neuromuscular Electrical Stimulation Conditions

Parameter	m-NMES	c-NMES
Current distribution	Multipath	Single path within two independent channels
Waveform	Biphasic Square	Biphasic Square
Frequency	70 Hz	70 Hz
Pulse duration	400 µsec	400 µsec
Ramp	1 second up : 0.5 seconds down	1 second up : 0 seconds down*
On time / Off time	10 seconds / 50 seconds	10 seconds / 50 seconds
Stimulus intensity	mA required for 30% MVIC OR maximum comfortable	mA required for 30% MVIC OR maximum comfortable
Number of electrodes	4	4
Total area of electrodes	427 cm^2	360 cm^2*

Note. *In addition to current distribution method, the only systematic differences between the c-NMES and m-NMES parameters was the lack of a ramp-down using the c-NMES device, and slightly smaller c-NMES electrodes. It was not a possible to select a ramp-down of 0.5 seconds with this particular c-NMES device while also maintaining a similar ramp-up and hold time to the m-NMES device, thus a ramp-down was not included.

The c-NMES current was delivered via self-adherent electrodes centered over motor points to the extent possible without causing overlap with adjacent electrodes, and the appropriate motor points used to guide electrode placement were manually identified and marked using the pencil electrode method described in the literature (see pencil electrode section). [24,153]Authors of the aforementioned studies comparing c-NMES and m-NMES identified differences in the electrode sizes across conditions as a possible limitation of their observed results.[21,27] In an effort to reduce the plausibility of electrode

size confounding the results, electrodes as similar in size as possible to the electrodes integrated into the m-NMES garment were used during the c-NMES condition. Two 5 cm x 9 cm self-adherent electrodes (Metron^{TM,} Bolingbrook, IL) were centered over the proximal and distal vastus lateralis motor points. A 10.79 cm x 17.78 cm electrode (TENS Products, Grand Lake, CO) was be centered over the rectus femoris motor point, and a 7 cm x 14 cm electrode (SME INC., Wilmington, NC) was centered over the distal vastus medialis motor point (Figure 5). The area covered by the c-NMES electrodes was roughly 360 cm^2, whereas the m-NMES electrodes covered an area of 427 cm^2 (Figure 6).[27] Although a difference in the area covered by the c-NMES and m-NMES electrodes remained, these electrodes were a significant improvement from the electrode configuration used during the c-NMES condition of previous studies; which consisted of three electrodes covering only 100 cm^2.[21,27] As has been done previously, a Kneehab® XP garment was placed over the thigh after the c-NMES electrodes had been applied to the quadriceps in an effort to blind the participants to the treatment condition.[27]

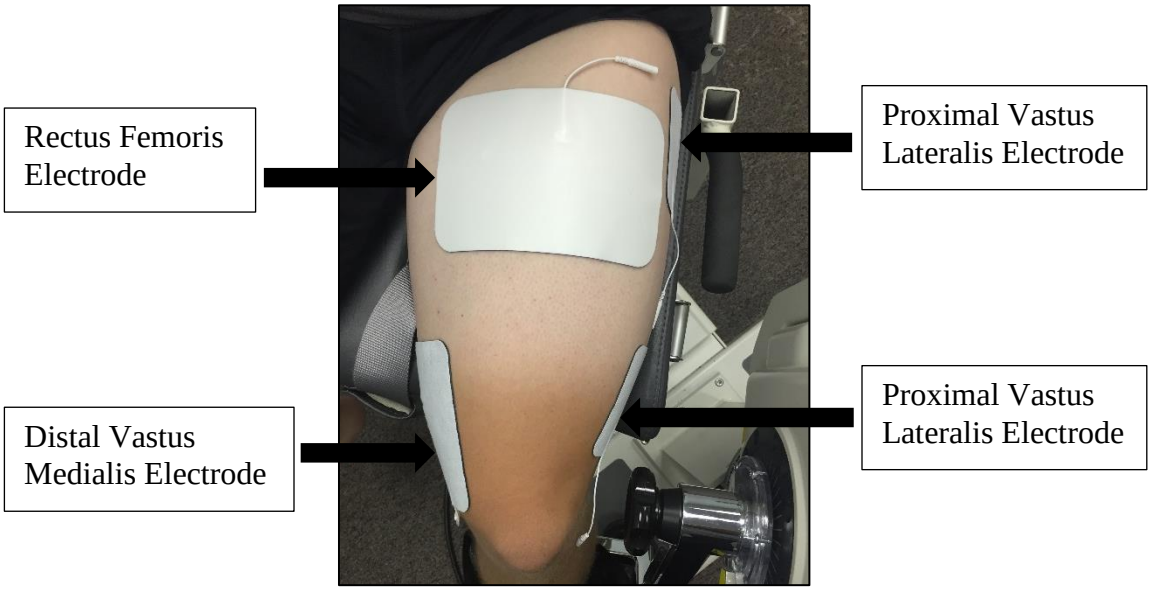


Figure 5. Conventional Electrode Configuration

Note. The left edge of the photo corresponds with the participant's medial leg.



Figure 6. Electrode Configuration Comparison

Note. c-NMES electrodes are on the left side of the photo and m-NMES electrodes are integrated into the neoprene garment on the right side of the photo.

Pencil Electrode

Based on the results of a recent study, which identified seven motor points of the quadriceps,[153] four commonly identified motor points were selected for use to guide the c-NMES electrode placement. Furthermore, the selected motor points allowed the c-NMES electrodes to be placed in a similar fashion to the m-NMES electrode configuration, as they were located on the proximal and distal vastus lateralis, proximal rectus femoris and distal vastus medialis.

The motor points were manually identified with a pencil electrode (Mettler Electronics XK2, Active Forever, Scottsdale, AZ; Figure 7). To locate the motor points the stimulus intensity of an Intelect Legend XT (Chattanooga Group, Inc., Hixon, TN) electrical stimulator was set to a level that only elicited a motor response when the pencil electrode passed over a motor point.[24,153] After each motor point was located it was

marked with a marker (Figure 7). The results of Botter et al.[153] indicate that the selected motor points may not be present or identifiable in every individual. Consequently, in the event that the primary investigator was unable to locate a motor point during the allotted 8 minute time frame, the motor point was considered unidentifiable and the average position reported by Botter et al. was utilized to guide the corresponding electrode placement.

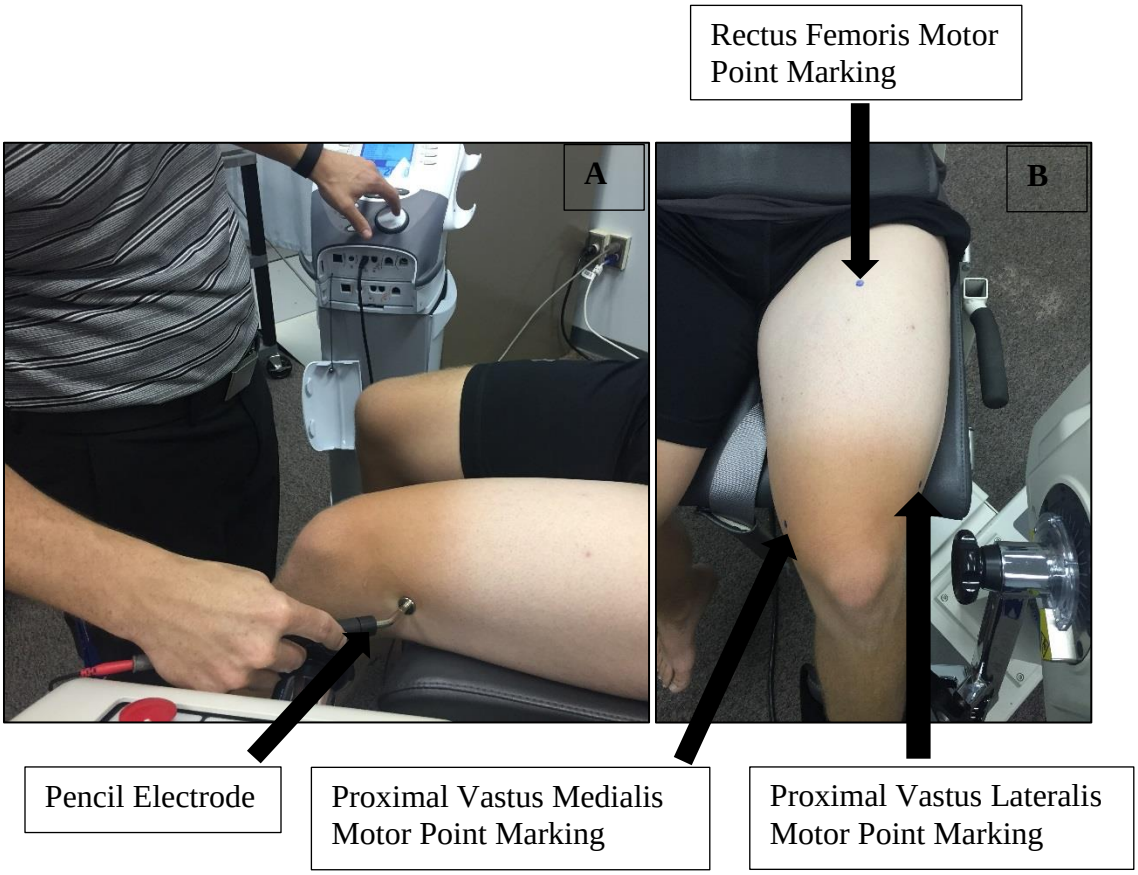


Figure 7. Motor Point Identification

Note. The photo on the left (A) illustrates the pencil electrode method for manually identifying motor points. The photo on the right (B) illustrates the motor point markings.

Motor points were identified with the participant in the position in which the treatment was applied, as has been recommended.[24] Since using an anatomical chart may not adequately locate motor points,[24] the motor points were manually identified via the pencil electrode method to exclude electrode placement as a confounding variable. Furthermore, NMES-induced discomfort levels may differ when an atlas is used for motor point identification compared to manually identifying motor points.[39]

Multipath Neuromuscular Electrical Stimulation

All m-NMES treatments were applied using a Kneehab® XP stimulator (Theragen LLC, Leesburg, VA), with the stimulator parameters set by selecting program 6 (Table 5). The m-NMES electrodes were integrated into the neoprene garment and the garment was subsequently placed on the dominant thigh according to the manufacturer's recommendations.[167] Because the electrodes were integrated within the garment, motor point identification was not necessary prior to applying the garment to the thigh. However, motor points were still identified during m-NMES test sessions in an effort to blind participants to treatment condition.

Visual Analog Scale

A 100 mm horizontal visual analog scale (VAS) was used to measure self-reported discomfort levels during each NMES condition (see Appendix B). The descriptors at each end of the scale were "no discomfort" (0 mm) and "worst possible discomfort" (100 mm).[21,27,29,36,37,159,161] Participants were given a pen and asked to "rate your level of discomfort by making a vertical tick mark on the line" (Figure 8). The primary investigator scored each VAS by measuring the distance in millimeters (mm) from the "no discomfort" anchor to the vertical tick mark made on the horizontal line.

The VAS is considered to be a reliable and valid measurement tool,[159,168,171,172] thus the VAS has been frequently utilized in the literature as a measure of self-reported discomfort during NMES treatments.[21,23,27,29,36-38,69,72,159-161] When used to assess NMES-induced discomfort in a sample of healthy individuals, the VAS has been shown to have a high inter-session test-retest reliability (intraclass correlation coefficient [*ICC*]≥0.90).[159]

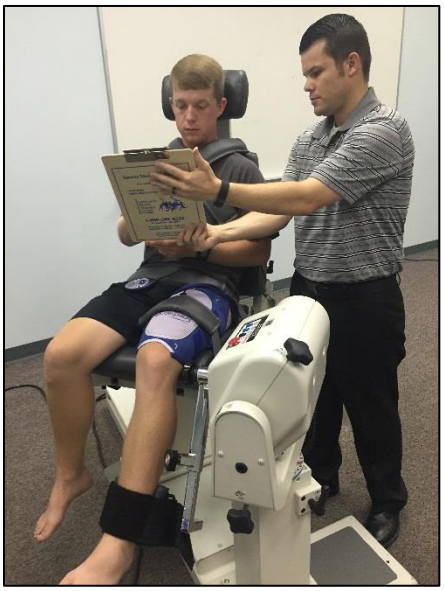


Figure 8. Participant Marking the Visual Analog Scale

Procedures

Participant Inclusion and Exclusion Criteria

In order to be included, participants were required to be: healthy, recreationally active, males, between the ages of 18-35. Healthy was defined as having no unresolved knee injuries or other injuries that would impact the lower-limb function of the leg of interest, as well as being free of all applicable electrotherapy contraindications.[152] Recreationally active was operationally defined as participation in some form of physical activity (e.g. strengthening related activities, jogging, running, cycling, swimming, tennis, etc.) for a minimum of two times per week for at least 20 minutes each time.

NMES tolerance and motor thresholds have been shown to differ between individuals with a body mass index (BMI) above and below 30 kg/m^2,[160] thus participants also had to have a BMI ≤30 kg/m^2 to be included. Females were excluded from participation because electrically induced discomfort levels have been shown to significantly differ over the course of the menstrual cycle.[162] This study was approved by the University's institutional review board (see Appendix C), and participants provided written informed consent (see Appendix D).

Overview

Participants reported at the same time of day (±2 hours) on six separate occasions. Each participant's dominant leg, which was operationally defined as the leg with which they would use to kick a soccer ball, served as the leg of interest throughout the study. In an effort to reduce electrical impedance, participants were instructed to shave the anterior thigh of their dominant leg prior to reporting each day. In the event that a participant reported to the laboratory unshaven, they were provided an unused razor and asked to shave the anterior thigh of their dominant leg. Participants were also asked to refrain from strenuous activities for 12 hours prior to reporting each day, and instructed to report well hydrated.

Each session lasted approximately 1 hour. The first two sessions (days 1 & 2) served as familiarization sessions and were separated by 24-48 hours, while the other sessions (days 3 through 6) served as test sessions and were separated by a 48-72 hour washout period. Participants began each session by completing a standardized warm-up that included: 5 minutes of cycling on a stationary bike at a self-selected pace, three 30 second bouts of dynamic quadriceps stretching and four isometric quadriceps

contractions while in the dynamometer chair (2 at 50%, 1 at 75% and 1 maximum contraction at 60° of knee flexion).[36] After completing the warm-up, participants stayed seated in the dynamometer chair throughout the remainder of the session. Table 6 provides a method by day summary to assist the reader.

Table 6

Method by Day Summary

Method	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Informed consent	X					
Participant health & activity questionnaire *(includes health, electrical stimulation contraindications and activity level questions)*	X					
Demographic data collected *(age, height, weight, gender, body mass index)*	X					
Participant assigned to test session sequence			X		X	
Standardized warm-up *(5 minutes of cycling, dynamic stretching, submaximal MVICs)*	X	X	X	X	X	X
8 minute rest after warm-up *(leg cleansed, motor points identified for c-NMES electrode placement)*	X	X	X	X	X	X
Participants allowed to practice performing MVICs *(minimum of six repetitions)*	X					
3-6 pre-test MVICs *(performed at 60° of knee flexion, separated by 2 minutes of rest, three consecutive repetitions required to be within 10% of one another)*		X	X	X	X	X
5 minute rest after maximum voluntary isometric contractions	X	X	X	X	X	X
10 familiarization NMES-induced contractions using c-NMES and m-NMES *(participant controls the intensity and is encouraged to maintain a maximum comfortable stimulus intensity)*	X	X				

Table 6 (continued).

Method	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
NMES stimulus intensity determined *(30% of pre-test MVIC during days 3 & 4 OR maximum comfortable stimulus intensity on days 5 & 6)*			X	X	X	X
50 second rest after stimulus intensity determined			X	X	X	X
18 NMES-induced contractions with c-NMES OR m-NMES *(participant instructed to relax, and initial stimulus intensity was maintained constant)*			X	X	X	X
Self-reported discomfort levels measured *(100 mm VAS used after each NMES-induced contraction)*			X	X	X	X
1-2 post-test MVICs			X	X	X	X

Familiarization Sessions (Days 1-2)

After obtaining informed consent on the first day, participants were asked to complete a brief physical activity and health questionnaire and their demographic data was collected (height [cm], weight [kg], age [years], BMI [kg/m^2]) (see Appendix E); which allowed the primary investigator to determine that the previously discussed inclusion criteria were met. Participants then completed the warm-up, after which they were given an 8 minute rest period. During the rest period the motor points necessary for the c-NMES treatment condition were identified using the pencil electrode method previously described in the instrumentation section, and the leg of interest was cleansed with an alcohol free wipe. During the first familiarization session, participants were instructed on the maximum voluntary isometric contraction (MVIC) procedures and given an opportunity to practice performing MVICs of the quadriceps until they reported being comfortable with the procedures (discussed in detail in MVIC procedures section). An additional 5 minute rest period followed the MVICs, during which the c-NMES electrodes and an empty Kneehab® XP garment or the Kneehab® XP garment with integrated electrodes were appropriately placed over the participant's dominant thigh.

Participants were then exposed to both forms of NMES (c-NMES and m-NMES) by performing 10 NMES-induced quadriceps contractions using each NMES device (total of 20 contractions). The NMES parameters used during the familiarization sessions were similar to those used during test sessions (Table 5), with the exception that participants were allowed to control the stimulus intensity throughout the familiarization sessions. During previous studies in our laboratory it was observed that participants are willing to use greater stimulus intensities if they are allowed to control the intensity themselves.

Thus, during all familiarization sessions the stimulator controls were placed in a manner that allowed participants to control the amount of stimulus intensity used during all NMES-induced contractions. It was anticipated that participants would tolerate greater intensities as they became acclimated to the stimulus,[144] thus the primary investigator encouraged participants to increase the stimulus intensity between contractions as well as between familiarization sessions to maintain a maximum comfortable stimulus level during each contraction (e.g., highest stimulus intensity that does not cause pain).[77] The initial familiarization session allowed participants to practice the MVIC procedures and to acclimate to the NMES stimulus.[98,144]

The purpose of the second familiarization session was to allow the participants to further familiarize themselves with the MVIC procedures and acclimate to the NMES stimulus. In addition, the second familiarization session allowed the primary investigator to verify that each participant was capable of tolerating the stimulus intensity required during day 3 and day 4 test sessions. The procedures for the second familiarization day were similar to the first, with the exception that participants performed a series of pre-test MVICs following the standardized pre-test MVIC procedures (discussed in detail in MVIC procedures section). In addition, the order in which the devices were used during the NMES-induced contractions occurred in a reverse order from the pattern used during the initial session.

Participants were required to tolerate a stimulus intensity sufficient to produce a NMES-induced contraction of 30% MVIC during the subsequent day 3 and day 4 test sessions. To evaluate whether a participant was capable of tolerating the required stimulus intensity, the greatest peak torque observed over the 10 contractions of each

condition during the second familiarization session was identified by the primary investigator. The NMES-induced peak torque observed under each NMES condition was then expressed as a percentage of the participant's pre-test MVIC peak torque measured earlier during the second familiarization session (% MVIC; equation 1). If a participant was unable to tolerate a stimulus intensity sufficient to produce a NMES training intensity ≥30% MVIC, they were excluded from further participation.

[Equation 1]

$$\%\,\text{MVIC} = \left[\left(\frac{i_{NMES}}{\tau_{pre}}\right) \times 100\right]$$

Note. Where i_{NMES} = peak torque observed during the NMES-induced contraction of interest (e.g., repetition 1-18) and τ_{pre} = pre-test MVIC peak torque.

Test Sessions (Days 3-6)

Upon reporting for the first test session (day 3), participants were assigned via a probabilistic technique to one of two permutations designed to counterbalance the session order in which the c-NMES and m-NMES treatment conditions were performed on day 3 and day 4. Upon reporting for the third test session (day 5) participants were again assigned to one of two permutations designed to counterbalance the order in which the c-NMES and m-NMES conditions were performed on day 5 and day 6. Rather than using the term "random assignment", the term "probabilistic technique" is used to describe the method used for participant assignment because it ensured a similar number of participants were assigned to each permutation; which did not allow all participants to have the same non-zero chance of being assigned to each permutation. Participant assignment to test session order did not take place until day 3 and day 5 in an effort to limit the threat of post-randomization experimental mortality.[94] As previously

mentioned, this was likely an effective strategy since all participants assigned a test session order successfully completed all six sessions.

The procedures for all test sessions followed the same procedures previously outlined in detail with respect to the second familiarization session (Table 6); with the following exceptions:

1. Only 18 NMES-induced contractions were performed using a single NMES device (c-NMES or m-NMES) during each test session.
2. The stimulus intensity was standardized and not manipulated by the primary investigator or participant within each test session.
3. Participants performed post-test MVIC procedures immediately following the NMES-induced contractions during each test session (discussed in detail in the MVIC procedures section).

MVIC Procedures

Gandevia et al.[98] provided a series of recommendations that were incorporated during the MVIC procedures. Their recommendations that were incorporated throughout this project included the following:

1. Participants were given instructions and had an opportunity to practice.
2. Participants were provided visual feedback during all MVICs.
3. Standardized verbal encouragement was provided by the primary investigator during all MVICs.
4. Participants were given the opportunity to eliminate trials that they deemed to be submaximal.

In keeping with the first recommendation, participants were instructed on the MVIC procedures and given an opportunity to practice during the initial familiarization session (day 1). They were allowed to practice performing MVICs at a self-selected pace, and participants were allowed to practice until they reported being comfortable with the procedures. However, a minimum of six practice trials was required during the initial familiarization session.

All MVICs were 6 seconds in duration. Participants were instructed to gradually increase their effort during the initial portion of each contraction, with maximal effort being reached at roughly 3 seconds and maintained for the remaining 3 seconds. To facilitate participant understanding of a gradual increase, a figure showing an ideal MVIC curve was placed in the participants' view while performing all MVICs. Prior to each MVIC participants were instructed as follows: "cross your arms and prepare to push out with maximal effort". In addition, participants received real-time visual feedback of their torque production via the dynamometer computer screen and the primary investigator provided verbal encouragement throughout each repetition (Figure 4). Verbal encouragement began at the start of each MVIC and continued throughout the 6 second contraction. The primary investigator encouraged participants to "push out with maximal effort" in a loud clear voice, and in the event that a participant showed signs of fatigue the volume and intensity of the encouragement was gradually increased.[179] The primary investigator asked the participants to verify that their effort was maximal immediately following each repetition. In the event that a participant reported a submaximal effort, or the investigator judged an effort to be submaximal, the repetition was discarded and repeated following a 2 minute rest.

Participants performed standardized pre-test MVIC procedures during the second familiarization session and all subsequent test sessions (days 2-6). The standardized pre-test procedures consisted of a series of three MVICs, with each repetition separated by a 2 minute rest period in an effort to limit fatigue.[31,33] During the test sessions (days 3 through 6), the peak torque of the three pre-test trials was required to be within 10% or participants were asked to perform additional trials until three consecutive MVIC trials were within 10%; which is similar to a previous study.[27] To limit the possibility of fatigue as a confounding variable, participants were given a maximum of six contractions during each test session. If a participant was unsuccessful in completing the pre-test MVIC procedures, they were asked to return the following day for a second attempt. The trial with the greatest peak torque, from the three consecutive trials within 10%, was defined as the participant's pre-test MVIC value for that particular test session.

Pre-test MVICs performed during the second familiarization session (day 2) were primarily for the purpose of allowing the participant to further familiarize themselves with the MVIC procedures, and the peak torque observed during these repetitions also served as a reference value when verifying that each participant was able to tolerate an adequate stimulus intensity producing 30% MVIC. Consequently, three consecutive MVICs within 10% of one another during the second familiarization session (day 2) was not required for further participation, but participants were given an opportunity to perform up to six contractions in the event that three consecutive MVICs were not within 10%.

Post-test MVIC procedures were not performed at the conclusion of any of the familiarization sessions (days 1 & 2). However, during all test sessions (days 3-6) each

participant performed a single 6 second post-test MVIC immediately after completing the assigned NMES treatment condition. A single repetition was used in an effort to limit recovery from the NMES-induced contractions; but in the event that the participant or investigator deemed the post-test trial to be submaximal a second MVIC was performed after a 20 second rest period, in an effort to continue limiting recovery.[178]

An isometric contraction, rather than a dynamic contraction, was selected in an effort to limit the number of extraneous variables (e.g., joint stabilization and synergistic activity).[118] The inter-session and intra-session test-retest reliability of MVICs of the quadriceps in a sample of healthy individuals has been previously reported to be within acceptable limits ($ICC \geq 0.72$).[40,104,178,180-184] Furthermore, Place et al.[178] reported that the inter-session test-retest reliability of MVICs of the quadriceps immediately following a fatiguing protocol was also within acceptable limits ($ICC = 0.91$); which subsequently lead these authors to conclude that an MVIC performed after a fatiguing protocol is a reliable measure of muscle fatigue.

NMES and Stimulus Intensity Standardization Procedures

As mentioned in the instrumentation section, all NMES-induced contractions occurred with the participant seated in the dynamometer chair with the lever arm fixed to 60° (Figure 9). Although NMES may be applied during a relaxed state or superimposed during voluntary contraction, in order to better understand NMES-induced torque it has been recommended that the superimposed method be avoided during research studies.[17] Consequently, throughout each NMES-induced contraction participants were frequently encouraged by the investigator to "relax and allow the machine to do all the work".

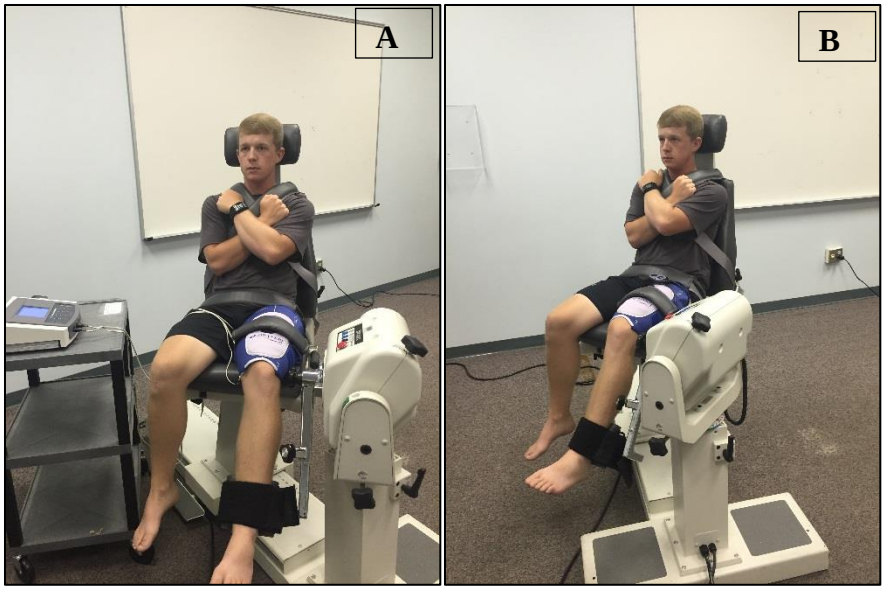


Figure 9. NMES Treatments

Note. The photo on the left (A) illustrates a c-NMES treatment with the Kneehab XP® garment placed over the c-NMES electrodes in an effort to blind participants to treatment condition. The photo on the right (B) illustrates a m-NMES treatment.

Prior to beginning each NMES condition during the first two test sessions (day 3 & day 4), the stimulus intensity required to produce a target torque output of 30% MVIC was determined by the investigator immediately after the 5 minute rest period that followed the pre-test MVICs. The primary investigator increased the stimulus intensity until the targeted torque output of 30% of the test session's pre-test MVIC was observed (equation 1), and held until the NMES-induced contraction was completed. If a participant reported reaching a maximum comfortable stimulus intensity prior to achieving a contraction of 30% MVIC the intensity was immediately decreased and the participant recovered for 30 seconds. This process was repeated a maximum of three times, and if the participant was unable to tolerate a sufficient stimulus intensity by the third attempt they were excluded from further participation. Following identification of the appropriate stimulus intensity participants rested for 50 seconds, after which the

assigned NMES condition was completed. The initial stimulus intensity on day 3 and on day 4 was not manipulated by the investigator or participant over the course of each NMES treatment condition. However, as previously discussed, occasionally the c-NMES device automatically reduced the stimulus output and in the event that this occurred the negatively influenced data were excluded from the appropriate statistical analyses.

The stimulus intensity was standardized across conditions on days 3 and 4 using a target training intensity of 30% MVIC because it lies within the therapeutic window identified in the literature.[144] This is a significant improvement from previous studies comparing c-NMES and m-NMES, as the intensity used during these studies does not fall within the aforementioned window and the authors identified their use of a low target training intensity (e.g., 20% MVIC) as a possible explanation for their non-significant results.[21,27] Furthermore, based on previous studies performed in our laboratory it is likely that most healthy individuals are able to tolerate a stimulus intensity required to produce a contraction of 30% MVIC, thus the target intensity was also selected in an effort to limit the threat of experimental mortality.[94]

Prior to beginning each NMES condition during the last two test sessions (day 5 & day 6), each participant's maximum comfortable stimulus intensity was determined instead of determining the stimulus intensity required to achieve a target training intensity of 30% MVIC. To determine each participant's maximum comfortable stimulus intensity, the stimulator was placed in a position that allowed each participant to control the stimulus intensity (Figure 10), and the investigator instructed the participant to gradually increase the stimulus intensity until they reported achieving a maximum comfortable stimulus; which was operationally defined as the highest stimulus intensity

that does not cause pain.[77] After identification of the appropriate stimulus intensity, the remaining NMES procedures were similar to the previously discussed day 3 and day 4 procedures.

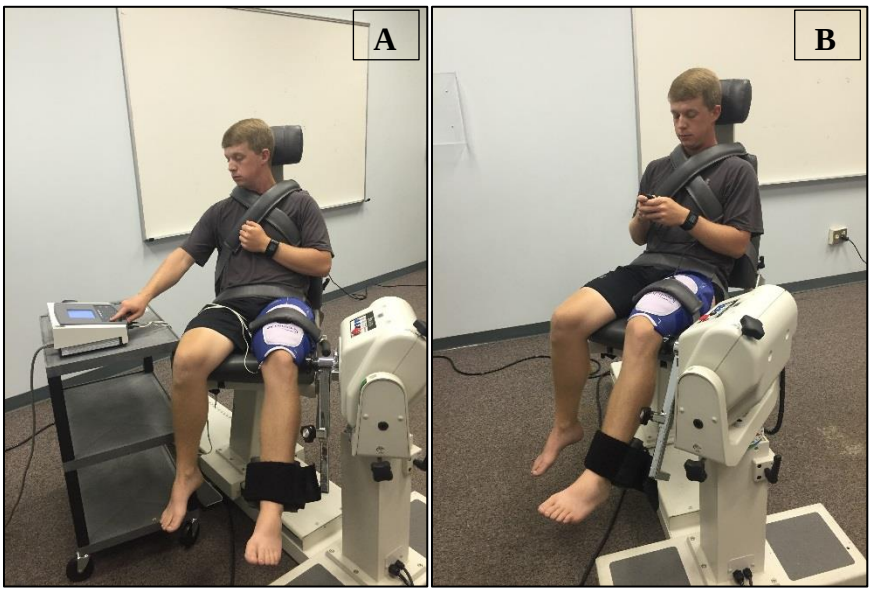


Figure 10. Setting Maximum Comfortable Stimulus Intensity

Note. Photo on the left (A) illustrates participant setting maximum comfortable stimulus intensity using c-NMES. Photo on the right (B) illustrates participant setting maximum comfortable stimulus intensity using m-NMES.

The purpose of the day 5 and day 6 test sessions was to address a limitation of the earlier test sessions. Standardizing the stimulus intensity based on a common initial NMES training intensity target (e.g., 30% MVIC), as done during day 3 and day 4 test sessions, was necessary to reduce the plausibility of alternative explanations for any observed differences with respect to fatigue related outcomes. For example, if participants were allowed to self-select an intensity under both conditions, it is plausible that an intensity sufficient to induce a contraction of 30% MVIC under one condition and an intensity sufficient to induce a contractions of 40% under the other condition could have been selected; thus greater fatigue observed during the latter condition may have

occurred solely because a higher initial training intensity was used. Therefore, due to the preliminary nature of this project, it was necessary to standardize the stimulus intensity based on a common initial NMES training intensity target to facilitate and strengthen any causal inferences corresponding to the fatigue related outcomes.[94]

Despite the fact that standardizing the stimulus intensity based on an initial NMES training intensity likely enhanced the experimental control, it also limited the generalizability of the subsequent results because the optimal stimulus intensity for NMES treatments occurring within clinical settings is a maximum comfortable intensity. Standardizing the stimulus intensity based on an initial NMES training intensity target of 30% MVIC also does not allow for inferences regarding which method of NMES, if any, allows for greater NMES training intensities while using a maximum comfortable stimulus intensity. The aforementioned limitation of the first two test sessions exemplifies the ever-present tradeoff between internal and external validity that often occurs in single studies.[94] Due to the necessary prioritization of internal validity on day 3 and day 4, the extent to which the results of the first test sessions hold true while using a clinically relevant maximum comfortable intensity would have remained unknown if this limitation was not addressed. Thus, in an effort to advance the generalizability of this project to clinical settings a maximum comfortable stimulus intensity was used under each NMES condition during the last two test sessions (day 5 & day 6). It is also important to note that the selected test session order, in which the test sessions using a maximum comfortable stimulus occurred after sessions that standardized the stimulus intensity based on an initial NMES training intensity target of 30% MVIC, was chosen because each of the earlier sessions (days 1-4) allowed for further acclimation to the

NMES stimulus; which likely allowed for greater maximum comfortable stimulus intensities during the day 5 and day 6 test sessions.[144]

Outcome Measures

Percent Decline in MVIC Torque

For the purposes of this study, muscle fatigue was operationally defined as an exercise-induced decrease in the quadriceps ability to produce force.[98,110] In accordance with this definition, the most often implemented measurement model is the assessment of peak torque production during an individual's MVIC prior to and immediately following fatigue inducing exercise.[105,110] A change in peak MVIC torque is also considered to be the gold standard for assessing fatigue.[105,110] This measurement model is often selected by researchers because it is representative of the aggregate chain of neuromuscular events required to produce a muscular contraction, thus it is considered to be a general assessment of fatigue without differentiating between central and peripheral fatigue.[110] Consequently, each participant's post-test MVIC peak torque was expressed as a percent decline relative to their pre-test MVIC peak torque via equation 2, and the subsequent percent decline served as a fatigue related outcome for this project.

[Equation 2]

$$\%\ \text{decline in MVIC} = \left[\left(\frac{\left(\tau_{post} - \tau_{pre}\right)}{\tau_{pre}}\right) \times 100\right]$$

Note. Where τ_{post} = post-test MVIC peak torque and τ_{pre} = pre-test MVIC peak torque.

Neyroud et al.[29] suggested that quantifying muscle fatigue solely by examining decreases in MVIC peak torque may underestimate fatigue when the intensity of NMES-induced contractions is substantially lower than that of an MVIC, because motor units not

recruited during NMES may be recruited during an MVIC; thus three additional measures were included as fatigue related outcome measures during this project.

Percent Decline in NMES-induced Torque

Failing to maintain a target force is another common method used to asses fatigue,[110] thus NMES-induced fatigue is also frequently assessed by measuring the decline in NMES-induced torque over the course of a treatment.[21,27,30-33,46,49,51,54,55,59,61,62,64,66,100] Accordingly, the peak torque produced during all 18 NMES-induced contractions of each test session (days 3-6) were recorded by the dynamometer, and these values were expressed as a percent decline relative to the peak torque produced during the initial NMES-induced contraction of each test session via equation 3.

[Equation 3]

$$\%\ \text{decline in NMES} = \left[\left(\frac{\left(i_{NMES} - \tau_{initid}\right)}{\tau_{initial}}\right) \times 100\right]$$

Note. Where i_{NMES} = ith number of contraction (e.g., i = contraction 2-18), $\tau_{initial}$ = NMES-induced peak torque of initial contraction.

Some authors[100] have suggested that simply using a decline in NMES-induced torque to represent fatigue is also flawed, as this may result in an overestimation of NMES-induced fatigue because other physiological factors (e.g., nerve accommodation) unrelated to the mechanisms of motor unit fatigue may influence these results. Consequently, the percent decline in MVIC measure was also included as a fatigue related outcome.

Percent Decline in Torque-time Integral

A decline in the TTI observed during NMES-induced contractions has also been used in a number of NMES studies as an index of NMES-induced fatigue,[23,29,47,115,116] as

it has been suggested to represent isometric work. Some of these authors used the decline of the TTI as the primary index of NMES-induced fatigue,[29] while others suggested that the TTI be used as a fatigue related outcome when longer stimulation trains are implemented.[47] Therefore, the data necessary to calculate the TTI (e.g., torque and duration of torque recording) was measured and recorded during the NMES-induced contractions by the dynamometer system and subsequently exported as a text file (sampling rate = 100 Hz, or one sample taken every 10 ms). The data files were imported into an analysis software package (Acqknowledge® 4, Biopac® Systems, Inc., Goleta, CA), which was used to calculate the TTI of each contraction via equation 4. The TTI of each NMES-induced contraction during the test sessions (days 3-6) was expressed as a percent decline via equation 5, and also served as a measure of NMES-induced fatigue.

[Equation 4]

$$\text{TTI} = \int_{a}^{b} f(t)dt$$

Note. Where a = lower limits, b = upper limits, $f(t)$ = function of the NMES-induced torque curve. Lower limits = sample immediately prior to observed torque onset, whereas upper limits = sample immediately following the observed torque offset. Onset was defined as the first sample with a torque recording above the gravity correction and offset was defined as the first sample that the torque recording returned to the gravity correction.

[Equation 5]

$$\%\ \text{decline in TTI} = \left[\left(\frac{\left(i_{NMESTTI} - \lambda_{NMESTTI}\right)}{\lambda_{NMESTTI}}\right) \times 100\right]$$

Note. Where $i_{NMESTTI}$ = the TTI of the ith number of the NMES-induced contraction (e.g., contractions 2-18), $\lambda_{NMESTTI}$ = the TTI of the initial NMES-induced contraction.

Total Torque-time Integral

The T-TTI was considered an index of the total amount of isometric work performed under each condition, and as such also represented a fatigue related outcome. The T-TTI for each condition was calculated by summing individual TTI data via equation 6.

[Equation 6]

$$\text{T-TTI} = \int_{a}^{b} f(t_i)dt + \ldots + \int_{a}^{b} f(t_n)dt$$

Note. Where a = lower limits, b = upper limits, $f(t)$ = function of the NMES-induced torque curve, i = ith number of contraction and n = total number of contractions. Lower limits = the sample immediately prior to the observed torque onset, whereas upper limits = the sample immediately following the observed torque offset. Onset was defined as the first sample with a torque recording above the gravity correction and offset was defined as the first sample that the torque recording returned to the gravity correction.

Self-Reported Discomfort

Participants were asked to rate their level of discomfort by making a vertical tick mark on a VAS immediately after each NMES-induced contraction performed during the test sessions (days 3-6). As previously mentioned, discomfort levels were obtained by measuring the distance from the "no discomfort" anchor to the vertical mark made on the horizontal line.

Maximum Comfortable Stimulus Intensity

The maximum comfortable stimulus intensity identified by each participant during the last two test sessions (day 5 & day 6) was manually recorded (see Appendix F) by the investigator and expressed in milliamps (mA), and it served as an additional outcome variable in manuscript 2 (Chapter V). The m-NMES device does not express the stimulus intensity in mA units, thus a conversion table provided by the manufacturer

was used to convert the observed m-NMES stimulus intensities into the appropriate units.[185] It is important to note that since this variable was measured during the initial contraction it was unaffected by any automatic decrease in the stimulus output by the c-NMES device that may have occurred over the course of the treatment, thus the maximum comfortable stimulus intensity data from each participant that successfully completed all sessions was included during the corresponding statistical analysis.

Initial Normalized NMES-induced Torque

The initial NMES-induced peak torque was also measured and recorded by the dynamometer during the last two test sessions (day 5 & day 6). However, in an effort to reduce inter-participant variability, initial NMES-induced peak torque values were normalized to each participant's body mass via equation 7 and expressed as Newton-meters per kilogram (Nm/kg), which has been done previously.[42,45] The initial normalized NMES-induced torque for each condition also served as an additional outcome variable in manuscript 2 (Chapter V). As with the maximum comfortable stimulus intensity, since this variable was measured during the initial contraction it was unaffected by any automatic decrease in the stimulus output by the c-NMES device that may have occurred over the course of the treatment, thus data from each participant that successfully completed all sessions was included during the corresponding statistical analysis.

[Equation 7]

$$\text{Normalized NMES-induced torque} = \frac{\tau_{initial}}{M}$$

Note. Where $\tau_{initial}$ = NMES-induced peak torque of initial contraction and M = body mass of the participant.

Statistical Analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corporation, Armonk, NY). Due to methodological differences across test sessions (Chapter I), data from the first two test sessions (day 3 & day 4; Table 9) were analyzed and reported separately from the data of the last two test sessions (day 5 and day 6; Table 10). However, many of the comparisons were made using the same analysis procedures because of the similar dependent and independent variables (Table 9 &10).

Data Screening

Prior to performing the statistical analyses the data were screened and the tenability of the applicable statistical assumptions was assessed (Tables 7 & 8). Due to the use of a rolling enrollment strategy and the nature of the outcome measures, there was very little missing data (less than 1%). Therefore, in the event that missing data was present the corresponding group mean was used to substitute missing values.[186] Missing data were identified via frequency tables produced via SPSS, and the investigator visually inspected each table to verify the presence of an appropriate valid *n* for each outcome variable. Any impacted analysis was run while including the substituted missing values and while excluding participants with missing data to verify that the mean substitution technique did not alter the results.[186]

Table 7

Tests for the Assumptions of a Dependent t-test

Assumption	Test
Matched pairs	valid *n* in frequency table for all outcome variables
Absence of outliers	difference score *z*-scores < ± 2.50
Difference scores of dependent variables are normally distributed	skewness and kurtosis *z*-scores < ± 3.29, visual inspection of histogram

Note. Assumptions and tests based on information provided in the literature.[186,187]

Table 8

Tests for the Assumptions of a Two-way Repeated Measures ANOVA

Assumption	Test
Matched pairs	valid N in frequency table
Absence of outliers	*z*-scores < ± 2.50
Dependent variables are normally distributed	skewness and kurtosis *z*-scores < ± 3.29, visual inspection of histogram
Matched pairs	valid *n* in frequency table
Sphericity	acceptable epsilon (ε) estimate (ε > 0.75)

Note. Assumptions and tests based on information provided in the literature.[186,187]

For this project, outliers were defined as having a *z*-score greater than ± 2.5.[188] A transformation procedure was used to change the original raw score of an observed outlier to a value ±1 unit larger than the next highest value. This procedure was recommended by Tabachnick and Fidell[186] because it allows the case identified as an outlier to remain deviant while also reducing the impact of an outlier. Prior to changing a raw score identified as an outlier, the raw data point was checked to verify that it was accurately entered.[186]

When dealing with smaller samples, such as the sample included in this project, it is recommended to perform skewness and kurtosis significance tests to determine normality.[186,187] Therefore, normality was assessed via converting skewness and kurtosis estimates reported by SPSS for each outcome variable into a *z*-score.[186,187] To determine if the sample distribution significantly deviated from a normal distribution, the resulting skewness and kurtosis *z*-scores were compared to a score of ±3.29; which corresponds to an alpha level of $p<0.001$ and is common practice.[186] It is important to note that with smaller samples, normality significance tests may lack the appropriate power to identify deviations in normality.[187] Consequently, the primary investigator also examined histograms to visually check normality, and the data were considered normally distributed if the shape approximated a normal bell curve shape.

To examine the plausibility of a systematic bias impacting the fatigue related outcomes of manuscript 1, a series of dependent *t*-tests were used to verify that a statistically significant difference did not occur with respect to the pre-test MVIC torque values, initial NMES training intensity and the initial TTI across the first two test sessions (day 3 & day 4). A dependent t-test was also used to verify that a statistically significant difference did not occur with respect to the pre-test MVIC torque values across the last two test sessions (day 5 & day 6). In addition, test-retest reliability estimates[189,190] (e.g., $ICC_{(2,1)}$) and measurement precision estimates[191] (e.g., SEM) with respect to the pre-test MVICs of manuscript 1 and 2 were also calculated. Due to the use of a cross-over study design, period effects were possible; thus the data were also examined using a tabular method provided in the literature for examining the presence of such effects (see Appendix G).[96,192]

Two-way Repeated Measures ANOVA

A series of two-way repeated measures ANOVA were used to examine the effect of NMES treatment condition and time on the percent decline in NMES-induced torque and percent decline in TTI variables, as well as on self-reported discomfort levels (Table 9 & 10). In the event of a low epsilon (ε) estimate (e.g., $\varepsilon<0.75$), the assumption of sphericity was determined to be violated and the Greenhouse-Geisser procedure for correcting degrees of freedom was followed; since it is a more conservative approach and has been recommended when $\varepsilon<0.75$.[187,193] In the event of a significant interaction effect (NMES condition*time), simple effects analysis was performed; which allowed the primary investigator to interpret the interaction effect by analyzing the effect of a single independent variable at each level of the other independent variable via a series of pairwise comparisons.[187] The procedures and syntax provided by Field[187] were used during all simple effects analyses, with the exception that a Bonferroni procedure was also included in the syntax to control the family-wise error rate during the multiple pairwise comparisons (see Appendix H). The investigator also examined the corresponding profile plot to aid the interpretation of any observed interaction effects.

In the event of a significant time main effect and the absence of an interaction effect, *post-hoc* pairwise comparisons using a Bonferroni procedure were performed. The Bonferroni procedure was selected because it is considered to be the most robust *post-hoc* method with respect to controlling the Type I error rate and power when sphericity has been violated.[187] Due to the number of NMES-induced contractions that occurred during each condition, in the event of a significant time main effect a large number of *post-hoc* pairwise comparisons existed (e.g.> 130). To simplify the results,

only the significant pairwise comparisons deemed to be clinically important were provided (e.g., first contraction to demonstrate a significant decline relative to the second contraction, and final contraction).

Dependent t-test

A series of dependent *t*-tests were used to compare the percent decline in MVIC, as well as the T-TII, across NMES treatment conditions (Table 9 & 10). In addition, dependent *t*-tests were used to compare the maximum comfortable stimulus intensity and initial normalized NMES-induced torque observed during the last two test sessions (days 5 & 6; Table 10).

Effect Sizes

To determine the magnitude of the observed differences, effect sizes corresponding with each of the statistical analyses were calculated and reported when appropriate (Table 9 & 10). Cohen's *d* effect sizes corresponding to the within groups comparisons were calculated using the equation suggested by Cumming[194] (equation 8), which uses the average standard deviation of the paired data as the standardizer (d_{sav}). Lakens[195] also recommended this approach when reporting Cohen's *d*, in an effort to facilitate cumulative science (e.g., future meta-analysis, future *a priori* power analysis). Since *d* statistics are believed to overestimate the population effect size, Cumming[194] recommended that an unbiased Cohen's *d* (d_{unb}) also be provided. Accordingly, d_{unb} effect sizes were also calculated via equation 9. All Cohen's *d* effect sizes ($d_{sav,}$ d_{unb}), as well as corresponding 95% confidence intervals, were obtained using the Exploratory Software for Confidence Intervals (ESCI Free Software) described by Cumming.[194] It is important to note that ESCI is unable to calculate confidence intervals when d_{unb} is larger

than ±2, thus they were not provided in the event that this occurred. Cohen's *d* effect sizes were interpreted as follows: *d* = 0.20-0.49 small, *d* = 0.50-0.79 medium and *d* ≥ 0.80 large.[176]

[Equation 8]

$$d_{sav} = \frac{M_{diff}}{S_{av}}$$

Note. Where M_{diff} = the difference between the paired means, and S_{av} = the average standard deviation of the paired data. Equation based on Cumming[194] text.

[Equation 9]

$$d_{unb} = \left[1 - \left(\frac{3}{4df - 1}\right)\right] \times d$$

Note. Where *df* = the degrees of freedom associated with the standardizer used in the denominator of the utilized Cohen's *d* equation (which is N-1 because the average standard deviation of the paired data was used[194]), and $d = d_{sav}$. Equation based on Cumming[194] text.

Cohen's *f* effect sizes were calculated using G*Power software, which converted partial eta squared values provided by SPSS to Cohen's *f* values (the conversion was also verified using the equation provided in the literature).[176] Although eta squared effect sizes are often reported with respect to the omnibus results of an ANOVA, Cohen's *f* values were provided in an effort to maintain consistency with respect to the interpretation of the reported effect sizes. In addition, providing Cohen's *f* values facilitates future *a priori* power analyses, as this is the effect size required by G*Power. Cohen's *f* values were interpreted as follows: *f* = 0.10-0.24 small, *f* = 0.25-0.39 medium and *f* ≥ 0.40 large.[176]

Table 9

Manuscript 1 Research Questions and Related Analyses

Research Question	Independent Variable	Dependent Variable	Analysis	Post-hoc / Effect Size
1. While implementing a 10:50 on:off ratio and an initial training intensity of 30% MVIC, does the percent decline in MVIC torque differ between the c-NMES and m-NMES conditions?	Treatment condition (m-NMES vs. c-NMES)	Percent decline in MVIC	Dependent *t*-test† (one-tailed)	**NA / Cohen's *d* (d_{sav}, d_{unb})

Table 9 (continued).

Research Question	Independent Variable	Dependent Variable	Analysis	Post-hoc / Effect Size
2. While implementing a 10:50 on:off ratio and an initial training intensity of 30% MVIC, does the percent decline in NMES-induced torque differ between the c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?	Treatment condition (m-NMES vs. c-NMES) & time (repetition number)	Percent decline in peak NMES-induced torque	Two-way repeated measures ANOVA† (main effects = one-tailed; interaction = two-tailed)	Simple effects analysis OR pairwise comparisons with Bonferroni procedure / Cohen's *f*, Cohen's *d* (d_{sav}, d_{unb})

Table 9 (continued).

Research Question	Independent Variable	Dependent Variable	Analysis	Post-hoc / Effect Size
3. While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does the percent decline in TTI differ between c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?	Treatment condition (m-NMES vs. c-NMES) & time (repetition number)	Percent decline in TTI	Two-way repeated measures ANOVA† (main effects = one-tailed; interaction = two-tailed)	Simple effects analysis OR pairwise comparisons with Bonferroni procedure / Cohen's *f*, Cohen's *d*(d_{sav}, d_{unb})

Table 9 (continued).

Research Question	Independent Variable	Dependent Variable	Analysis	Post-hoc / Effect Size
4. While implementing a 10:50 on:off ratio and an initial training intensity of 30% MVIC, does the T-TTI differ between the c-NMES and m-NMES conditions?	Treatment condition (m-NMES vs. c-NMES)	T-TTI (Nm*s)	Dependent *t*-test (one-tailed)	** NA / Cohen's *d* (d_{sav}, d_{unb})

Table 9 (continued).

Research Question	Independent Variable	Dependent Variable	Analysis	Post-hoc / Effect Size
5. While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does self-reported discomfort (mm) differ between the c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?	Treatment condition (m-NMES vs. c-NMES) & time (repetition number)	Self-reported discomfort level (mm)	Two-way repeated measures ANOVA (main effects = one-tailed; interaction = two-tailed)	Simple effects analysis OR pairwise comparisons with Bonferroni procedure / Cohen's *f*, Cohen's *d* (d_{sav}, d_{unb})

Note. †Dependent *t*-tests were also performed to verify that a statistically significant difference did not occur with respect to the pre-test MVIC torque, initial NMES training intensity and TTI across the two conditions. ***Post-hoc* tests not applicable because dependent *t*-test compares only two means

Table 10

Manuscript 2 Research Questions and Related Analyses

Research Question	Independent Variable	Dependent Variable	Analysis	Post-hoc / Effect Size
1. Does the maximum comfortable stimulus intensity differ between the c-NMES and m-NMES conditions?	Treatment condition (m-NMES vs. c-NMES)	Maximum comfortable stimulus intensity (mA)	Dependent *t*-test† (one-tailed)	**NA / Cohen's *d* (d_{sav}, d_{unb})
2. Does the initial normalized NMES-induced torque significantly differ between the c-NMES and m-NMES conditions while using a maximum comfortable stimulus intensity?	Treatment condition (m-NMES vs. c-NMES)	Initial normalized NMES-induced torque (Nm/kg)	Dependent *t*-test (one-tailed)	**NA / Cohen's *d* (d_{sav}, d_{unb})

Table 10 (continued).

Research Question	Independent Variable	Dependent Variable	Analysis	Post-hoc / Effect Size
3. While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does the percent decline in MVIC torque differ between the c-NMES and m-NMES	Treatment condition (m-NMES vs. c-NMES)	Percent decline in MVIC	Dependent *t*-test* (one-tailed)	**NA / Cohen's *d* (d_{sav}, d_{unb})

Table 10 (continued).

Research Question	Independent Variable	Dependent Variable	Analysis	Post-hoc / Effect Size
4. While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does the percent decline in NMES-induced torque differ between the c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?	Treatment condition (m-NMES vs. c-NMES) & time (repetition number)	Percent decline in peak NMES-induced torque	Two-way repeated measures ANOVA (main effects = one-tailed; interaction = two-tailed)	Simple effects analysis OR pairwise comparisons with Bonferroni procedure / Cohen's *f*, Cohen's *d* (d_{sav}, d_{unb})

Table 10 (continued).

Research Question	Independent Variable	Dependent Variable	Analysis	Post-hoc / Effect Size
5. While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does the percent decline in TTI differ between c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?	Treatment condition (m-NMES vs. c-NMES) & time (repetition number)	Percent decline in TTI	Two-way repeated measures ANOVA (main effects = one-tailed; interaction = two-tailed)	Simple effects analysis OR pairwise comparisons with Bonferroni procedure / Cohen's *f*, Cohen's *d* (d_{sav}, d_{unb})

Table 10 (continued).

Research Question	Independent Variable	Dependent Variable	Analysis	Post-hoc / Effect Size
6. While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does the T-TTI differ between the c-NMES and m-NMES conditions?	Treatment condition (m-NMES vs. c-NMES)	T-TTI (Nm*s)	Dependent *t*-test (one-tailed)	**NA / Cohen's *d* (d_{sav}, d_{unb})
7. While implementing a 10:50 on:off ratio and a maximum comfortable stimulus intensity, does self-reported discomfort differ between the c-NMES and m-NMES conditions and over time? In addition, does the rate of change over time differ based upon NMES condition?	Treatment condition (m-NMES vs. c-NMES) & time (repetition number)	Self-reported discomfort level (mm)	Two-way repeated measures ANOVA (main effects = one-tailed; interaction = two-tailed)	Simple effects analysis OR pairwise comparisons with Bonferroni procedure / Cohen's *f*, Cohen's *d* (d_{sav}, d_{unb})

Note. †A dependent t-test was also performed to verify that a statistically significant difference did not occur with respect to the pre-test MVIC torque. ***Post-hoc* tests not applicable because dependent t-test compares only two means.

CHAPTER IV – STUDY 1 MANUSCRIPT

Abstract

Context

Multipath NMES (m-NMES) employs a novel multipath current distribution method that is marketed as having the potential to positively impact NMES outcome measures, such as NMES-induced fatigue and discomfort. Relative to conventional NMES (c-NMES), previous studies have reported some improved outcomes during m-NMES treatments. However, due to methodological limitations, the mechanisms by which m-NMES outperformed c-NMES remain unclear.

Objective

To compare the effects of m-NMES and c-NMES on fatigue and discomfort related outcomes.

Design

Single-blind counterbalanced cross-over study.

Setting

Research laboratory.

Patients or Other Participants

We included data from 14 recreationally active males that successfully completed all sessions.

Interventions

Participants performed NMES-induced contractions under two conditions: m-NMES and c-NMES. Eighteen contractions were completed during each condition.

Main Outcome Measure(s)

We measured percent decline in MVIC torque, percent decline in NMES-induced torque, percent decline in torque-time integral and total torque-time integral with an isokinetic dynamometer. Participants reported discomfort levels using a visual analog scale.

Results

Percent declines in MVIC, NMES-induced torque and torque-time integral were not significantly different across conditions ($P>0.05$). Self-reported discomfort levels did not significantly differ across conditions ($P>0.05$). The total torque-time integral was significantly greater under the c-NMES condition ($P>0.05$). Percent declines in NMES-induced torque and torque-time integral were significantly greater over time, while self-reported discomfort levels significantly decreased over time ($P<0.05$).

Conclusion

The novel multipath current distribution method did not positively impact fatigue related outcomes or self-reported discomfort. Therefore, it does not appear that the multipath current distribution method influenced the outcomes in a clinically meaningful manner.

Key Words

Multipath, NMES, quadriceps

Introduction

Several studies have demonstrated that neuromuscular electrical stimulation (NMES) may lead to increased quadriceps strength in healthy[7,8] and injured[9-11] populations. NMES training intensity, which is most often defined as the ratio of NMES-induced torque to torque produced during a maximum voluntary isometric contraction

(expressed as % MVIC),[5] is thought to be the primary determinant of the effectiveness of NMES treatments.[16,17] This belief is based on the established dose-response relationship, which indicates that NMES training intensity is positively related to strength gains.[7-10,18-20] Consequently, clinicians should maximize NMES training intensity to the extent possible,[17] but it is difficult to achieve and maintain a sufficient NMES training intensity due to a series of limitations; which include: muscle fatigue,[17,21-23] spatially limited motor unit recruitment[17,21,24] and patient discomfort associated with the electrical stimulus and subsequent involuntary contraction.[5,17,21,23-27] Strategies with the potential to minimize these limitations have been examined extensively.[28-33,36-39,42-45] Unfortunately, many of the techniques supported by empirical evidence cannot be easily incorporated or are inaccessible within clinical settings,[24] thus additional strategies are needed.[22]

The Kneehab® XP (Theragen LLC, Leesburg, VA) is an electrical stimulator that incorporates a novel clinically applicable strategy referred to as multipath™ technology. The stimulator is marketed to enhance motor unit recruitment via improved patient comfort and spatial distribution of the stimulus leading to stronger NMES-induced contractions, while also minimizing muscle fatigue.[84,88,92] Conventional NMES (c-NMES) devices transmit an electrical current from one electrode to another via a single fixed path, while the novel device transmits an electrical current with altered pulse durations between four large electrodes integrated within a neoprene thigh garment via two separate channels.[21,27,84,88,91] Due to its unique current distribution method this device has been referred to as multipath NMES (m-NMES), and it has gained a significant amount of attention in the literature.[21,27,84,86-90]

Feil et al.[87] observed greater quadriceps strength six weeks after an ACL repair in a group of patients receiving m-NMES treatments compared to a group receiving c-NMES treatments. However, these authors reported that the mechanisms by which m-NMES outperformed c-NMES during their study remain unclear. Two basic studies[21,27] were subsequently performed to determine if the mechanisms responsible for the outcomes observed by Feil et al. were the proposed benefits of the m-NMES device, and both of these studies reported some improved outcomes under the m-NMES condition. The m-NMES and c-NMES conditions during these studies differed substantially with respect to two distinct factors, current distribution method (e.g., multipath vs. single fixed path) and electrode size (e.g., large vs. small). Therefore, as acknowledged by the authors, these previous studies[21,27] were limited in their ability to determine if the novel multipath current distribution method was the primary mechanism responsible for the improved outcomes they observed while using the m-NMES device.

Evidence-based practice requires that clinicians incorporate current best evidence addressing the efficacy of therapeutic interventions, along with their clinical expertise, when making clinical decisions.[93] Therefore, scientific examination of commercially available modalities, such as the novel m-NMES device, is needed to provide evidence that can be used by clinicians when making decisions with respect to therapeutic interventions (e.g., which NMES device to purchase). Further investigation of the m-NMES device, with an emphasis on examining the influence of its novel current distribution method, is warranted in order to advance the evidence-based decision-making process with respect to NMES treatments. Therefore, the purpose of our study

was to compare the effects of m-NMES and c-NMES on fatigue related outcomes and discomfort.

Methods

Design

We performed a single-blind counterbalanced cross-over study with 2 independent variables (NMES condition at 2 levels: m-NMES and c-NMES; time at 17 or 18 levels: based on number of NMES-induced repetitions) and 5 dependent variables (percent decline in MVIC, percent decline in NMES-induced torque, percent decline in torque-time integral, total torque-time integral, self-reported discomfort). We assigned participants to one of two permutations designed to counterbalance the session order in which the c-NMES and m-NMES treatment conditions were performed (see Appendix A, Figure A1).

Participants

We determined a target sample size via *a priori* power analyses using G*Power software (version 3.1.9.2).[175] For each of the planned statistical analyses, we determined the number of participants required to maintain adequate power ($1\text{-}\beta = 0.80$) and detect a medium to large effect size ($d = 0.650$, $f = 0.325$).[176] We selected a medium to large effect size as we believe that any statistically significant differences with small to medium effect sizes would lack clinical relevance with respect to the outcomes of our study. It is also important to note that other authors have selected a large effect size (e.g., Cohen's $f = 0.68$) for an *a priori* power analysis during a NMES study examining similar dependent variables,[37] which further supports our selection of a medium to large effect size as appropriate for this study. The dependent *t*-test power analysis revealed a target

sample size requirement of 17 participants, while only 12 participants were required for the two-way repeated measures ANOVA.

A convenience sample of 21 participants from the university and community completed all four study sessions. Although we initially exceeded our target sample of 17 participants, we determined that seven of the participants' data needed to be excluded due to a unique limitation of the c-NMES unit (discussed in detail in procedures section). As a result of this limitation our final sample consisted of 14 participants (age = 23.7 ± 4.8 years, height = 175.3 ± 6.4 cm, mass = 78.7 ± 11.6 kg, BMI = 25.4 ± 2.8 kg/m^2).

To be included participants were required to be healthy, recreationally active, males, between the ages of 18-35. We defined healthy as having no unresolved knee injuries or other injuries that would impact lower-limb function, as well as being free of all applicable electrotherapy contraindications. We defined recreationally active as participation in some form of physical activity (e.g. strengthening activities, jogging, running, cycling, swimming, tennis, etc.) for a minimum of two times per week for at least 20 minutes each time. NMES tolerance and motor thresholds have been shown to differ between individuals with a body mass index (BMI) above and below 30 kg/m^2,[160] thus participants also had to have a BMI ≤30 kg/m^2 to be included. We chose to exclude females from participation because electrically-induced discomfort levels have been shown to significantly differ over the course of the menstrual cycle.[162] This study was approved by the University's institutional review board, and participants provided written informed consent. To facilitate participant recruitment, we incentivized participants via a lottery for a chance to win one of four $50 gift cards.

Instrumentation

We used a Quickset 4 Biodex dynamometer (Biodex Medical Systems Inc., Shirley, New York) to measure and record isometric knee extension torque during all voluntary and NMES-induced contractions, at a sampling rate of 100 Hz. Participants removed their shoe from the dominant foot prior to being seated on the dynamometer. During all contractions participants were seated in the dynamometer chair with the seat back tilt at 85° and the dominant leg secured within a lever arm fixed at 60°. We aligned the axis of rotation of the dynamometer to the anatomical axis of the test knee, and the lower leg was secured in the fixed lever arm via an ankle strap placed 2-3 cm above the lateral malleolus.[27] We calibrated the dynamometer to the manufacturer's specifications prior to beginning the study to ensure reliable measurements. In addition, we used stabilization straps to prevent undesired movement of the upper body and asked participants to cross their arms over their chest while performing all voluntary and NMES-induced contractions (Figure 4).[27,178]

We applied all c-NMES treatments using the same Sonicator® Plus 940 stimulator (Mettler Electronics® Corp., Anaheim, CA). To maintain consistency across the two NMES conditions, we set the c-NMES parameters as similar as possible to the parameters used with the Kneehab® XP program 6 (Table 5). Although our sample size was negatively impacted by an unforeseen feature of the Sonicator® Plus 940, it is important to note that we selected this stimulator because it allowed us to more closely match the parameters of the m-NMES device.

We used four self-adherent electrodes to deliver the c-NMES current (two- 5 cm x 9 cm [Metron™, Bolingbrook, IL], one- 10.79 cm x 17.78 cm [TENS Products, Grand

Lake, CO], one- 7 cm x 14 cm electrode [SME INC., Wilmington, NC]; Figure 5). To guide the placement of the c-NMES electrodes, we manually identified motor points using a pencil electrode (Mettler Electronics XK2, Active Forever, Scottsdale, AZ) following the procedures outlined in the literature (Figure 7).[24] Based on the results of a recent study, which identified seven motor points of the quadriceps,[153] we selected four commonly identified motor points to guide the c-NMES electrode placement. Furthermore, the motor points we selected allowed us to place the c-NMES electrodes in a similar fashion to the m-NMES electrode configuration, as they were located on the proximal and distal vastus lateralis, proximal rectus femoris and distal vastus medialis (Figure 7).

We applied all m-NMES treatments using the same Kneehab® XP stimulator (Theragen LLC, Leesburg, VA), however we assigned each participant a separate Kneehab® XP garment with integrated electrodes. We integrated the m-NMES electrodes into the neoprene garment and subsequently placed the garment on the dominant thigh according to the manufacturer's recommendations (Figure 9).[167] We set the stimulator parameters to program 6 during all m-NMES treatments (Table 5).

Procedures

Participants reported at the same time of day (±2 hours) on four separate occasions and each session lasted approximately 1 hour. Each participant's dominant leg, which we defined as the leg with which they would use to kick a soccer ball, served as the leg of interest throughout the study (13 right, 1 left). In an effort to reduce electrical impedance, participants shaved their anterior thigh each day. We also instructed

participants to report well hydrated and to refrain from strenuous activities for 12 hours prior to reporting.

The first two sessions served as familiarization sessions and were separated by 24-48 hours, while the last two sessions served as test sessions and were separated by 48-72 hours. Participants began each session by completing a standardized warm-up that included: 5 minutes of cycling on a stationary bike at a self-selected pace, three 30 second bouts of dynamic quadriceps stretching and four isometric quadriceps contractions while in the dynamometer chair (2 at 50%, 1 at 75% and 1 maximum contraction at 60° of knee flexion).[36] Participants rested for 8 minutes following the warm-up, during which we identified the motor points using the pencil electrode method and cleaned the leg of interest with an alcohol free wipe. Although motor point identification was not necessary for the m-NMES condition because the electrodes were integrated within the garment, we still identified motor points during all sessions in an effort to blind participants to treatment condition.

Participants performed maximum voluntary isometric contractions (MVICs) of the quadriceps and all MVICs were 6 seconds in duration. Participants gradually increased their effort during the initial portion of each contraction, with maximal effort being reached at roughly 3 seconds and maintained for the remaining 3 seconds. To facilitate participant understanding of a gradual increase, we placed a figure showing an ideal MVIC curve in the participants' view while performing all MVICs. We instructed the participants by saying "cross your arms and prepare to push out with maximal effort". Based on the recommendations provided in the literature,[98] we instructed participants on the MVIC procedures and gave them an opportunity to practice during the initial

familiarization session. In addition, the dynamometer computer screen provided participants with real-time visual feedback of their torque production and we provided verbal encouragement throughout each repetition (Figure 4).[98] We encouraged participants to "push out with maximal effort" in a loud clear voice, and in the event that a participant showed signs of fatigue we gradually increased the volume and intensity.[179] We asked the participants to verify that their effort was maximal immediately following each repetition.[98] In the event that a participant reported a submaximal effort, or we judged an effort to be submaximal, the repetition was discarded and repeated.

We allowed participants to practice performing MVICs at a self-selected pace during the initial familiarization session until they reported being comfortable with the procedures, but a minimum of six practice trials was required. Participants performed standardized pre-test MVIC procedures during the second familiarization session and all test sessions. These procedures consisted of a series of three MVICs, with each repetition separated by a 2 minute rest period.[31,33] During the test sessions (day 3 and day 4), the peak torque of the three pre-test trials was required to be within 10% or we asked participants to perform additional trials until three consecutive MVIC trials were within 10%.[27] To limit the possibility of fatigue as a confounding variable, we gave participants a maximum of six contractions during each test session. If a participant was unsuccessful in completing the pre-test MVIC procedures, we asked them to return the following day for a second attempt. We defined the trial with the greatest peak torque, from the three consecutive trials within 10%, as the participant's pre-test MVIC value for that particular test session.

Although the standardized pre-test MVIC procedures were followed during the second familiarization session (day 2), this was done for the purpose of allowing the participant to further familiarize themselves with the MVIC procedures. The peak torque observed during these repetitions only served as a reference value when verifying that each participant was able to tolerate an adequate stimulus intensity producing 30% MVIC. Consequently, three consecutive MVICs within 10% of one another during the second familiarization session was not required for further participation, but we gave participants an opportunity to perform up to six contractions in the event that three consecutive MVICs were not within 10%.

Participants rested for 5 minute prior to performing the NMES procedures, during which we placed the Kneehab® XP garment with integrated electrodes or the c-NMES electrodes over the participant's dominant thigh. We also placed an empty Kneehab® XP garment over the c-NMES electrodes in an effort to blind participants to treatment condition (Figure 9).[27] Participants were exposed to both forms of NMES (c-NMES and m-NMES) during the two familiarization sessions by performing 10 NMES-induced quadriceps contractions using each NMES device (total of 20 contractions). The NMES parameters we used during the familiarization sessions mirrored those used during the test sessions, with the exception that we allowed participants to control the stimulus intensity throughout the familiarization sessions. During previous studies in our laboratory we have observed that participants are willing to use greater stimulus intensities if they are allowed to control the intensity themselves. Thus, during all familiarization sessions we placed the stimulator controls in a manner that allowed participants to control the amount of stimulus intensity used during all NMES-induced

contractions. We anticipated that participants would tolerate greater intensities as they became acclimated to the stimulus,[144] thus we encouraged participants to increase the stimulus intensity between contractions as well as between familiarization sessions to maintain a maximum comfortable stimulus level during each contraction (e.g., highest stimulus intensity that does not cause pain).[77]

The NMES procedures for the second familiarization day were similar to the first, with the exception that the devices were used in a reverse order from the pattern used during the initial session. Participants were required to tolerate a stimulus intensity sufficient to produce a NMES-induced contraction of 30% MVIC during the subsequent test sessions (day 3 and day 4). To evaluate whether a participant was capable of tolerating the required stimulus intensity, we identified the greatest peak torque observed over the 10 contractions of each condition during the second familiarization session. We expressed the NMES-induced peak torque observed under each NMES condition as a percentage of the participant's pre-test MVIC peak torque measured earlier during the second familiarization session (equation 1). If a participant was unable to tolerate a stimulus intensity sufficient to produce a NMES training intensity ≥30% MVIC, we excluded them from further participation.

The NMES procedures during the two test sessions were similar to the familiarization sessions, with the following exceptions: participants only performed 18 NMES-induced contractions using a single NMES device (c-NMES or m-NMES) during each test session; we standardized the stimulus intensity using a target training intensity of 30% MVIC and did not manipulate it within each test session; and participants

performed post-test MVIC procedures immediately following the NMES-induced contractions during each test session.

Prior to beginning each test session's NMES condition we determined the stimulus intensity required to produce a target torque output of 30% MVIC, which we selected because it falls within the therapeutic window identified in the literature.[144] We increased the stimulus intensity until the targeted torque output of 30% of the test session's pre-test MVIC was reached. If a participant reported reaching a maximum comfortable intensity prior to this point we immediately decreased the intensity and the participant recovered for 30 seconds. We repeated this process a maximum of three times and if the participant was unable to tolerate a sufficient stimulus intensity by the third attempt we excluded them from further participation. After 50 seconds of rest participants completed the assigned NMES condition, and we frequently encouraged participants to "relax and allow the machine to do all the work" during all NMES-induced contractions. We did not manipulate the initial stimulus intensity used during the test sessions over the course of each NMES treatment condition. However, the c-NMES device we used has an obscure setting that may automatically reduce the stimulus output when the unit senses a change in impedance over the course of the NMES-induced contractions.[177] As expected, we observed that the outcome measures were influenced when this occurred, ultimately resulting in an undesired systematic bias in c-NMES test sessions during which the stimulus output was automatically reduced by the unit. Consequently, when this automatic step-down in output occurred we excluded the negatively influenced data from the statistical analyses.

Immediately after completing the assigned NMES treatment condition participants performed a single 6 second post-test MVIC. We elected to use a single repetition in an effort to limit recovery from the NMES-induced contractions. However, in the event that we deemed the post-test trial to be submaximal, or the participant deemed it to be submaximal, a second MVIC was performed after a 20 second rest period in an effort to continue limiting recovery.[178]

Outcome Measures

Percent Decline in MVIC Torque

A change in peak MVIC torque is considered to be the gold standard for assessing fatigue.[105,110] Therefore, we expressed each participant's post-test MVIC peak torque as a percent decline relative to their pre-test MVIC peak torque (equation 2), and the subsequent percent decline served as a fatigue related outcome measure for our study.

Percent Decline in NMES-induced Torque

Failing to maintain a target force is another common method used to asses fatigue,[110] thus NMES-induced fatigue is also frequently assessed by measuring the decline in NMES-induced torque over the course of a treatment.[21,27,30-33,46,49,51,54,55,59,61,62,64,66,100] Accordingly, we expressed the peak torque produced during all 18 NMES-induced contractions for each test session as a percent decline relative to the peak torque produced during the initial NMES-induced contraction of each test session (equation 3)

Percent Decline in Torque-time Integral

A decline in the torque-time integral (TTI) observed during NMES-induced contractions has also been used in a number of studies as an index of NMES-induced

fatigue, as it has been suggested to represent isometric work.[23,29,47,115,116] Therefore, the data necessary to calculate the TTI (e.g., torque and duration of torque recording; expressed as Newton-meter seconds [Nm*s]) was measured and recorded during the NMES-induced contractions and subsequently exported as a text file (sampling rate = 100 Hz, or one sample taken every 10 ms). We imported the data files into an analysis software package (Acqknowledge® 4, Biopac® Systems, Inc., Goleta, CA), which we used to calculate the TTI of each contraction (equation 4). We expressed the TTI of each NMES-induced contraction during the test sessions as a percent decline (equation 5), which also served as an outcome measure of NMES-induced fatigue.

Total Torque-time Integral

We considered the total torque-time integral (T-TTI) to be an index of the total amount of isometric work performed under each condition, and as such it also represented a fatigue related outcome. We calculated the T-TTI for each condition by summing individual TTI data (equation 6).

Self-reported Discomfort

We used a 100 mm horizontal visual analog scale (VAS) to measure self-reported discomfort levels during each NMES condition. As is common during NMES studies, the descriptors at each end of the scale were "no discomfort" (0 mm) and "worst possible discomfort" (100 mm).[21,27,29,36,37,159,161] We gave the participants a pen and asked them to "rate your level of discomfort by making a vertical tick mark on the line" following each NMES induced contraction (Figure 8). We obtained self-reported discomfort levels by measuring the distance (mm) from the "no discomfort" anchor to the vertical mark made on the horizontal line. When used to assess NMES-induced discomfort in a sample of

healthy individuals, the VAS has been shown to have a high inter-session test-retest reliability (intraclass correlation coefficient [*ICC*] ≥0.90).[159]

Statistical Analysis

We analyzed the data using the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corporation, Armonk, NY). We performed a series of two-way repeated measures analysis of variance (ANOVA) on three of the outcome measures (percent decline in NMES-induced torque, percent decline in TTI, and self-reported discomfort). In the event that the assumption of sphericity was determined to be violated, we followed the Greenhouse-Geisser procedure for correcting degrees of freedom. In the event of a significant time main effect, we performed *post-hoc* pairwise comparisons using a Bonferroni procedure to maintain family-wise error rate. Due to the number of NMES-induced contractions, we performed a large number of *post-hoc* pairwise comparisons (e.g., >130). To simplify the results, we provided only the significant pairwise comparisons deemed to be clinically important (e.g., first contraction to demonstrate a significant decline relative to the second contraction, and final contraction).

We performed a dependent *t*-test on the other outcome measures (percent decline in MVIC, T-TTI). In addition, we performed dependent *t*-tests to examine any potential baseline differences between the two conditions with respect to pre-test MVIC, initial NMES training intensity (% MVIC), and initial TTI. We also calculated test-retest reliability ($ICC_{(2,1)}$)[189,190] and measurement precision (SEM)[191] estimates for pre-test MVIC measurements using the equations provided in the literature.

To examine the magnitude of the differences, we calculated Cohen's *f* and *d* effect sizes.[176] We calculated Cohen's *d* effect sizes corresponding to within groups comparisons using the equation suggested by Cumming[194] (equation 8), which uses the average standard deviation of the paired data as the standardizer (d_{sav}). Since *d* statistics are believed to overestimate the population effect size, Cumming[194] recommended that an unbiased Cohen's *d* (d_{unb}) also be provided. Accordingly, we calculated d_{unb} values using the equation provided by Cumming[194] (equation 9). We interpreted Cohen's *f* values as follows: f = 0.10-0.24 small, f = 0.25-0.39 medium and $f \geq 0.40$ large; whereas we interpreted Cohen's *d* effect sizes as follows: d = 0.20-0.49 small, d = 0.50-0.79 medium and $d \geq 0.80$ large.[176]

Results

Prior to analyzing the data, we assessed the tenability of the applicable statistical assumptions. We defined outliers as any raw score with a corresponding *z*-score >2.5.[188] For outliers, we transformed the score by changing the original raw score to a value ±1 unit larger than the next highest value, as has been recommended because this allows the case to remain deviant while also reducing the impact of an outlier.[186] There was a small amount of missing data (<1%), which we replaced using the corresponding group mean.[186] We assessed the normality of the data via skewness and kurtosis *z*-scores, and the data were considered to be normally distributed. There were no significant differences with respect to baseline measurements across the two conditions (Table 11), and the test-retest reliability and measurement precision estimates of the pre-test MVICs were within acceptable limits ($ICC_{(2,1)}$ = 0.957; 95% CI: 0.855, 0.983; SEM = 13.35

Nm).[184] Furthermore, these values are similar to those reported in the literature when assessing healthy populations.[181,183]

Table 11

Baseline Comparisons Across Conditions

	NMES condition			
Variable	m-NMES	c-NMES	*P* Value	*d*
Pre-test MVIC (Nm)	221.9 ± 56.5	217.021 ± 66.1	0.420	0.079
Initial NMES Training Intensity (% MVIC)	31.0 ± 6.0	32.1 ± 5.0	0.653	-0.17
Initial TTI (Nm*s)	541.2 ± 146.8	653.5 ± 260.4	0.122	-0.531

Note. Values represent mean ± 1SD.

Percent Decline in MVIC Torque

The dependent *t*-test revealed that the percent decline in MVIC torque following the NMES treatments was not significantly different across conditions (t_{13} =1.086; P = 0.149; d = 0.310; 95% CI for effect size: -0.276, 0.884; d_{unb} = 0.292; Figure 11)

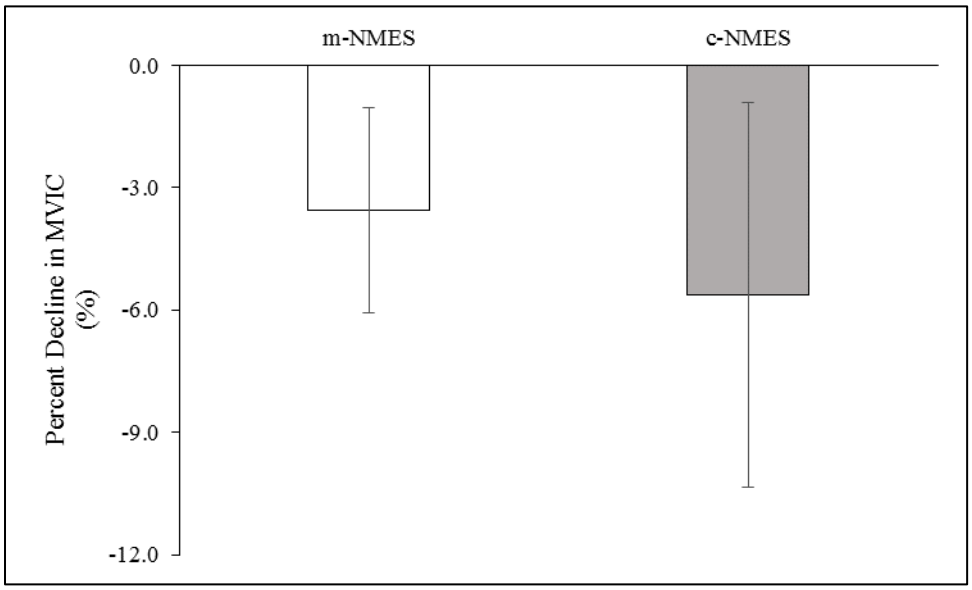


Figure 11. Percent Decline in MVIC Torque

Note. Error bars indicate 95% confidence intervals calculated using a critical *t*-value as has been recommended.[194]

Percent Decline in NMES-induced Torque

For the percent decline in NMES-induced torque, the repeated-measures ANOVA revealed no significant condition by time interaction ($F_{2.6, 34}$ = 0.849; P = 0.464; f = 0.255) or condition main effect ($F_{1, 13}$ = 0.052; P = 0.411; f = 0.063). However, there was a significant time main effect ($F_{2.9, 38.1}$ = 192.156; P<0.001; f = 3.857; Figure 12). *Post-hoc* analysis revealed that the decline was significantly greater by the sixth contraction (difference = 10.9 ± 7.5%; P<0.001; d =1.559; 95% CI for effect size: 0.978, 2.125; d_{unb} = 1.515) and it remained significantly greater for each of the subsequent contractions (18th contraction, difference = 54.0 ± 13.7%; P<0.001; d = 5.004; d_{unb} = 4.863)

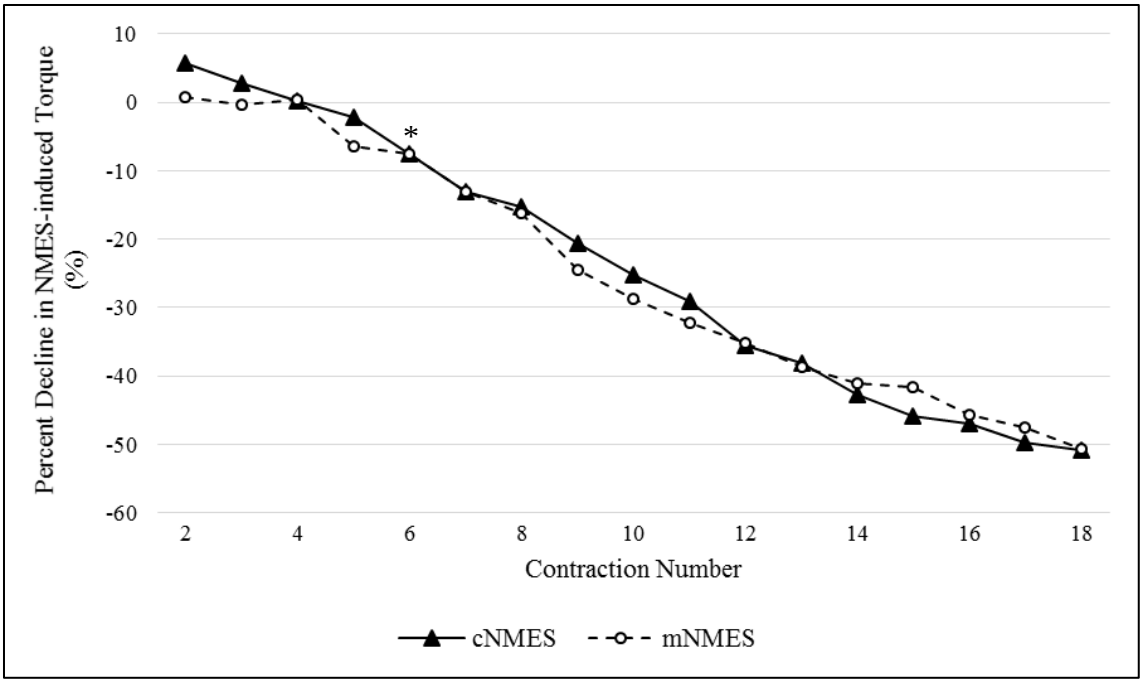


Figure 12. Percent Decline in NMES-induced Torque

Note. *First contraction with a significantly greater decline relative to contraction 2 (P<0.001).

Percent Decline in Torque-time Integral

For the percent decline in TTI, the repeated-measures ANOVA revealed no significant condition by time interaction ($F_{2.4, 30.6}$ = 1.223; P = 0.313; f = 0.306) or condition main effect ($F_{1, 13}$ = 0.182; P = 0.338; f = 0.119). However, there was a significant time main effect ($F_{2.7, 34.8}$ = 276.330, P<0.001; f = 4.607; Figure 13). *Post-hoc* analysis revealed that the decline was significantly greater by the fifth contraction (difference = 9.04±7.7%; P =0.004; d =1.123; 95% CI for effect size: 0.654, 1.579; d_{unb} = 1.091) and it remained significantly greater for each of the subsequent contractions (18th contraction, difference = 59.0±14.0%; P<0.001; d = 5.48; d_{unb} = 5.326).

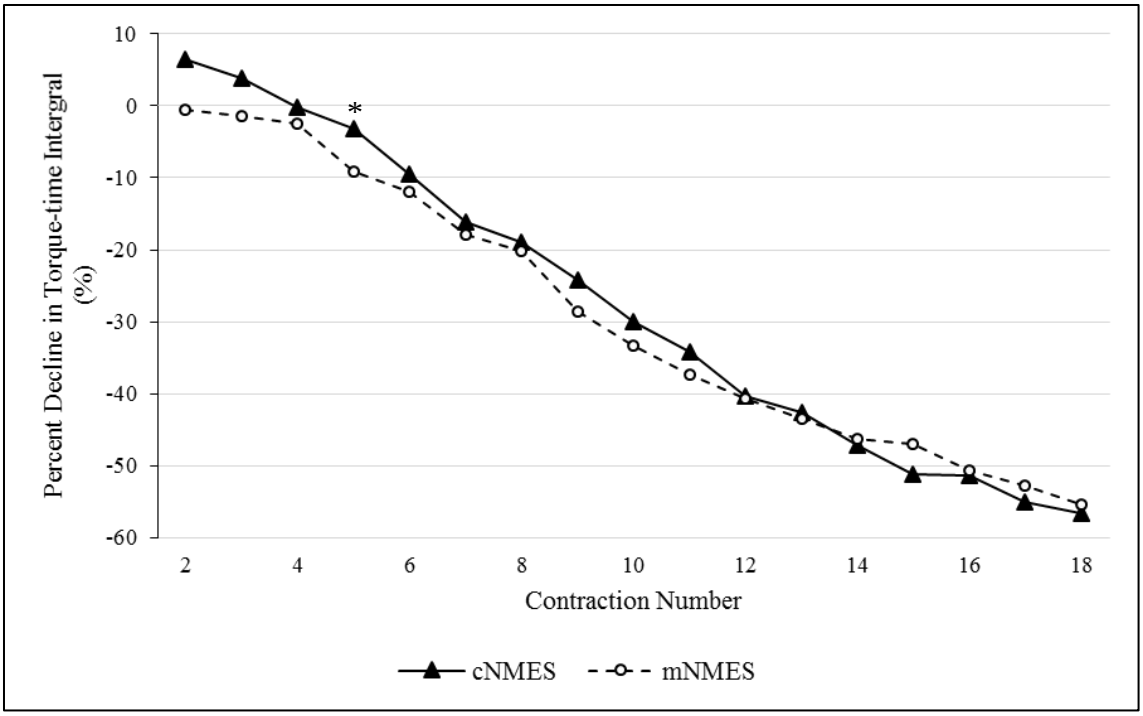


Figure 13. Percent Decline in Torque-time Integral

Note. *First contraction with a significantly greater decline relative to contraction 2 (P=0.004).

Total Torque-time Integral

The dependent *t*-test revealed that the T-TTI was significantly greater during the c-NMES condition (t_{13} = -2.068, P =0.0295; d = -0.391; 95% CI for effect size: -0.783, 0.015; d_{unb} = -0.368; Figure 14).

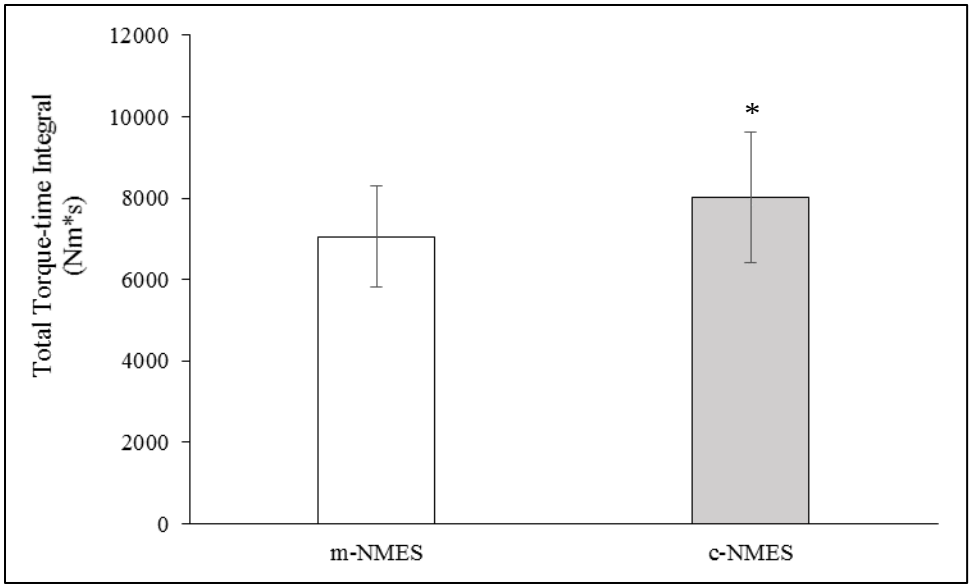


Figure 14. Total Torque-time Integral

Note. *Significantly greater T-TTI (P = 0.0295). Error bars indicate 95% confidence intervals calculated using a critical t-value as has been recommended.[194]

Self-reported Discomfort

The repeated-measures ANOVA revealed that there was no significant condition by time interaction ($F_{2.7, 35.7}$ = 0.963; P = 0.415; f = 0.272) or condition main effect ($F_{1, 13}$ = 0.419; P = 0.265; f = 0.179) for self-reported discomfort levels. However, there was a significant time main effect ($F_{1.92, 25}$ = 3.60; P = 0.022; f = 0.526; Figure 15), but *post-hoc* analyses did not reveal any significant differences (P >0.05).

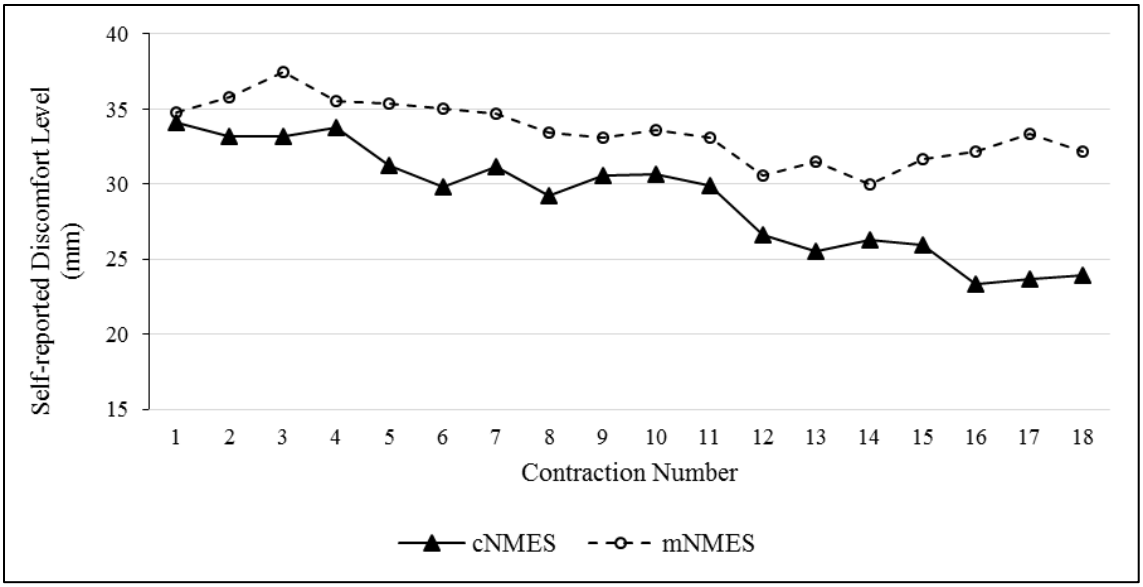


Figure 15. Self-reported Discomfort

Discussion

Overall, while using similar stimulus parameters and electrode configurations, the findings of our study indicate that m-NMES was not significantly better on any of the outcome measures when compared to c-NMES. To the best of our knowledge m-NMES and c-NMES have not been previously compared while implementing similar electrode configurations. Therefore, we believe these findings are important because our approach allowed us to better isolate the influence of the novel multipath current distribution method on fatigue and discomfort related outcomes.

The findings of our study also indicate that some of the outcome measures significantly changed over time, irrespective of the stimulator. The decline in NMES-induced torque and decline in the TTI were significantly greater over time (Figure 12 & 13), while self-reported discomfort levels significantly decreased over time (Figure 15). We believe these findings are also important as they illustrate the need for additional

strategies to prevent the decline in NMES-induced torque in order to maintain a sufficient NMES training intensity over the course of a treatment.

Studies examining NMES-induced fatigue of the quadriceps, while using a variety of stimulus parameters, have reported declines in MVIC ranging from 7-33%.[21,27,30,99,104,121,145] For the m-NMES and c-NMES conditions in our study we observed declines of only 3.6 ± 4.4% and 5.6 ± 8.2%, respectively. It is unclear why the declines in MVIC we observed are lower than the values reported in the aforementioned studies, but it may be attributable to a variety of methodological differences (e.g., total number of NMES-induced contractions, duration of contractions, rest time, initial NMES training intensity). Despite the fact that the declines in MVIC we observed were lower than expected, our observation that the declines were not significantly different between the two NMES conditions is in agreement with the results of Maffiuletti et al.[21]; but contradicts the results of Morf et al.[27] One possible explanation is that Maffiuletti et al. also used healthy participants while Morf et al. used individuals who had recently undergone a knee replacement.

Although the results for decline in MVIC of our study and those of Maffiuletti et al.[21] are in direct contrast to those reported by Morf et al.[27], the mean differences observed between the NMES conditions and corresponding effect sizes are similar across all three studies. While reporting a statistically significant difference, Morf et al. observed a mean difference between the two NMES conditions of only 3.0%, which is similar to the non-significant 2.6% difference observed by Maffiuletti et al. and the 2.1% non-significant difference we observed. Corresponding Cohen's *d* effect sizes for each study were considered to be small because they were 0.383, 0.311 and 0.310,

respectively. Since all three studies observed similar small mean differences between the two conditions, as well as small corresponding effect sizes, we believe the more likely explanation for the inconsistent statistical results is the difference in sample size across the three studies; which were $n = 20$, $n = 10$ and $n = 14$, respectively. Furthermore, the comparable small effect sizes consistently observed across each of these studies suggests, irrespective of the presence or absence of statistical significance, that m-NMES does not reduce the decline in MVIC in a clinically meaningful way. Although, with a large enough sample statistically significant differences may be observed.[27]

Others have observed declines in NMES-induced torque ranging from 8-61% while using a variety of treatment parameters.[21,27,31,46,51,61,62,99] Our observation that NMES-induced torque significantly declined over the course of each treatment condition is in agreement with these previous studies. Likewise, our observation that the decline in NMES-induced torque was not significantly different between the two NMES conditions is in agreement with the two earlier studies comparing c-NMES and m-NMES.[21,27] Morf et al.[27] hypothesized that they did not observe a significant difference between conditions due to their use of short contraction durations and a low target training intensity, which may have limited potential differences between the two conditions. Consequently, we chose to include a longer contraction duration (10 seconds) and higher target training intensity (30% MVIC), but we observed a similar result for decline in NMES-induced torque so our results do not appear to support their hypothesis.

In addition to longer contraction durations, we implemented a longer rest period than earlier studies.[21,27] We elected to use an on:off ratio of 10:50 rather than 5:10 because a meta-analysis[6] addressing NMES efficacy for quadriceps strengthening

revealed that a 10:50 ratio was used most often by the included randomized controlled trials. Despite implementing a longer rest period between contractions, which has been shown to reduce the decline in NMES-induced torque,[61,62] we observed a mean decline in NMES-induced torque of roughly 50% under each condition. This was much greater than the declines of roughly 20-25% observed by Maffiuletti et al.[21] and Morf et al.[27]. The declines we observed were likely larger because we implemented a higher initial target training intensity and our NMES-induced contractions were twice the duration.

Declines in NMES-induced torque over the course of a treatment pose a significant clinical problem because this subsequently reduces the NMES training intensity, which is considered to be the primary determinant of NMES treatment efficacy.[16,17] Therefore, we believe that the substantial difference in the declines in NMES-induced torque we observed versus those reported during the two earlier studies[21,27] warrants further investigation. The smaller percent declines observed during the two earlier studies may indicate that shorter contraction durations result in smaller percent declines in NMES-induced torque, but because our target training intensity was higher and our rest intervals were longer it is difficult to directly compare our results to those of earlier studies. To the best of our knowledge the decline in NMES-induced torque while implementing different contraction durations has yet to be examined. We believe differences in contraction duration warrant further examination, particularly because the previously mentioned training study[87] reporting improved patient outcomes while using m-NMES implemented on:off ratios of 10:20 and 5:10 for the c-NMES and m-NMES conditions, respectively. Therefore, different contraction durations may have

been a confounding factor, but due to a lack of research in this area the extent to which different contraction durations influenced these results remains unclear.

Declines in NMES-induced torque are often used as a measure of muscle fatigue,[21,27,31,46,51,59,61,62] but caution should be exercised while interpreting these results since declines in NMES-induced torque may be a combination of muscle fatigue and accommodation of the motor nerves.[99,100] Alon and Smith[144] defined accommodation as the transient process by which the threshold required to excite the nerve increases in response to the electrical stimulus. Accommodation has been suggested as a contributing factor to declines in NMES-induced torque output because an increased nerve threshold has the potential to result in a diminished number of recruited motor units.[28,77,100] We did not directly measure accommodation in our study, thus we are unable to confidently differentiate between muscle fatigue and accommodation. However, we did observe two interesting patterns that warrant discussion.

We observed a much larger decline in NMES-induced torque (roughly 50%) relative to the decline in MVIC (roughly 5%), irrespective of the NMES condition. A similar pattern has also been observed in recent studies attempting to examine accommodation.[99,100] Matkowski et al.[99] hypothesized that their observed pattern of a larger decline in NMES-induced torque relative to a much smaller decline in MVIC, which we also observed in our study, is primarily attributable to accommodation occurring during the NMES-induced contractions. In addition to declines in NMES-induced torque, we also observed a significant decline in self-reported discomfort over the course of the treatments (Figure 15). Randolph et al.[145] also observed a decline in discomfort which can be attributed to accommodation in sensory nerves, but the authors

also hypothesized that accommodation affected motor nerves therefore contributing to their observed declines in NMES-induced torque. Although we are unable to definitively determine the relative contributions of accommodation and fatigue, based on our observed pattern of a smaller decline in MVIC relative to the decline in NMES-induced torque, as well as a corresponding decline in self-reported discomfort, we hypothesize that accommodation was the primary contributing factor to the large declines in NMES-induced torque.[99,100,145]

We achieved our target training intensity of 30% MVIC, but due to the large declines in NMES-induced torque the mean NMES training intensity was only 15.2 ± 4.6% MVIC during the final contraction; which is well below the therapeutic window of 25-50% MVIC .[144] The low NMES training intensities of the final NMES-induced contractions may offer an additional explanation for the observed minimal decreases in post-test MVIC torque. Since participants were producing very little torque during the final NMES-induced contractions their quadriceps were not heavily taxed during these contractions, which may have allowed them to fully recover from earlier more intense contractions prior to performing post-test MVICs. This observation, as well as our hypothesis that accommodation was the primary contributing factor to the large declines in NMES-induced torque that we observed, highlights a need for researchers to develop clinically applicable strategies focused on combating accommodation rather than muscle fatigue. One such strategy that has been successfully used is systematically increasing the stimulus intensity over the course of the treatment.[44]

Relative to others who have compared m-NMES and c-NMES,[21,27] our study is unique in that we also compared TTI data between the two conditions. Due to a lack of

similar comparisons in the literature it is difficult to compare the declines in TTI that we observed to earlier studies. However, a previous NMES study has observed a different TTI response when comparing two NMES conditions.[29] The declines we observed in TTI mirrored the declines we observed in NMES-induced torque, as the decline in the TTI was not significantly different between the two conditions but it was significantly greater over time. The decline in TTI reached 55.4 ± 15.4% and 56.7 ± 12.9% during the 18th contraction for m-NMES and c-NMES, respectively. Since the declines in TTI mirrored the declines in NMES-induced torque during our study, including TTI comparisons may seem repetitive. However, we elected to include TTI comparisons because it has been suggested as a better determinant of fatigue during longer duration NMES-induced contractions like those performed during our study.[47] We also felt that it was important to examine the decline in TTI because peak torque alone does not provide an adequate summary of the entire 10 second contraction. For example, it is plausible that similar peak torque values could be observed during two contractions, but if the amount of time during which the contraction is held at or near peak torque differed substantially then the TTI of each contraction would differ.

We also examined the T-TTI, which is representative of the total amount of isometric work performed under each condition. Despite non-significant baseline differences with respect to the initial NMES training intensity or initial TTI, as well as similar declines in NMES-induced torque and in TTI during each condition, the T-TTI was significantly greater during the c-NMES condition. However, it is important to acknowledge that this difference reached statistical significance due to our use of a one-tailed test, but was contrary to our hypothesized direction. This may be due to an

observed mean torque recording duration of 8.6 ± 0.2 seconds during the m-NMES condition while the mean duration of the torque recording during c-NMES was 10.3 ± 0.2 seconds. Since each device used similar "on" times this was unexpected. The isokentic dynamometer is only capable of recording torque when the contraction intensity is sufficient to overcome the force of gravity and cause the lower leg to push against the fixed lever arm. Therefore, it appears that the amount of time the NMES-induced contractions exceeded gravity during the m-NMES condition was roughly 1.7 seconds shorter than during c-NMES. This difference may be attributable to the multipath current distribution method of the m-NMES device. Research into NMES has primarily focused on NMES-induced peak torque, which influences the total amount of work performed during a particular session but is not the only determining factor of isometric work. To the best of our knowledge there are no published studies that have examined the impact that the amount of work done during NMES sessions has on the effectiveness of the treatments, thus we believe that future research in this area is warranted.

Our observation that self-reported discomfort levels were similar across the two NMES conditions is contrary to the results of the earlier studies comparing m-NMES and c-NMES.[21,27] Morf et al.[27] observed that VAS scores during c-NMES were 39% higher over the course of their treatment, and Maffiuletti et al. observed a similar 35% difference prior to the 20th contraction. We believe this discrepancy in our results was due to the similarly sized electrodes that we used across the two conditions. Both Morf et al. and Maffiuletti et al. acknowledged that using different electrode configurations during the NMES conditions limited their ability to attribute the improved outcomes observed during the m-NMES condition to the novel multipath current distribution method.

Therefore, we used similarly sized electrodes during each condition in our study. The c-NMES electrodes covered a surface area of roughly 360 cm^2, whereas the m-NMES electrodes covered an area of 427 cm^2 (Figure 6).[27] Although there was a small difference in the area covered by c-NMES and m-NMES, our electrodes were more similar than the electrode configuration used during the c-NMES condition of the previous studies; which consisted of three electrodes covering only 100 cm^2.[21,27]

Although we observed a significant time main effect indicating a decline in self-reported discomfort levels over the course of the NMES treatment conditions, we did not observe any statistically significant *post-hoc* pairwise comparisons. This likely occurred because of our use of the conservative Bonferroni correction to maintain family-wise error rate. The largest mean differences we observed during the pairwise comparisons were minimal, ranging from 6.4-7.6 mm. During a previous NMES study performed in our laboratory we defined a 13 mm threshold for determining clinically significant differences with respect to self-reported discomfort levels.[36] Therefore, we do not believe that the mean differences we observed during the pairwise comparisons represented clinically significant differences, which is in agreement with our non-significant findings during the *post-hoc* analyses.

Although outcome measures for m-NMES were not significantly better than c-NMES in our study the devices did perform similarly, which we believe is also an important observation. The m-NMES device is portable and because the electrodes are integrated within a neoprene sleeve patients can easily apply the treatment themselves. Feil et al.[87] observed greater compliance during the m-NMES condition of their training study, which they suggested may have occurred due to the convenience of the m-NMES

device. Therefore, in agreement with previous authors,[21] we believe that the m-NMES device may serve as a clinically useful option because it performed similarly to c-NMES and is easier to use.

Limitations

The extent to which the findings of our study hold true with respect to females and injured populations remains unclear, thus excluding females and using only healthy participants may be viewed as limitations of our study. We did not use females because the menstrual cycle has been shown to influence self-reported discomfort levels,[162] thus due to our study design requiring repeated measurements over time we felt it was necessary to exclude females. In addition, during exploratory NMES studies, similar in nature to our study, it is common practice to use healthy participants.[21,26,29,30,33,37,44,62,77,99,104,144,145,159]

Despite achieving an original sample size larger than our *a priori* power analyses indicated, we excluded the data of seven participants due to a limitation of the c-NMES device that we used. Therefore, our sample size was smaller than desired which may have allowed a type II error. However, due to the fact that each of our non-significant observations also had corresponding effect sizes that were below the medium to large threshold used during our *a priori* power analyses, we believe that our sample size was adequate for the purposes of our study; which we designed to focus on clinically meaningful differences.

A maximum comfortable stimulus intensity, which may vary across NMES conditions and individuals, should be utilized in clinical settings. Therefore, another limitation of our study was that we used a fixed target training intensity of 30% MVIC,

which does not permit inferences regarding which NMES method allows for greater initial training intensities or regarding fatigue and discomfort related outcomes while using a clinically applicable stimulus intensity. A maximum comfortable stimulus intensity is self-selected by each individual and so baseline differences in NMES-induced torque may occur across conditions or individuals; whereas using a target training intensity is likely to prevent a baseline difference. Therefore, we standardized the stimulus intensity by using a target training intensity of 30% MVIC in an effort to enhance experimental control and subsequently facilitate our interpretation of the results. Future studies should compare the two NMES conditions while using a clinically relevant maximum comfortable stimulus intensity. Although participants were required to shave their dominant thigh each day prior to reporting and the leg was cleansed with a non-alcoholic wipe in an effort to standardize electrical impedance across test sessions, we did not make an attempt to standardize skin impedance via an objective measure. Since skin impedance may vary on a daily basis,[97] this may also be considered a limitation of our study.

Conclusions

Based on our results, it does not appear that the novel multipath current distribution method positively impacts NMES-induced fatigue and discomfort in a clinically meaningful manner. Since we used similar electrode configurations across conditions and did not observe any significant differences across the two conditions in favor of m-NMES, we believe it is likely that a contributing factor for the improved outcomes observed during previous similar studies[21,27] is the larger electrodes integrated into the garment of the m-NMES device rather than the novel current distribution

method. Although we did not directly measure accommodation, we hypothesize that the large declines in NMES-induced torque we observed during both NMES-conditions are primarily attributable to motor nerve accommodation. Therefore, the novel multipath current distribution method does not appear to positively influence motor nerve accommodation in a clinically meaningful manner, thus additional strategies are needed.

CHAPTER V – STUDY 2 MANUSCRIPT

Abstract

Context

During a previous study performed in our laboratory comparing multipath NMES (m-NMES) and conventional NMES (c-NMES) we standardized the stimulus intensity using a target training intensity. However, standardizing the stimulus intensity by using a maximum comfortable stimulus intensity is a more clinically relevant approach warranting examination.

Objective

To compare the effects of m-NMES and c-NMES on NMES related outcomes, while addressing a methodological limitation of our previous study.

Design

Single-blind counterbalanced cross-over study.

Setting

Research laboratory.

Patients or Other Participants

We included data from 21 recreationally active males that successfully completed all sessions.

Interventions

Participants performed 18 NMES-induced contractions while using a maximum comfortable stimulus intensity under two conditions: m-NMES and c-NMES.

Main Outcome Measure(s)

We manually recorded maximum comfortable stimulus intensity (mA). We measured initial normalized NMES-induced torque (Nm/kg), percent decline in MVIC torque, percent decline in NMES-induced torque, percent decline in torque-time integral and total torque-time integral (Nm*s) with an isokinetic dynamometer. Participants self-reported discomfort levels using a visual analog scale (mm).

Results

Maximum comfortable stimulus intensity was significantly greater under the m-NMES condition ($P<0.05$), but the subsequent normalized NMES-induced torque was not significantly different across conditions ($P>0.05$). Percent declines in MVIC, NMES-induced torque and torque-time integral, as well as the total torque-time integral, were not significantly different across conditions ($P>0.05$). Percent declines in NMES-induced torque and torque-time integral were significantly greater over time ($P<0.05$). Depending on the level of time, self-reported discomfort levels significantly differed across conditions ($P<0.05$).

Conclusion

Although m-NMES resulted in a significantly greater maximum comfortable stimulus intensity, it did not subsequently result in significantly greater NMES-induced torque production. It also did not positively impact NMES-induced fatigue and discomfort related outcomes. Therefore, it does not appear that the multipath current distribution method influenced the outcomes in a clinically meaningful manner.

Key Words

Multipath, NMES, quadriceps

Introduction

Neuromuscular electrical stimulation (NMES) treatments are common in orthopedic clinical settings as they can be used for a variety of purposes.[1-5] Despite this versatility, NMES is most often used for the specific goal of enhancing quadriceps strength.[6] The effectiveness of NMES for this purpose is believed to be primarily determined by the NMES training intensity,[16,17] which is often defined as the ratio of NMES-induced torque to torque produced during a maximum voluntary isometric contraction (expressed as % MVIC).[5] Accordingly, clinicians are encouraged to maximize NMES training intensities to the degree possible,[17] but the ability to achieve and maintain appropriate NMES training intensities is limited by a variety of factors; which include: patient discomfort,[5,17,21,23-27] muscle fatigue[17,21-23] and spatially limited motor unit recruitment.[17,21,24]

The Kneehab® XP (Theragen LLC, Leesburg, VA) is an electrical stimulator that has received substantial attention in the literature,[21,27,84,86-90] because it implements a novel strategy marketed to address the primary factors limiting NMES training intensity.[84,88,92] The stimulator uses multipath™ technology, which distributes the electrical current between four large electrodes integrated within a neoprene thigh garment via two separate channels while also altering pulse durations[21,27,84,88,91]; thus it is referred to as multipath NMES (m-NMES).[21,27] In contrast, conventional NMES (c-NMES) stimulators distribute the electrical current in each channel via a single fixed path between a pair of electrodes.[84]

Studies comparing m-NMES and c-NMES have reported improved outcomes while using m-NMES, but due to limitations of these studies the authors acknowledged

that the mechanisms by which m-NMES outperformed c-NMES remain unclear.[21,27,87] For example, two basic studies[21,27] comparing m-NMES and c-NMES used substantially different electrode configurations across conditions, thus the authors' ability to attribute improved NMES-induced torque, as well as fatigue and discomfort related outcomes, to the novel multipath current distribution method was limited. Consequently, we performed a similar basic study in our laboratory comparing the influence of m-NMES and c-NMES on fatigue and discomfort related outcomes while using similar electrode configurations (Chapter IV). We believe this approach allowed us to better examine the influence of the novel multipath current distribution method on these outcomes.

When comparing fatigue and discomfort related outcomes, as we did in our previous study, it is necessary to standardize the NMES stimulus intensity across conditions. However, different standardizing methods exist in the literature. Two common methods of standardizing the stimulus intensity are using a maximum comfortable or maximum tolerable intensity (e.g., mA) identified by each individual,[23,37,45,62,73,75,77] or by using a target NMES training intensity (e.g., % MVIC) set by the investigator.[21,27,30,38] Since the maximum comfortable or maximum tolerable intensity approach uses self-selected stimulus intensities by each individual, it may allow significant baseline differences in the initial NMES-induced torque production to occur across conditions or individuals. The target training intensity approach is more likely to limit these baseline differences, thus it provides greater experimental control. Therefore, since we felt that any systematic baseline differences across conditions could influence the outcomes, which would make the results difficult to interpret, we elected to standardize the stimulus intensity by using a target training intensity of 30% MVIC

during our previous study. Although we believe this approach allowed for greater experimental control, it also limited the generalizability of our results because each individual's maximum comfortable stimulus intensity should be used within clinical settings. In addition, this approach limited our previous study because we were unable to determine which current distribution method, if any, allowed for greater maximum comfortable stimulus intensities and subsequent NMES-induced torque production. Each of these outcome measures are clinically relevant and warrant further investigation, as they ultimately impact the NMES training intensity. Therefore, the primary purpose of our study was to compare the effects of m-NMES and c-NMES on the clinically relevant maximum comfortable stimulus intensity and the subsequent NMES-induced torque. Our secondary purpose was to compare fatigue and discomfort related outcomes across the two NMES conditions while using a maximum comfortable stimulus intensity.

Methods

Design

We performed a single-blind counterbalanced cross-over study with 2 independent variables (NMES condition at 2 levels: m-NMES and c-NMES; time at 17 or 18 levels: based on the number of NMES-induced contraction repetition) and 7 dependent variables (maximum comfortable stimulus intensity, initial normalized NMES-induced torque, percent decline in MVIC, percent decline in NMES-induced torque, percent decline in torque-time integral, total torque-time integral, self-reported discomfort). We assigned participants to one of two permutations designed to counterbalance the session order in which the c-NMES and m-NMES treatment conditions were performed (see Appendix A, Figure A2).

Participants

We performed *a priori* power analyses using G*Power software (version 3.1.9.2)[175] to determine a target sample size. We determined a target sample size of 17 participants in order to maintain adequate power ($1\text{-}\beta = 0.80$) and detect a medium to large effect size ($d = 0.650$, $f = 0.325$).[176] We selected medium to large effect sizes for the power analyses because we believe that any statistically significant differences with corresponding effect sizes smaller than this threshold would lack clinical relevance for the outcomes included in our study. It is also important to note that other authors have selected a large effect size (e.g., Cohen's $f = 0.68$) for an *a priori* power analysis during a NMES study examining similar dependent variables,[37] which further supports our selection of a medium to large effect size as appropriate for this study. The dependent *t*-test power analysis revealed a target sample size requirement of 17 participants, while only 12 participants were required for the two-way repeated measures ANOVA.

A convenience sample of 21 participants (age = 23.9 ± 5.1 years, height = 175.1 ± 7.4 cm, mass = 78.1 ± 11.7 kg, BMI = 25.3 ± 2.6 kg/m^2) from the university and community completed the two study sessions. As has been done previously,[26] participants in our current study had prior NMES experience due to their participation in an earlier study performed in our laboratory intended to address related research questions, but using a different methodologic approach. We elected to use participants from our previous study comparing m-NMES and c-NMES because an individual's tolerance to NMES is likely to improve over the first few exposures to NMES treatments.[144] We believe that through their participation in our previous study, which exposed participants to NMES during four sessions, participants were appropriately

familiarized with the NMES stimulus prior to participating in our current study. This likely reduced the "fear of the unknown" that often accompanies initial NMES treatments, which we believe allowed participants to self-select a more accurate maximum comfortable stimulus intensity.

Data from all 21 participants were included during the maximum comfortable stimulus intensity and initial normalized NMES-induced torque comparisons. However, we determined that 10 of the participants' data needed to be excluded from the fatigue and discomfort related outcome comparisons due to a unique limitation of the c-NMES unit (discussed in detail in procedures section). As a result of this limitation, data from 11 of the 21 participants were included when comparing the fatigue and discomfort related outcomes (age = 24.5 ± 5.2 years, height = 174.8 ± 5.7 cm, mass = 77.1 ± 12.4 kg, BMI = 25.1 ± 3.1 kg/m^2).

Participants were required to be healthy, recreationally active, males, between the ages of 18-35. We defined recreationally active as participation in some form of physical activity (e.g. strengthening activities, jogging, running, cycling, swimming, tennis, etc.) for a minimum of two times per week for at least 20 minutes each time. We defined healthy as having no unresolved knee injuries or other injuries that would impact lower-limb function, as well as being free of all applicable electrotherapy contraindications. We excluded females from participation because electrically induced discomfort levels have been shown to significantly differ over the course of the menstrual cycle.[162] Participants also had to have a body mass index (BMI) ≤30 kg/m^2 to be included, as NMES tolerance and motor thresholds have been shown to differ between individuals with a BMI above and below 30 kg/m^2.[160] To be included in our current study

participants had to tolerate a NMES training intensity of at least 30% MVIC during the second session of our previous study. This study was approved by the University's institutional review board and participants provided written informed consent. To facilitate participant recruitment, we incentivized participants via a lottery for a chance to win one of four $50 gift cards.

Instrumentation

We used a Quickset 4 Biodex dynamometer (Biodex Medical Systems Inc., Shirley, New York) to measure and record isometric knee extension torque during all NMES-induced and voluntary contractions, at a sampling rate of 100 Hz. Participants removed their shoe from the dominant foot prior to being seated on the dynamometer. During all contractions participants were seated in the dynamometer chair with the seat back tilt at 85° and the dominant leg secured within a lever arm fixed at 60°. We aligned the axis of rotation of the dynamometer to the anatomical axis of the test knee, and the lower leg was secured in the fixed lever arm via an ankle strap placed 2-3 cm above the lateral malleolus.[27] We calibrated the dynamometer to the manufacturer's specifications prior to beginning the study to ensure reliable measurements. In addition, we used stabilization straps to prevent undesired movement of the upper body and asked participants to cross their arms over their chest while performing all voluntary and NMES-induced contractions (Figure 4).[27,178]

We applied all c-NMES treatments using the same Sonicator® Plus 940 stimulator (Mettler Electronics® Corp., Anaheim, CA). To maintain consistency across the two NMES conditions, we set the c-NMES parameters as similar as possible to the parameters used with the Kneehab® XP program 6 (Table 5). Although our sample size

was negatively impacted by an unforeseen feature of the Sonicator® Plus 940, it is important to note that we selected this stimulator because it allowed us to more closely match the parameters of the m-NMES device.

We used four self-adherent electrodes to deliver the c-NMES current (two- 5 cm x 9 cm [Metron™, Bolingbrook, IL], one- 10.79 cm x 17.78 cm [TENS Products, Grand Lake, CO], one- 7 cm x 14 cm electrode [SME INC., Wilmington, NC]; Figure 5). To guide the placement of the c-NMES electrodes, we manually identified motor points using a pencil electrode (Mettler Electronics XK2, Active Forever, Scottsdale, AZ) following the procedures outlined in the literature (Figure 7).[24] Based on the results of a recent study, which identified seven motor points of the quadriceps,[153] we selected four commonly identified motor points to guide the c-NMES electrode placement. Furthermore, the motor points we selected allowed us to place the c-NMES electrodes in a similar fashion to the m-NMES electrode configuration, as they were located on the proximal and distal vastus lateralis, proximal rectus femoris and distal vastus medialis (Figure 7).

We applied all m-NMES treatments using the same Kneehab® XP stimulator (Theragen LLC, Leesburg, VA), however we assigned each participant a separate Kneehab® XP garment with integrated electrodes. We integrated the m-NMES electrodes into the neoprene garment and subsequently placed the garment on the dominant thigh according to the manufacturer's recommendations.[167] We set the stimulator parameters to program 6 during all m-NMES treatments (Table 5).

Procedures

Following completion of our previous study, which consisted of four sessions, participants reported at the same time of day (±2 hours) on two additional occasions and each session lasted approximately 1 hour. Each participant's dominant leg, which we defined as the leg with which they would use to kick a soccer ball, served as the leg of interest throughout the study (20 right, 1 left). In an effort to reduce electrical impedance, participants shaved their anterior thigh each day. We also instructed participants to report well hydrated and to refrain from strenuous activities for 12 hours prior to reporting.

Our previous study served as familiarization sessions for our current study, which consisted of two additional test sessions separated by 48-72 hours. Each session began with the participants completing a standardized warm-up that included: 5 minutes of cycling on a stationary bike at a self-selected pace, three 30 second bouts of dynamic quadriceps stretching and four isometric quadriceps contractions while in the dynamometer chair (two- at 50%, one- at 75% and one- maximum contraction at 60° of knee flexion).[36] Participants rested for 8 minutes following the warm-up, during which we identified the motor points using the pencil electrode method and cleaned the leg of interest with an alcohol free wipe. Although motor point identification was not necessary for the m-NMES condition because the electrodes were integrated within the garment, we still identified motor points during both sessions in an effort to blind participants to treatment condition.

Participants performed maximum voluntary isometric contractions (MVICs) of the quadriceps and all MVICs were 6 seconds in duration. Participants gradually

increased their effort during the initial portion of each contraction, with maximal effort being reached at roughly 3 seconds and maintained for the remaining 3 seconds. To facilitate participant understanding of a gradual increase, we placed a figure showing an ideal MVIC curve in the participants' view while performing all MVICs. We instructed the participants by saying "cross your arms and prepare to push out with maximal effort". We familiarized participants with the MVIC procedures during our previous study. We provided verbal encouragement throughout each repetition while the dynamometer computer screen provided real-time visual feedback of the participant's torque production (Figure 4).[98] We encouraged participants to "push out with maximal effort" in a loud clear voice, and in the event that a participant showed signs of fatigue we gradually increased the volume and intensity.[179] We asked the participants to verify that their effort was maximal immediately following each repetition.[98] In the event that a participant reported a submaximal effort, or we judged an effort to be submaximal, the repetition was discarded and repeated.

Pre-test MVIC procedures consisted of a series of three MVICs, with each repetition separated by a 2 minute rest period.[31,33] The peak torque of the three pre-test trials was required to be within 10% or we asked participants to perform additional trials until three consecutive MVIC trials were within 10%.[27] To limit the possibility of fatigue as a confounding variable, we gave participants a maximum of six contractions during each session. If a participant was unsuccessful in completing the pre-test MVIC procedures, we asked them to return the following day for a second attempt. We defined the trial with the greatest peak torque, from the three consecutive trials within 10%, as the participant's pre-test MVIC value for that particular session.

Participants rested for 5 minutes prior to performing the NMES procedures, during which we placed the Kneehab® XP garment with integrated electrodes or the c-NMES electrodes over the participant's dominant thigh. We also placed an empty Kneehab® XP garment over the c-NMES electrodes in an effort to blind participants to treatment condition (Figure 9).[27] Participants performed 18 NMES-induced contractions using a single NMES device (c-NMES or m-NMES) during each session while using a self-selected maximum comfortable stimulus intensity, which is defined as the highest intensity that does not cause pain.[77]

To determine maximum comfortable stimulus intensity, we placed the stimulator controls in a manner that allowed the participants to increase the stimulus intensity (Figure 10). We instructed them to "increase the intensity until reaching the highest intensity that does not cause pain". Participants completed the NMES-induced contractions after 50 seconds of rest. We frequently encouraged participants to "relax and allow the machine to do all the work", and we did not adjust the self-selected maximum comfortable stimulus intensity over the course of each NMES treatment condition. However, the c-NMES device we used has an obscure setting that may automatically reduce the stimulus output when the unit senses a change in impedance over the course of the NMES-induced contractions.[177] As expected, we observed that the fatigue and discomfort related outcome measures were negatively impacted when this occurred, resulting in an undesired systematic bias during c-NMES test sessions in which the stimulus output was automatically reduced by the unit. Consequently, when this automatic step-down in output occurred we excluded the participant's fatigue and discomfort related data from the corresponding statistical analyses.

Immediately after completing the assigned NMES treatment condition participants performed a single 6 second post-test MVIC. We elected to use a single repetition in an effort to limit recovery from the NMES-induced contractions. A second MVIC was performed after a 20 second rest period in an effort to continue limiting recovery in the event that we deemed the post-test trial to be submaximal, or the participant deemed it to be submaximal.[178]

Outcome Measures

Maximum Comfortable Stimulus Intensity

We manually recorded the maximum comfortable stimulus intensity selected by each participant, which was expressed in milliamps (mA). The m-NMES device does not express the stimulus intensity in mA units, thus a conversion table provided by the manufacturer was used to convert the observed m-NMES stimulus intensities into the appropriate units.[185] Since maximum comfortable stimulus intensity was recorded during the initial contraction it was unaffected by the automatic step-down function of the c-NMES device, thus we included data from each participant during the statistical analysis of this outcome measure.

Initial Normalized NMES-induced Torque

The isokinetic dynamometer measured and recorded the initial NMES-induced peak torque under each condition. In an effort to reduce inter-participant variability, we normalized the initial NMES-induced peak torque values to each participant's body mass (see equation 7), which converts the unit of measure to Newton-meters per kilogram (Nm/kg) and has been done previously.[42,45] Initial normalized NMES-induced torque was also unaffected by the automatic step-down function of the c-NMES device because

it was recorded during the first contraction. Therefore, we included the data from each participant during the statistical analysis of this outcome measure.

Percent Decline in MVIC Torque

A change in peak MVIC torque is considered to be the gold standard for assessing fatigue.[105,110] Therefore, we expressed each participant's post-test MVIC peak torque as a percent decline relative to their pre-test MVIC peak torque (equation 2), and the subsequent percent decline served as a fatigue related outcome measure for our study.

Percent Decline in NMES-induced Torque

Failing to maintain a target force is another common method used to asses fatigue,[110] thus NMES-induced fatigue is also frequently assessed by measuring the decline in NMES-induced torque over the course of a treatment.[21,27,30-33,46,49,51,54,55,59,61,62,64,66,100] Accordingly, we expressed the peak torque produced during all 18 NMES-induced contractions for each test session as a percent decline relative to the peak torque produced during the initial NMES-induced contraction of each test (equation 3).

Percent Decline in Torque-time Integral

A decline in the torque-time integral (TTI) observed during NMES-induced contractions has also been used in a number of studies as an index of NMES-induced fatigue, as it has been suggested to represent isometric work.[23,29,47,115,116] Therefore, the data necessary to calculate the TTI (e.g., torque and duration of torque recording; expressed as Newton-meter seconds [Nm*s]) was measured and recorded during the NMES-induced contractions and subsequently exported as a text file (sampling rate = 100 Hz, or one sample taken every 10 ms). We imported the data files into an analysis

software package (Acqknowledge® 4, Biopac® Systems, Inc., Goleta, CA), which we used to calculate the TTI of each contraction (equation 4). We expressed the TTI of each NMES-induced contraction during the test sessions as a percent decline (equation 5), which also served as an outcome measure of NMES-induced fatigue.

Total Torque-time Integral

We considered the total torque-time integral (T-TTI) to be an index of the total amount of isometric work performed under each condition, and as such it also represented a fatigue related outcome. We calculated the T-TTI for each condition by summing individual TTI data (equation 6).

Self-reported Discomfort

We used a 100 mm horizontal visual analog scale (VAS) to measure self-reported discomfort levels during each NMES condition. As is common during NMES studies, the descriptors at each end of the scale were "no discomfort" (0 mm) and "worst possible discomfort" (100 mm).[21,27,29,36,37,159,161] We gave the participants a pen and asked them to "rate your level of discomfort by making a vertical tick mark on the line" following each NMES induced contraction (Figure 8). We obtained self-reported discomfort levels by measuring the distance (mm) from the "no discomfort" anchor to the vertical mark made on the horizontal line. When used to assess NMES-induced discomfort in a sample of healthy individuals, the VAS has been shown to have a high inter-session test-retest reliability *(ICC*$\geq$0.90).[159]

Statistical Analysis

We used the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corporation, Armonk, NY) to analyze the data. We performed a series of two-way

repeated measures analysis of variance (ANOVA) on three of the outcome measures (percent decline in NMES-induced torque, percent decline in TTI, and self-reported discomfort). We followed the Greenhouse-Geisser procedure for correcting degrees of freedom when the assumption of sphericity was determined to be violated. We performed simple effects analysis in the event of a significant interaction effect (NMES condition*time), which allowed us to analyze the effect of NMES condition at each level of time via a series of pairwise comparisons.[187] We used the procedures and syntax provided by Field[187] to perform the simple effects analysis, with the exception that a Bonferroni procedure was also included in the syntax to control the family-wise error rate (Appendix H). In the event of a significant time main effect and the absence of an interaction effect, we performed *post-hoc* pairwise comparisons and we again used a Bonferroni procedure to maintain family-wise error rate. Due to the number of NMES-induced contractions, we performed a large number of *post-hoc* pairwise comparisons (e.g., >130). To simplify the results, we provided only the significant pairwise comparisons deemed to be clinically important (e.g., first contraction to demonstrate a significant decline relative to the second contraction, and final contraction).

We performed a dependent *t*-test on the other four outcome measures (maximum comfortable stimulus intensity, initial normalized NMES-induced torque, percent decline in MVIC, T-TTI). In addition, we performed a dependent *t*-test to examine any potential baseline differences between the two conditions with respect to pre-test MVIC. We also calculated test-retest reliability ($ICC_{(2,1)}$)[189,190] and measurement precision (SEM)[191] estimates for pre-test MVIC measurements using the equations provided in the literature.

To examine the magnitude of the differences, we calculated Cohen's *f* and *d* effect sizes.[176] We calculated Cohen's *d* effect sizes corresponding to within groups comparisons using the equation suggested by Cumming[194] (equation 8), which uses the average standard deviation of the paired data as the standardizer (d_{sav}). Since *d* statistics are believed to overestimate the population effect size, Cumming[194] recommended that an unbiased Cohen's *d* (d_{unb}) also be provided. Accordingly, we calculated d_{unb} values using the equation provided by Cumming[194] (equation 9). We interpreted Cohen's *f* values as follows: f = 0.10-0.24 small, f = 0.25-0.39 medium and $f \geq 0.40$ large; whereas we interpreted Cohen's *d* effect sizes as follows: d = 0.20-0.49 small, d = 0.50-0.79 medium and $d \geq 0.80$ large.[176]

Results

Prior to analyzing the data, the tenability of the applicable statistical assumptions was assessed. We defined outliers *a priori* as any raw score with a corresponding *z*-score >2.5.[188] For outliers, we transformed the corresponding raw score by changing the original raw score to a value ±1 unit larger than the next highest value, as has been recommended because this allows the case identified as an outlier to remain deviant while also reducing the impact of an outlier.[186] We assessed the normality of the data via skewness and kurtosis *z*-scores, and the data were considered to be normally distributed. There was no significant difference with respect to the pre-test MVIC measurements across the two conditions (t_{10} = 1.985; P = 0.075; d = 0.123; 95% CI for effect size: -0.012, 0.254; d_{unb} = 0.114). In addition, the corresponding test-retest reliability and measurement precision were within acceptable limits ($ICC_{(2,1)}$ = 0.975; 95% CI: 0.934,

0.990; SEM = 10.02 Nm),[184] and these estimates are similar to those reported in the literature when assessing healthy populations.[181,183]

Maximum Comfortable Stimulus Intensity

The dependent *t*-test revealed that the maximum comfortable stimulus intensity (mA) was significantly higher during the m-NMES condition (t_{20} = 2.817; P = 0.006; d = 0.581; 95% CI for effect size: -0.133, 1.018; d_{unb} = 0.559; Figure 16).

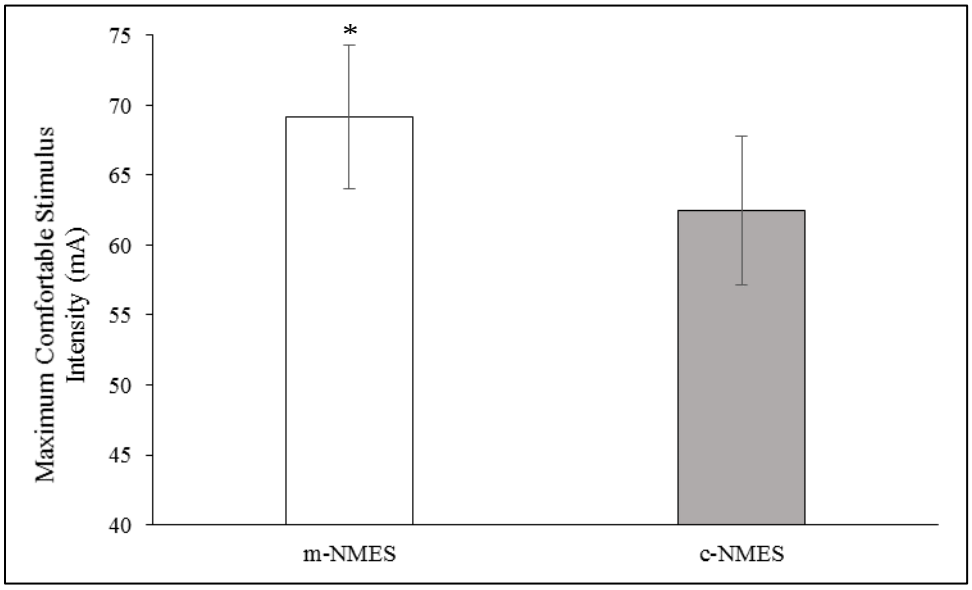


Figure 16. Maximum Comfortable Stimulus Intensity

Note. *Significantly greater maximum comfortable stimulus intensity (P = 0.006). Error bars indicate 95% confidence intervals calculated using a critical *t*-value as has been recommended.[194]

Initial Normalized NMES-induced Torque

The dependent *t*-test revealed that the initial normalized NMES-induced torque (Nm/kg) was not significantly different across conditions (t_{20} = 1.397; P = 0.089; d = 0.282; 95% CI for effect size: -0.125, 0.683; d_{unb} = 0.272; Figure 17).

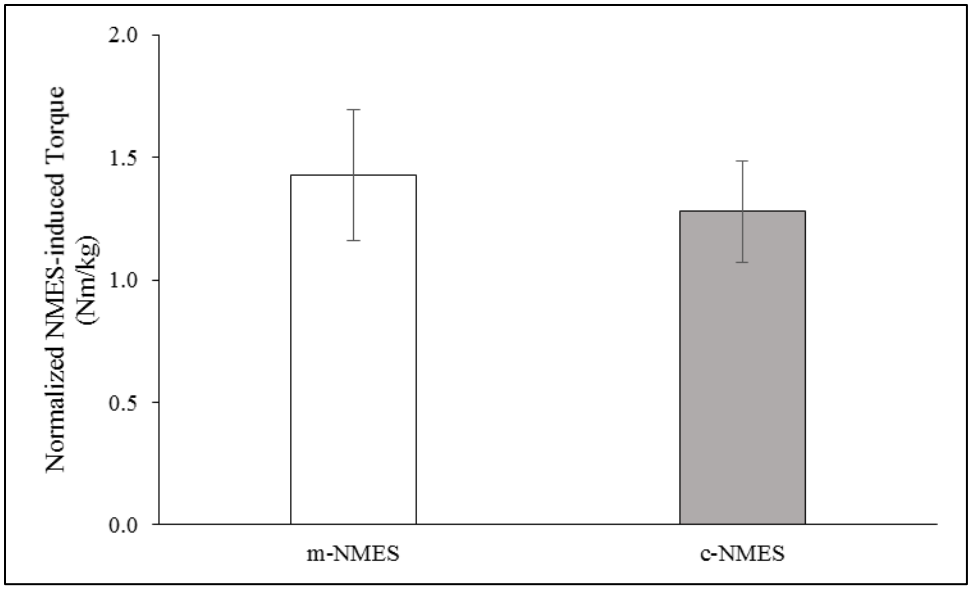


Figure 17. Initial Normalized NMES-induced Torque

Note. Error bars indicate 95% confidence intervals and were calculated using a critical *t*-value as has been recommended.[194]

Percent Decline in MVIC Torque

The dependent *t*-test revealed that the percent decline in MVIC torque following the NMES treatments was not significantly different across conditions ($t_{10} = 1.186$; $P = 0.132$; $d = 0.385$; 95% CI for effect size: -0.269, 1.022; $d_{unb} = 0.356$; Figure 18)

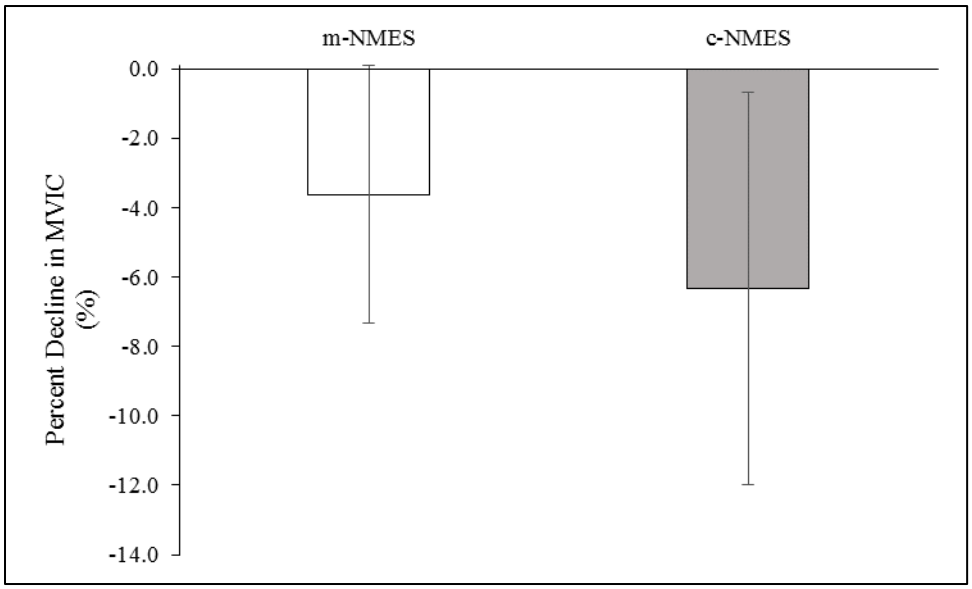


Figure 18. Percent Decline in MVIC

Note. Error bars indicate 95% confidence intervals calculated using a critical *t*-value as has been recommended.[194]

Percent Decline in NMES-induced Torque

For percent decline in NMES-induced torque, the repeated measures ANOVA revealed no significant condition by time interaction ($F_{2.2, 22} = 2.106$; $P = 0.142$; $f = 0.459$) or condition main effect ($F_{1, 10} = 0.225$; $P = 0.322$; $f = 0.150$). However, there was a significant time main effect ($F_{2, 20.4} = 245.502$; $P<0.001$; $f = 4.964$; Figure 19). *Post-hoc* analysis revealed that the decline was significantly greater by the fourth contraction (difference = 5.1 ± 3.5%; $P = 0.004$; $d = 0.866$; 95% CI for effect size: 0.504, 1.218; $d_{unb} = 0.834$) and it remained significantly greater for each of the subsequent contractions (18th contraction, difference = 51.9 ± 10.7%; $P<0.001$; $d = 5.745$; $d_{unb} = 5.537$).

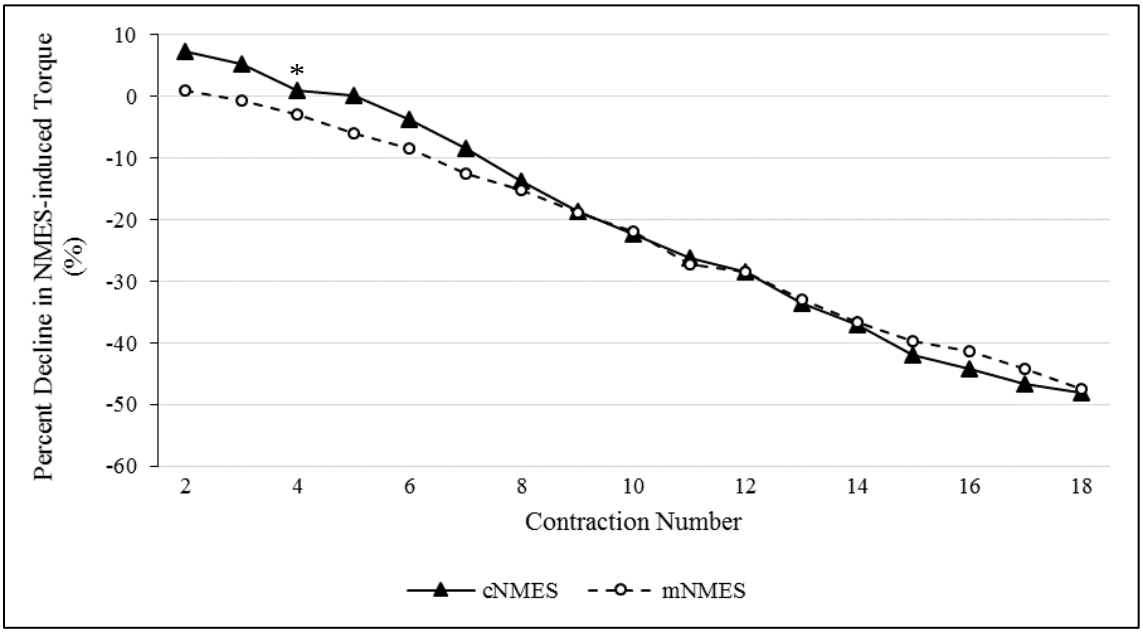


Figure 19. Percent Decline in NMES-induced Torque

Note. *First contraction with a significantly greater decline relative to contraction 2 (P=0.004).

Percent Decline in Torque-time Integral

For percent decline in TTI, the repeated measures ANOVA revealed no significant condition by time interaction ($F_{2.3, 23.1} = 3.263$; $P = 0.050$; $f = 0.571$) or a condition main effect ($F_{1, 10} = 0.431$; $P = 0.263$; $f = 0.207$). However there was a significant time main effect ($F_{1.8, 18.3} = 274.311$; $P<0.001$; $f = 5.251$; Figure 20). *Post-hoc* analysis revealed that the decline was significantly greater by the fourth contraction (difference = 5.0 ± 3.1%; $P = 0.001$; $d = 0.598$; 95% CI for effect size: 0.356, 0.834; $d_{unb} = 0.576$) and it remained significantly greater for each of the subsequent contractions (18th contraction, difference = 57.3 ± 12.3%; $P<0.001$; $d = 5.509$; $d_{unb} = 5.309$).

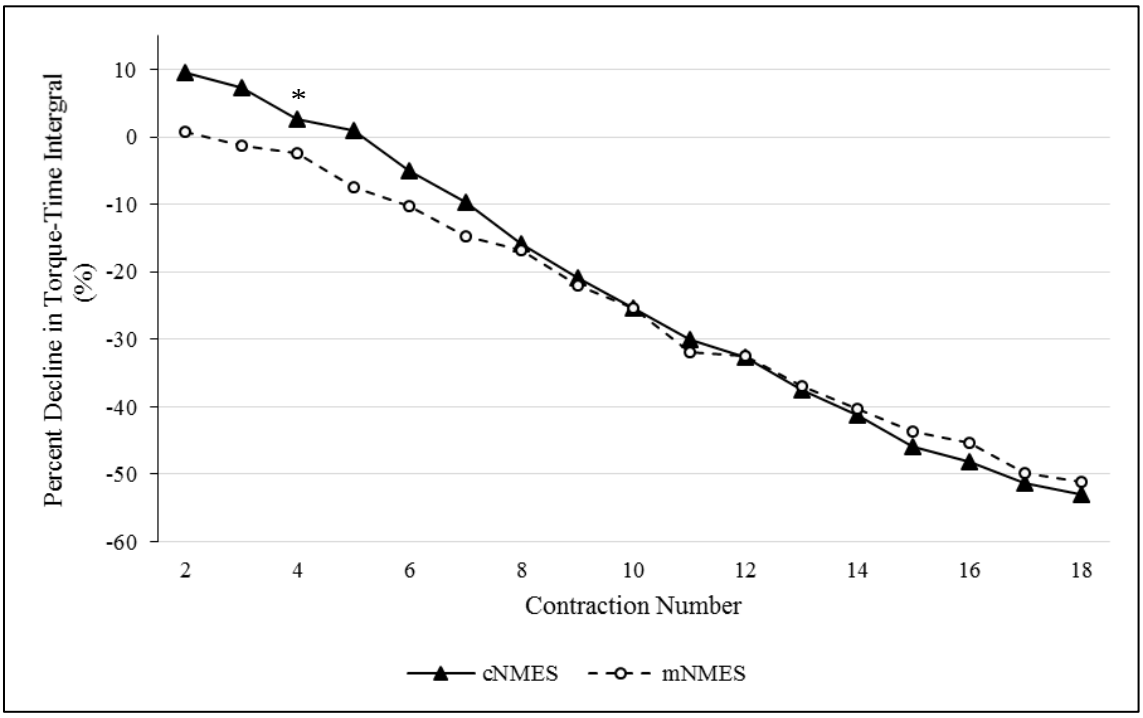


Figure 20. Percent Decline in Torque-time Integral

Note. *First contraction with a significantly greater decline relative to contraction 2 (P=0.001).

Total Torque-time Integral

The dependent *t*-test revealed that the T-TTI was not significantly different across conditions (t_{10} = 0.074; *P* =0.471; *d* = 0.027; 95% CI for effect size: -0.682, 0.734; d_{unb} = 0.025; Figure 21).

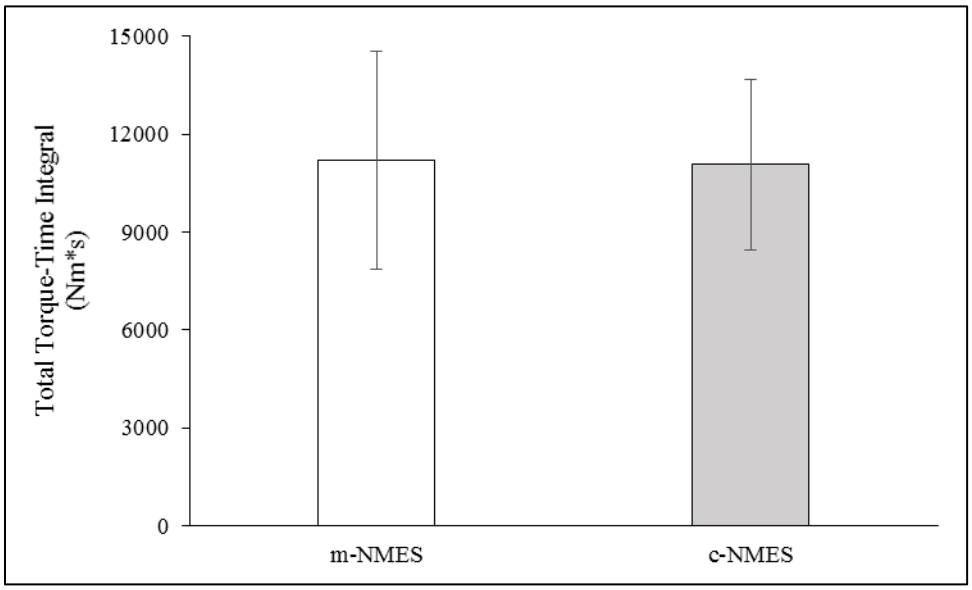


Figure 21. Total Torque-time Integral

Note. Error bars indicate 95% confidence intervals calculated using a critical *t*-value as has been recommended.[194]

Self-reported Discomfort

The repeated measures ANOVA revealed that there was no significant condition main effect ($F_{1, 10}$ = 1.526; *P* = 0.123; *f* = 0.390) for self-reported discomfort levels. However there was a significant condition by time interaction ($F_{2.3, 22.6}$ = 3.999, *P* = 0.029; *f* = 0.633; Figure 22) and time main effect ($F_{2.5, 24.8}$ = 5.662; *P* = 0.003; *f* = 0.753). The simple-effects analysis revealed significantly lower discomfort during the c-NMES condition but only during the final three contractions (16th difference = 14.9 ± 20.0 mm; *P* = 0.033; *d* = 0.536; 95% CI for effect size: 0.042, 1.010; d_{unb} = 0.495, 17th difference = 16.5 ± 19.8 mm; *P* = 0.020; *d* = 0.625; 95% CI for effect size: 0.094, 1.133; d_{unb} = 0.577,

18th difference = 15.5 ± 20.8 mm; P = 0.033; d = 0.556 ; 95% CI for effect size: 0.044, 1.047; d_{unb} = 0.513).

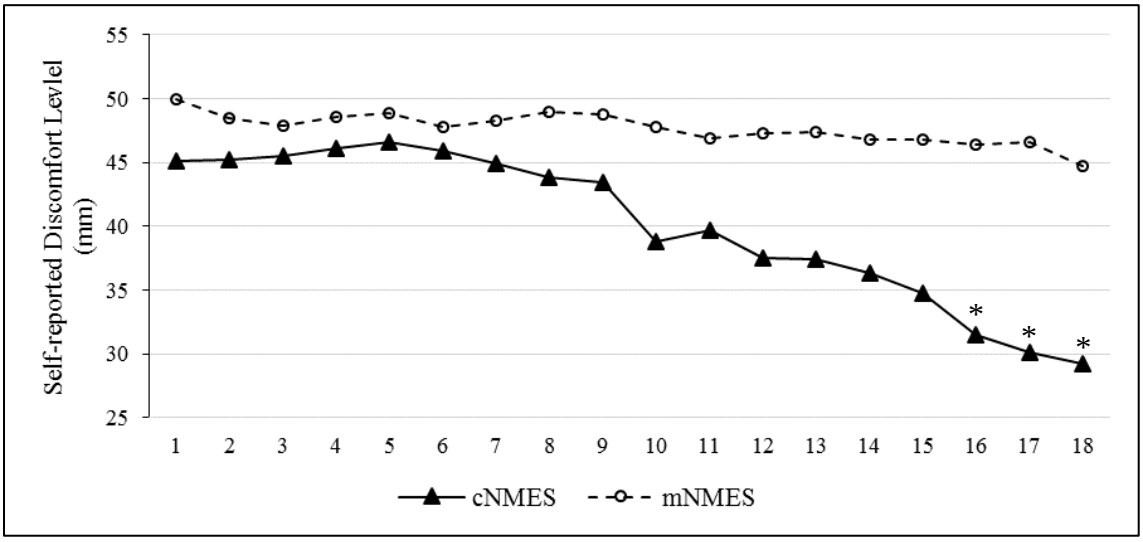


Figure 22. Self-reported Discomfort

Note. *Significantly lower self-reported discomfort levels during the c-NMES condition (P<0.05).

Discussion

Overall, while using similar electrode configurations, the findings of our study indicate that the maximum comfortable stimulus intensity was significantly higher under the m-NMES condition. However, the higher stimulus intensity did not subsequently result in significantly greater normalized NMES-induced torque during the initial contraction. Due to the positive linear relationship between stimulus intensity and NMES-induced torque,[17,50,51] the primary clinical objective of using higher stimulus intensities is to enhance the NMES training intensity by increasing NMES-induced torque production. Therefore, the greater maximum comfortable stimulus intensity that we observed during the m-NMES condition does not appear to be clinically meaningful. Despite our efforts to standardize the electrode configurations, the m-NMES electrodes covered an area of 427 cm^2 while the c-NMES electrodes covered a surface area of

roughly 360 cm^2 (Figure 6).[27] Since the current was spread over a greater area during the m-NMES condition, the current density (mA/cm^2) was subsequently lower during this condition while using the same amount of current (e.g., mA).[4] Therefore, the small difference in electrode sizes is a possible explanation as to why we did not observe significantly greater NMES-induced torque during the m-NMES condition. Although a small difference in the area covered by c-NMES and m-NMES remained during our study, the c-NMES electrode configurations used during previous studies consisted of three electrodes covering only 100 cm^2.[21,27]

The findings of our current study also indicate that the declines in NMES-induced torque and in TTI were significantly greater over time, irrespective of the type of NMES used. These results are similar to those of our previous study comparing m-NMES and c-NMES while standardizing the NMES training intensity at 30% MVIC, as well as those of previous studies using a training intensity of 20% MVIC.[21,27] To the best of our knowledge, our current study is the first to compare the influence of m-NMES and c-NMES on fatigue and discomfort related outcomes while using similar electrode configurations and a maximum comfortable stimulus intensity. Therefore, we believe our findings are important because our approach allowed us to better isolate the influence of the novel multipath current distribution method while also using a more clinically relevant stimulus intensity.

The maximum comfortable stimulus intensities we observed under the m-NMES and c-NMES conditions were 69.1 ± 11.3 mA and 62.5 ± 11.6 mA, respectively. During a similar study Maffiuletti et al.[21] reported values of 92 ± 25 mA and 53 ± 25 mA during their m-NMES and c-NMES conditions. Despite the fact that both of these studies

observed significantly greater stimulus intensities under the m-NMES condition, the mean stimulus intensity we observed during m-NMES is much smaller. Two likely explanations for this difference are that Maffiuletti et al. used a maximum tolerable stimulus intensity and a modified m-NMES device that allowed a maximum current output of 200 mA. We elected to use a lower threshold maximum comfortable stimulus intensity because it has been suggested to be more clinically relevant.[77] In addition, Maffiuletti et al. acknowledged that their use of a modified version of the m-NMES device was a limitation of their study, so we elected to use the clinically available m-NMES device with a maximum output of only 79.2 mA.

During the initial contraction of the m-NMES and c-NMES conditions we observed normalized NMES-induced torque values of 1.4 ± 0.6 Nm/kg and 1.3 ± 0.5 Nm/kg, respectively. It is difficult for us to directly compare these values to some similar studies comparing m-NMES and c-NMES because normalized torque values were not reported, and these studies did not use a maximum comfortable stimulus intensity.[21,27] However, while using a c-NMES device and a maximum comfortable stimulus intensity a comparable normalized NMES-induced torque value of 1.1 ± 0.7 Nm/kg was reported during a previous study.[42] In order to facilitate the comparison of our results to previous studies, we converted the initial normalized NMES-induced torque values to NMES training intensities. The subsequent training intensities were 47.9 ± 17.1% MVIC and 43.6 ± 13.8% MVIC for m-NMES and c-NMES, respectively. Our observed values are comparable to other NMES studies examining a variety of parameters while using maximum comfortable stimulus intensities, with reported values in the literature ranging from 20-48% MVIC.[42,45,62,75,77] Although previous studies[21,27] comparing peak torque

output across m-NMES and c-NMES conditions used a higher threshold maximum tolerable stimulus intensity, the NMES training intensities we observed are also comparable to values reported during these studies; which ranged from roughly 35-45% MVIC. Despite using a lower threshold maximum comfortable stimulus intensity, we believe that comparable NMES training intensities observed during our study are due to the participation in the four familiarization sessions, as this likely allowed participants to better acclimate to the NMES stimulus prior to participation in our current study.[144] In contrast, one[21] of the other studies comparing m-NMES and c-NMES did not incorporate familiarization sessions and the other[27] included a single familiarization session.

Although we observed a significantly greater maximum comfortable stimulus intensity under the m-NMES condition, we did not observe a significant difference with respect to the initial normalized NMES-induced torque across the two conditions. This observation is contrary to the results of Maffiuletti et al.[21] and Morf et al.,[27] as they reported significantly greater stimulus intensities and NMES-induced torque during their m-NMES conditions. One possible explanation for the inconsistency of our results is that we used a lower threshold maximum comfortable stimulus intensity. Additional methodological differences between our study and the previous studies also warrant further discussion.

Maffiuletti et al.[21] and Morf et al.[27] hypothesized that a possible mechanism for the significantly greater NMES-induced torque they observed during m-NMES was the novel multipath current distribution method. Maffiuletti et al. suggested that relative to the fixed single path current distribution method of c-NMES, a larger number of motor units may have been recruited during the m-NMES condition due to its greater spatial

distribution of the stimulus. However, the m-NMES and c-NMES conditions during these studies differed in two systematic ways, which were the current distribution method and electrode configuration. Morf et al. indicated that as a result of these two systematic differences, it is unclear whether the greater NMES-induced torque they observed was primarily attributable to the multipath current distribution method, larger electrodes or a combination of these factors. Consequently, we standardized the electrode configuration across conditions to the extent possible during our study, as we believe this approach allowed us to better isolate the influence of current distribution method on NMES-induced torque. Since we did not observe significantly greater NMES-induced torque under the m-NMES condition, our results do not support the hypothesis of Maffiuletti et al. and Morf et al. that the multipath current distribution method is a possible mechanism by which m-NMES resulted in greater NMES-induced torque during their studies.

Maffiuletti et al.[21] also suggested that the higher maximum tolerable stimulus intensity they observed under the m-NMES condition may have increased the number of motor units recruited, and as such they hypothesized that the difference in stimulus intensity across the two conditions was another possible mechanism by which NMES-induced torque was greater during the m-NMES condition. This hypothesis is reasonable due to the aforementioned positive linear relationship between stimulus intensity and NMES-induced torque.[17,50,51] Nonetheless, our results do not support the hypothesis of Maffiuletti et al., because we also observed significantly greater stimulus intensities under the m-NMES condition but this did not result in significantly greater NMES-induced torque. It is possible that our results do not support this hypothesis because the difference in stimulus intensity we observed across the two conditions is much smaller in

magnitude than that observed by Maffiuletti et al. (d = 0.581 vs. d = 1.560). We do not know why the magnitude of our effect is substantially smaller, but it may be attributable to the different target stimulus intensity and/or c-NMES electrode configuration that we used. Another possible explanation for the inconsistent effect size is that we used a clinically available version of the m-NMES device that has a maximum output capacity of only 79.2 mA, whereas Maffiuletti et al. used a modified unit with a maximum output capacity of 200 mA. Eight participants reached the output capacity of the m-NMES device prior to achieving their maximum comfortable threshold during our study. This likely prevented these participants from reaching their true maximum comfortable stimulus intensity during the m-NMES condition, and this may have subsequently reduced the magnitude of our observed effect. Furthermore, the smaller effect size we observed corresponding to the difference in stimulus intensity across conditions may also explain why we did not observe significantly greater NMES-induced torque under the m-NMES condition.

The declines in MVIC we observed following m-NMES and c-NMES treatments were 3.6 ± 5.6% and 6.3 ± 8.5%, respectively (Figure 18). The corresponding mean difference across the conditions was 2.7 ± 7.5% lower following m-NMES, but this did not reach statistical significance. We observed a similar non-significant 2.6% mean difference during our previous study and Maffiuletti et al.[21] likewise reported a 2.1% non-significant mean difference. In contrast to each of these studies, Morf et al. reported a significantly lower decline in MVIC following m-NMES but the corresponding mean difference was only 3.0%. Despite inconsistent results, the magnitude of the observed effects during each of these studies are consistently small (e.g., d = 0.385, d = 0.310, d =

0.383, 0.311), suggesting that a large sample size is required in order to detect any potential effect with a statistical test. This observation is a likely explanation as to why Morf et al. reported a significant difference while the other studies, including our current study, observed a non-significant difference (n = 20 vs. n = 11, n = 14, n = 10). Interestingly, each of the reported effect sizes are consistently positive, which indicates a consistent tendency for the decline in MVIC to be lower following m-NMES treatments. However, based on the non-significant results of our current study, as well as our observation that the reported effect sizes are consistently small throughout the literature, it does not appear that m-NMES limits the decline in MVIC in a clinically meaningful manner.

We observed significantly greater declines in NMES-induced torque and in TTI over time during our current study that were similar to those we observed during our previous study, even though the initial NMES training intensities of our current study were more than 10% higher relative to those of our previous study. Although declines in these outcomes are commonly attributed to muscle fatigue, caution should be exercised as these declines may be attributable to a combination of fatigue and accommodation of the motor nerve[99,100]; which is defined as the transient process by which the threshold required to excite a nerve increases in response to the electrical stimulus.[144] Since we did not included a direct measure of accommodation we are unable to determine if muscle fatigue or accommodation is the primary mechanism responsible for the large declines that we observed. However, we hypothesize that accommodation is the primary mechanism responsible for these declines. This hypothesis is based on our observation that the percent declines in NMES-induced torque production were much larger than the

decline in MVIC (e.g., roughly 50% vs. 5%), which has been suggested by others to be a pattern that may indicate motor nerve accommodation as the primary mechanism responsible for declines in NMES-induced torque.[99,100]

The previously mentioned initial NMES training intensities of our current study were near the upper margin of the proposed therapeutic window of 25%-50% MVIC.[144] However, due to the large declines in NMES-induced torque, the mean NMES training intensities fell below the therapeutic window with values of 24.4% MVIC and 20.9% MVIC occurring during the final contraction of the m-NMES and c-NMES conditions. Based on our hypothesis that the declines in NMES-induced torque we observed are primarily attributable to accommodation, this observation demonstrates that after achieving adequate NMES training intensities clinicians also need to implement strategies that limit the impact of accommodation to prevent declines in NMES-induced torque; such as systematically increasing the stimulus intensity.[44] This observation also demonstrates the need for future research toward additional strategies capable of preventing these declines, as m-NMES did not appear to be an effective strategy during our studies or during previous studies.[21,27]

The T-TTI, which is primarily determined by the magnitude and duration of the torque recording, was not significantly different across the two conditions. This finding is contrary to that of our previous study, during which we observed a greater T-TTI under the c-NMES condition. We do not know why the results of our two studies are inconsistent, but it may be attributable to the fact that we observed a significantly higher stimulus intensity under the m-NMES condition during our current study. We expected the duration of the torque recordings to be similar across conditions because the

stimulator "on" times were comparable. However, the isokinetic dynamometer is only able to record torque output when the contraction intensity surpasses the force of gravity, and as a result torque recording durations may differ across conditions while using similar "on" times. The duration of the torque recordings was roughly 1.7 seconds shorter during the m-NMES condition of our previous study, which we hypothesized was a possible explanation for the greater T-TTI we observed during the c-NMES condition. We observed a smaller 1.4 second difference in the torque recordings during our current study, which may explain why we did not observe a difference in favor of c-NMES. We do not know why the torque recording duration is consistently shorter during the m-NMES condition of our studies, but it may be due to the fact that the m-NMES device is designed to deliver the output only to the medial channel during initiation and termination of each contraction cycle to focus on stimulating the vastus medialis.[92]

The significant condition by time interaction for the self-reported discomfort levels is a unique finding of our current study, and it indicates that while using a maximum comfortable stimulus intensity the effect of NMES condition on self-reported discomfort depended upon the level of time. Figure 22 illustrates that a greater rate of decline in self-reported discomfort occurred over time during the c-NMES condition. This greater rate of decline subsequently resulted in significantly lower levels of self-reported discomfort under the c-NMES condition, but only during the last three NMES-induced contractions. Interestingly, the self-reported discomfort levels were not significantly different across the two conditions until the difference was greater than 13 mm, which has been identified as the threshold for determining clinically meaningful differences during a previous NMES study.[36] The primary mechanism responsible for our

observed interaction effect is unknown, but similar to NMES-induced torque the decline in self-reported discomfort levels may be attributable to sensory nerve accommodation.[145,147] Therefore, the greater rate of decline in self-reported discomfort levels we observed during the c-NMES condition may have occurred because the multipath current distribution method resulted in less sensory nerve accommodation, but future research is needed to verify this hypothesis. We are unaware of a clinical benefit for preventing sensory nerve accommodation during NMES treatments, but a greater decline in self-reported discomfort levels may prove beneficial when combating the negative effects of motor nerve accommodation on NMES-induced torque by systematically increasing the stimulus intensity.[44]

The results of our study do not indicate that the novel multipath current distribution method improves any of the outcomes included in our study in a clinically meaningful manner. However, the m-NMES device did perform similarly, which we also believe to be an important observation. Alon[196] has indicated a need for more patient friendly NMES stimulators, which the m-NMES device accomplishes because it is portable and the electrodes are integrated within a neoprene sleeve. The convenience of the m-NMES device may improve patient compliance, which has been proposed as a mechanism by which m-NMES outperformed c-NMES during a recent training study.[87] Therefore, in agreement with previous authors,[21] we believe that the m-NMES device may still serve as a clinically useful option.

Limitations

Using the clinically available m-NMES device with a maximum output of only 79.2 mA likely prevented eight participants from achieving their maximum comfortable stimulus intensity during this condition, and this may have limited our results. Although incorporating the clinically available device during our study may be viewed as a limitation, we feel that it ultimately enhances the clinical applicability of our findings. Despite achieving an original sample size larger than our *a priori* power analyses indicated, we excluded the data of 11 participants during the fatigue and discomfort related comparisons due to a limitation of the c-NMES device that we used. Therefore, our sample size was smaller than desired during five of our comparisons, which may have resulted in a type II error. Excluding the interaction effects for the decline in NMES-induced torque and decline in TTI outcomes, our non-significant observations also had corresponding effect sizes that were below the medium to large threshold used during our *a priori* power analyses. Therefore, we believe that our sample size was adequate for the purpose of our study, which we designed to focus on clinically meaningful differences.

The extent to which our results are generalizable is unclear, due to our use of healthy participants and exclusion of females. The menstrual cycle has been shown to influence self-reported discomfort levels,[162] thus due to our study design requiring repeated measurements over time we felt it was necessary to exclude females. In addition, during exploratory NMES studies, similar in nature to our study, it is common practice to use healthy participants.[21,26,29,30,33,37,44,62,77,99,104,144,145,159] Although participants were required to shave their dominant thigh each day prior to reporting and

the leg was cleansed with a non-alcoholic wipe in an effort to standardize electrical impedance across test sessions, we did not make an attempt to standardize skin impedance via an objective measure. Since skin impedance may vary on a daily basis,[97] this may also be considered a limitation of our study.

Conclusion

Although the novel multipath current distribution method resulted in a significantly greater maximum comfortable stimulus intensity, this did not subsequently result in significantly greater NMES-induced torque production. Therefore, we do not believe the difference in stimulus intensity we observed to be clinically meaningful. Based on our results, it also does not appear that the novel multipath current distribution method positively impacts NMES-induced fatigue and discomfort in a clinically meaningful manner. We believe future NMES research and device development should focus specifically on counteracting motor nerve accommodation in an effort to limit the decline in NMES-induced torque. Similar previous studies[21,27] have observed improved outcomes when comparing m-NMES and c-NMES, but because we did not observe any clinically meaningful differences while using similar electrode configurations across conditions, we believe it is likely that a contributing factor for their improved outcomes is the difference in electrode configuration rather than the novel current distribution method.

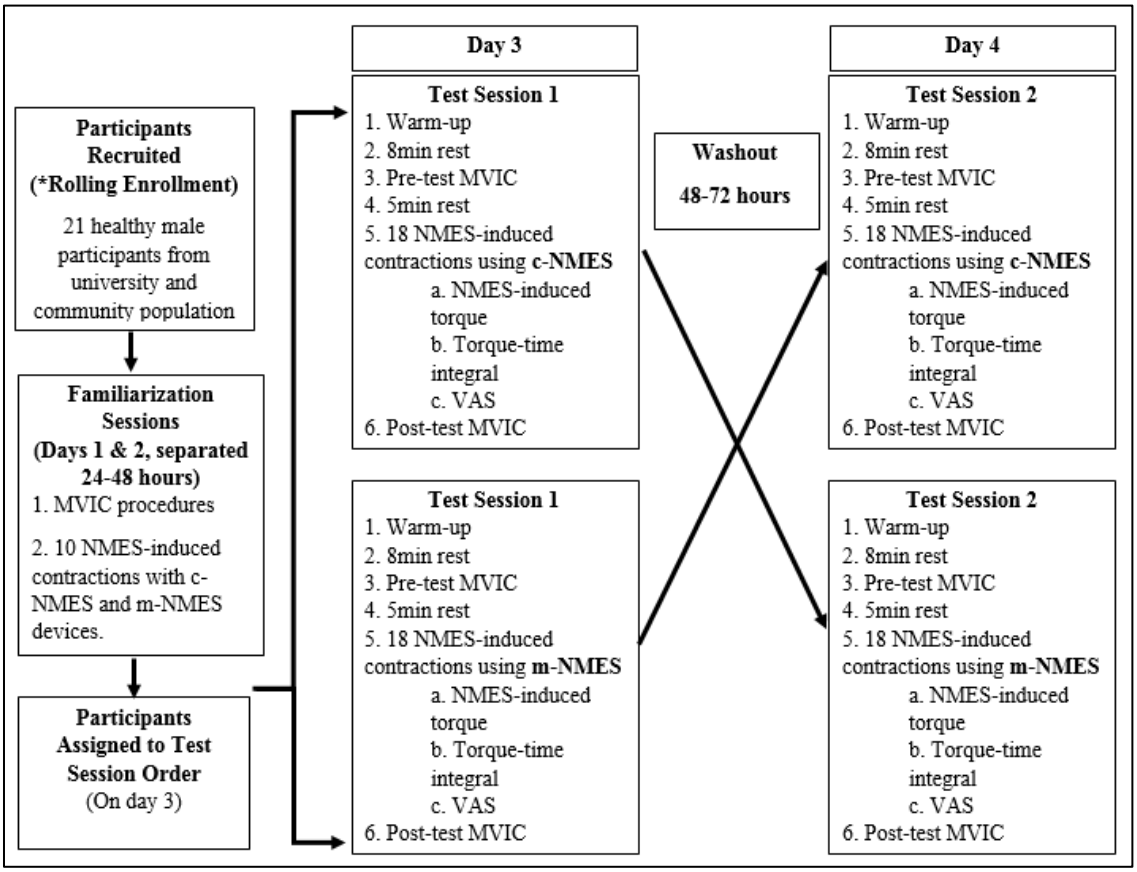


Figure A1. Manuscript 1 Design

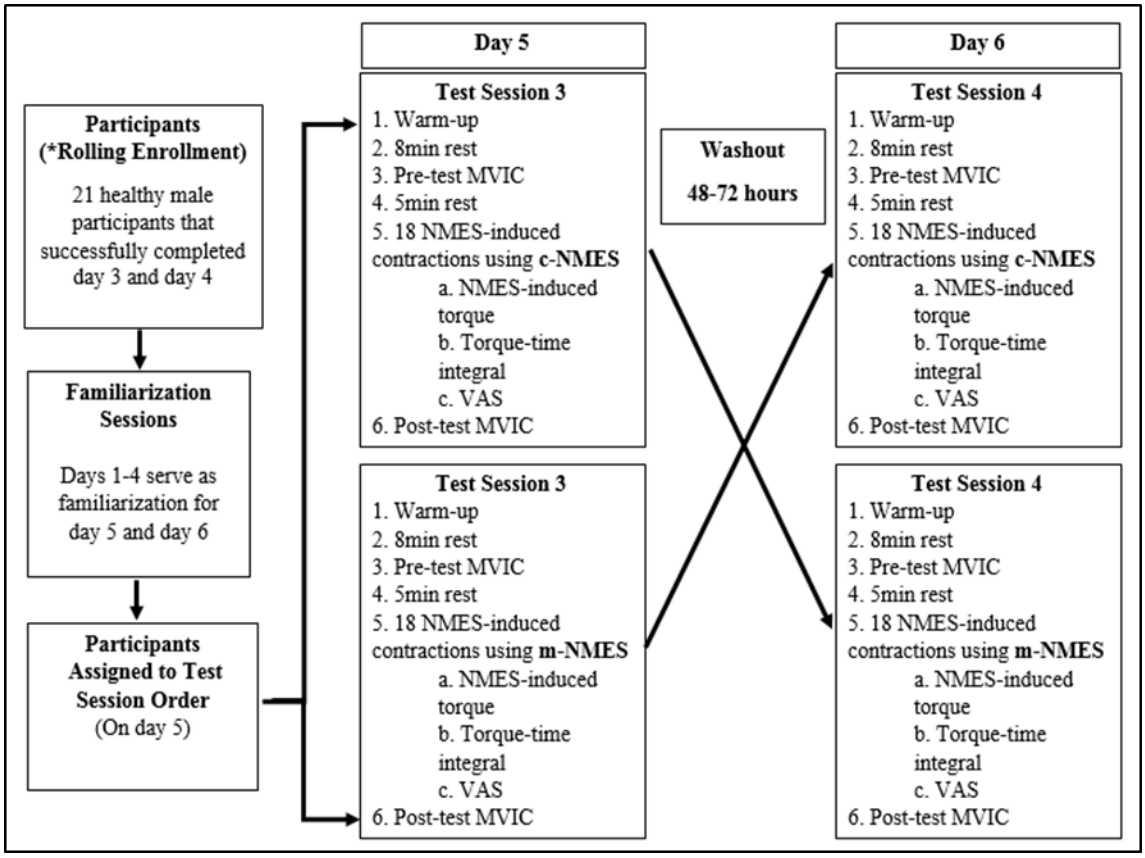


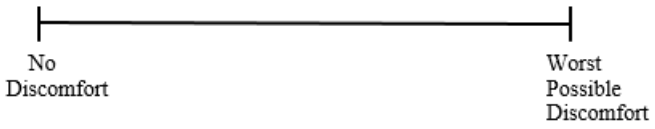
Figure A2. Manuscript 2 Design

APPENDIX B – Visual Analog Scale

Participant #: **Diss2016-_____** Contraction #:

Condition: **m-NMES OR c-NMES**

Day: 3 4 5 6



_____ mm.

APPENDIX C – IRB Approval Letter



THE UNIVERSITY OF
SOUTHERN MISSISSIPPI

INSTITUTIONAL REVIEW BOARD
118 College Drive #5147 | Hattiesburg, MS 39406-0001
Phone: 601.266.5997 | Fax: 601.266.4377 | www.usm.edu/research/institutional.review.board

NOTICE OF COMMITTEE ACTION

The project has been reviewed by The University of Southern Mississippi Institutional Review Board in accordance with Federal Drug Administration regulations (21 CFR 26, 111), Department of Health and Human Services (45 CFR Part 46), and university guidelines to ensure adherence to the following criteria:

- The risks to subjects are minimized.
- The risks to subjects are reasonable in relation to the anticipated benefits.
- The selection of subjects is equitable.
- Informed consent is adequate and appropriately documented.
- Where appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure the safety of the subjects.
- Where appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of all data.
- Appropriate additional safeguards have been included to protect vulnerable subjects.
- Any unanticipated, serious, or continuing problems encountered regarding risks to subjects must be reported immediately, but not later than 10 days following the event. This should be reported to the IRB Office via the "Adverse Effect Report Form".
- If approved, the maximum period of approval is limited to twelve months.
  Projects that exceed this period must submit an application for renewal or continuation.

PROTOCOL NUMBER: 16021902
PROJECT TITLE: A Comparison of Multipath and Conventional Neuromuscular Electrical Stimulation
PROJECT TYPE: New Project
RESEARCHER(S): Cody Bremner
COLLEGE/DIVISION: College of Health
DEPARTMENT: School fo Kinesiology
FUNDING AGENCY/SPONSOR: N/A
IRB COMMITTEE ACTION: Expedited Review Approval
PERIOD OF APPROVAL: 02/26/2016 to 02/25/2017
Lawrence A. Hosman, Ph.D.
Institutional Review Board

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI

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NOTICE OF COMMITTEE ACTION

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- The risks to subjects are minimized.
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- If approved, the maximum period of approval is limited to twelve months.
  Projects that exceed this period must submit an application for renewal or continuation.

PROTOCOL NUMBER: CH16021902
PROJECT TITLE: A Comparison of Multipath and Conventional Neuromuscular Electrical Stimulation
PROJECT TYPE: Change to a Previously Approved Project
RESEARCHER(S): Cody Bremner
COLLEGE/DIVISION: College of Health
DEPARTMENT: School of Kinesiology
FUNDING AGENCY/SPONSOR: N/A
IRB COMMITTEE ACTION: Expedited Review Approval
PERIOD OF APPROVAL: 04/12/2016 to 04/11/2017
Lawrence A. Hosman, Ph.D.
Institutional Review Board

APPENDIX D – Informed Consent

ORI | Office of Research Integrity

INSTITUTIONAL REVIEW BOARD
LONG FORM CONSENT

LONG FORM CONSENT PROCEDURES
This completed document must be signed by each consenting research participant. • The Project Information and Research Description sections of this form should be completed by the Principal Investigator before submitting this form for IRB approval. • Signed copies of the long form consent should be provided to all participants. Last Edited August 28th, 2014

Today's date:		
PROJECT INFORMATION		
Project Title: A comparison of multipath and conventional neuromuscular electrical stimulation		
Principal Investigator: Cody Bremner	Phone: 601-266-5996	Email: Cody.Bremner@eagles.usm.edu
College: Health	Department: School of Kinesiology	

RESEARCH DESCRIPTION

1. **Purpose**:

The primary purpose of this study is to compare fatigue and comfort related outcomes while using multipath and conventional neuromuscular electrical stimulation. This study is being performed to provide insight regarding the efficacy of multipath neuromuscular electrical stimulation during rehabilitation. All data collected may be used for the prinicipal investigator's dissertation, as well as for publications and/or presentations.

2. **Description of Study:**

• Up to 30 male participants between the age of 18 to 35 with no unresolved injuries to the dominant knee joint (leg with which I would choose to kick a soccer ball) or other injuries (e.g., muscular or neurological) that would impact function of the dominant leg. Participants must also report the absence of electrotherapy contraindications (see health and physical activity questionnaire), and must be recreationally active (see physical activity questionnaire).

• If I decide to participate in the study, I will be asked to report to the laboratory for approximately 1 hour on six days each separated by 24 to 72 hours. I will be asked to report at the same time of day (±2 hours) on each occasion. The first two days will serve as orientation days and the last four for testing.
  o During the first day my height, weight, body mass index (BMI) and age will be measured and/or recorded. I will also be asked to complete a brief health and physical activity questionnaire.

• I will be prepared in a similar fashion on each day. I will be asked to shave the front thigh of my dominant leg prior to arriving at my assigned time. In the event that I report to the laboratory unshaven, I will be provided an unused razor and asked to shave the front thigh of my dominant leg. I will be asked to refrain from strenuous activities for 12 hours prior to my reporting each day, and I will be instructed to report well hydrated.

• I will perform a standard warm-up each day, which will include:
  o 5-minutes of cycling on a stationary bike using a self-selected pace.
  o Three 30-second bouts of dynamic quadriceps stretching.
  o Four knee extension muscle actions, in which no movement occurs, (2 at 50%, 1 at 75% and 1 maximum

contraction) on the Biodex dynamometer.

□ I will be positioned with my dominant knee in 60° of knee flexion and asked to remove the shoe from my dominant foot.

□ The axis of rotation of the dynamometer will be aligned to the axis of my dominant knee and my dominant ankle will be strapped tightly in the fixed lever arm (Figure 1).

□ To ensure reliable measurements, straps will be used to prevent unwanted movement.

□ I will be required to keep my hands free from gripping any of the equipment, and I will receive visual feedback and verbal encouragement during testing.

o I will remain seated on the Biodex chair for the remainder of the session.

• Each day following the warm-up I will rest for 8-minutes.

o The front thigh of my dominant leg will be cleaned with a non-alcoholic cleansing wipe.

o A pencil electrode will be used to locate four motor points, defined as the location of skin by which the motor nerve is most hypersensitive to stimulation, on the front of my dominant thigh (Figure 2). The electrical stimulator's intensity will be set to a level that will only cause a muscle contraction when the pencil electrode is passed over the motor point.

o After motor points have been identified they will be marked with a marker (Figure 2)

□ In the event that a motor point is deemed unidentifiable the average position will be marked. The average position of the motor points is as follows: proximal vastus lateralis = 22.5 cm from the anterior superior iliac spine, distal vastus lateralis = 9.5 cm from the superolateral border of the knee cap, proximal rectus femoris motor point is 24.8 cm from the superior border of the knee cap, distal vastus medialis = 10.3 cm from the superomedial border of the knee cap.

• After the 8-minute rest I will perform a series of pre-test maximum voluntary quadriceps contractions (with no knee movement occurring) on the dynamometer using my dominant leg, and each repetition will last 6-seconds. The dynamometer will measure and record the amount of force produced during each contraction, as well as the amount of time force is being produced.

o On day 1 I will be instructed on how to perform the maximum voluntary contractions and will be given an opportunity to practice performing the contractions at a self-selected pace.

□ I will be allowed to practice until I report being comfortable with the procedures, but I will be required to complete a minimum of 6 practice trials.

o On days 2 through 6 I will perform 3 to 6 maximum voluntary quadriceps contractions, each separated by a 2-minute rest period.

□ On days 2 through 6 I will perform maximum voluntary contractions until the peak force of three consecutive repetitions is within 10%, with a maximum of six contractions during each session.

• In the event that I am unable to successfully complete the pre-test maximum contraction procedures on days 3 through 6, I will be asked to return the following day for a second attempt.

• In the event that I am unable to successfully complete the pre-test maximum contraction procedures during the second attempt, I will be excluded from further participation.

o During all pre-test maximum quadriceps contractions I will be:

□ Instructed to gradually increase my effort during the initial portion of each contraction, with maximal effort being reached at roughly 3-seconds and maintained throughout the remaining 3-seconds.

• A picture of an ideal maximum quadriceps contraction curve will be placed in my view to help me understand a gradual increase.

□ Instructed to cross my arms and prepare to push out against the fixed lever arm with maximal effort prior to performing each repetition.

□ Receive real-time visual feedback of my force production via the Biodex computer screen.

□ Receive verbal encouragement from the investigator throughout each repetition.

• Verbal encouragement will begin at the start of each maximum quadriceps contraction and will continue throughout the 6-second repetition.

• Verbal encouragement will be provided in a loud clear voice, and in the event that I show signs of fatigue the loudness and intensity of the encouragement will be gradually increased.

□ Asked to verify that my effort was maximal immediately following each repetition.

• In the event that I or the investigator deem my effort to be submaximal, the repetition will be discarded and repeated following a 2-minute rest.

• Following the pre-test maximum voluntary contractions I will rest for 5-minutes.

o During this rest period self-adherent electrodes and a neoprene garment, or a neoprene garment with integrated electrodes will be placed over the front of my dominant thigh.

□ The self-adherent electrodes will be centered over the previously marked motor points to the extent possible without causing overlap, or the neoprene garment with integrated electrodes will be placed

over my anterior thigh. The self-adherent electrodes will be centered over the previously marked motor points to the extent possible without causing overlap (See Figure 3).

• I will then perform a series of neuromuscular electrical stimulation induced (NMES-induced) quadriceps contractions with my dominant leg. During all NMES-induced contractions I will be asked to "relax my thigh muscles and let the machine do the work", and the investigator will observe each contraction to insure compliance with these instructions. Each NMES-induced contractions will last roughly 10-seconds and I will be given a 50-second rest period between contractions. The dynamometer will measure and record the amount of force produced during each contraction, as well as the amount of time force is being produced.
  o All NMES treatments will be delivered using two different devices (conventional NMES and multipath NMES). The device parameters will be similar between the two devices, and the selected parameters are commonly used within clinical settings.
  o On days 1 and 2 I will be given an opportunity to familiarize myself with both forms of NMES by performing 10 NMES-induced quadriceps contractions using each device (20 total).
    □ The stimulator controls will be placed in a manner that allows me to control the amount of stimulus intensity used during all NMES-induced contractions.
    • I will be instructed to gradually increase the stimulus intensity until I feel a "tingling" sensation. I will then increase the intensity until a muscular response is observed and I report a maximum comfortable stimulus intensity (defined as the highest intensity that does not cause pain).
    • It is anticipated that I will acclimate to the NMES stimulus throughout the NMES-induced contractions on days 1 and 2. Therefore, the investigator will encourage me to increase the stimulus intensity between contractions as well as between sessions to maintain a maximum comfortable stimulus for each repetition.
    □ On day 2 the investigator will verify that I am able to tolerate a sufficient stimulus intensity for testing on days 3 and 4.
    • If it is determined that I am unable to tolerate an adequate stimulus intensity for testing on days 3 and 4, I will be excluded from further participation.
  o On days 3 and 4 I will perform 18 NMES-induced quadriceps contractions using only one NMES device each day.
    □ Prior to performing the NMES-induced contractions the investigator will determine the stimulus intensity required to produce a force output ≥30% of my pre-test maximum voluntary quadriceps contraction.
    • The investigator will gradually increase the stimulus intensity until a NMES-induced contraction of ≥30% of my pre-test maximum voluntary contraction is observed, and this will be held until the 10-second NMES-induced contraction is completed.
      o In the event that I reach a maximum comfortable intensity prior to achieving an adequate contraction, the investigator will immediately decrease the intensity and I will recover for 30-seconds. This process will be repeated a maximum of three times, and if I am unable to tolerate a sufficient stimulus intensity by the third attempt I will be excluded from further participation.
    □ I will be given a 50-second rest period following the identification of the required stimulus intensity.
    □ The identified stimulus intensity will be recorded and will be used (held constant) throughout the 18 NMES-induced contractions.
    □ I will be asked to provide a rating of my perceived comfort by making a vertical mark on the comfort scale immediately following each NMES-induced contraction.
  o On days 5 and 6 I will perform 18 NMES-induced quadriceps contractions using only one NMES device each day.
    □ Prior to performing the NMES-induced contraction I will determine my maximum comfortable stimulus intensity.
    • The stimulator will be placed in a position that will allow me to control the stimulus intensity. The investigator will instruct me to gradually increase the stimulus intensity until I report reaching a maximum comfortable stimulus, and this will be held until the 10-second NMES-induced contraction is completed
    □ I will be given a 50-second rest period following the identification of my maximum comfortable stimulus intensity for days 5 and 6.
    □ The identified stimulus intensity will be recorded and will be used (held constant) throughout the 18 NMES-induced contractions.
    □ I will be asked to provide a rating of my perceived comfort by making a vertical mark on the comfort scale immediately following each NMES-induced contraction.

• Immediately after completing the NMES-induced contractions on days 3 through 6 I will perform a post-test

maximum voluntary quadriceps contraction (with no knee movement occurring) on the dynamometer, and the same procedures used during the pre-test contractions will be followed.

o In general I will only perform a single post-test repetition. However, in the event that I or the investigator deem the contraction to be submaximal I will be given a 10-second rest period and a second post-test maximum voluntary quadriceps repetition will be performed.

3. **Benefits:**

There may not be direct benefits to me in this study. However, I will be exposed to the lab, diagnostic equipment and the modality being tested. I may learn my maximum strength capability during knee extension. In addition, as a participant in this study I will be entered into a lottery for a chance to win one of four $50 Best Buy gift certificates.

4. **Risks:**

One potential risk is that of burns or irritation from electrical current passing through the skin. However, electrical stimulation with these parameters is commonly used in the management of athletic injuries and the amount of research using electrical stimulation is substantial. Therefore, risks of burns or irritation is minimal. There is also minimal risk of knee injury during maximum contractions. I may experience some muscle soreness or muscle injury due to the forceful contractions, but no more than is common with physical activity. If I do experience soreness I should fully recover in a few days. Due to the minimal risks involved in this study there are no treatment procedures.

5. **Confidentiality:**

Any personal identifying records will be locked in a file drawer. Participant data collected by the Biodex computer system will be coded to ensure anonymity (e.g., Diss16-1, Diss16-2).

6. **Alternative Procedures:**

There are no alternative procedures. However, if I no longer wish to participate I may withdraw at any time.

7. **Participant's Assurance:**

This project has been reviewed by the Institutional Review Board, which ensures that research projects involving human subjects follow federal regulations.

Any questions or concerns about rights as a research participant should be directed to the Chair of the IRB at 601-266-5997. Participation in this project is completely voluntary, and participants may withdraw from this study at any time without penalty, prejudice, or loss of benefits.

Any questions about the research should be directed to the Principal Investigator using the contact information provided in Project Information Section above.

CONSENT TO PARTICIPATE IN RESEARCH

Participant's Name: _____

Consent is hereby given to participate in this research project. All procedures and/or investigations to be followed and their purpose, including any experimental procedures, were explained to me. Information was given about all benefits, risks, inconveniences, or discomforts that might be expected.

The opportunity to ask questions regarding the research and procedures was given. Participation in the project is completely voluntary, and participants may withdraw at any time without penalty, prejudice, or loss of benefits. All personal information is strictly confidential, and no names will be disclosed. Any new information that develops during the project will be provided if that information may affect the willingness to continue

participation in the project.

Questions concerning the research, at any time during or after the project, should be directed to the Principal Investigator with the contact information provided above. This project and this consent form have been reviewed by the Institutional Review Board, which ensures that research projects involving human subjects follow federal regulations. Any questions or concerns about rights as a research participant should be directed to the Chair of the Institutional Review Board, The University of Southern Mississippi, 118 College Drive #5147, Hattiesburg, MS 39406-0001, (601) 266-5997.

Include the following information only if applicable. Otherwise delete this entire paragraph before submitting for IRB approval: The University of Southern Mississippi has no mechanism to provide compensation for participants who may incur injuries as a result of participation in research projects. However, efforts will be made to make available the facilities and professional skills at the University. Participants may incur charges as a result of treatment related to research injuries. Information regarding treatment or the absence of treatment has been given above.

Research Participant	**Person Explaining the Study**
Date	Date

APPENDIX E – Physical Activity and Health Questionnaire

1. With which leg would you choose to kick a soccer ball (circle one)?

   Right **Left**

2. To the best of your knowledge, do you have any unresolved knee injuries or other injuries (e.g., muscular or neurological) that would impact leg function of the leg with which you would choose to kick a soccer ball (circle one)?

   Yes **No**

   *If yes, please identify the injury:

3. Do you currently participate in some form of physical activity (e.g. strengthening related activities, jog, run, cycle, swim, tennis etc.) for at least 20 minutes, two times per week (circle one)?

   Yes **No**

4. To the best of your knowledge do you have any of the following conditions (circle one)?
   a. Cardiac disability
   b. Pacemaker
   c. Arterial disease
   d. Blood clots
   e. Pregnancy
   f. Cancer
   g. History of seizures
   h. Sensory impairment
   i. Unstable fractures
   j. Exposed metal implants

   Yes **No**

Height: ___ (cm) / ___ (m) **Weight:** ___ (kg) **Age:** ___ (yrs) **BMI:** ___ / ___ (Kg/m^2)

Participant status: **accept** **reject** Reason for rejection (if applicable):

Participant #: Diss2016-_____

APPENDIX F – Data Collection Sheets

PARTICIPANT DATA COLLECTION SHEET-DAY 1

Participant #: Diss2016-_____

Number of practiced MVICs: 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Repetition with greatest peak torque_____
Peak MVIC (peak torque) @ 60° _____Nm

Order of NMES:

c-NMES/m-NMES OR m-NMES/c-NMES

Stimulus intensity used with c-NMES contraction #1: _____mA
Stimulus intensity used with c-NMES contraction #2: _____mA
Stimulus intensity used with c-NMES contraction #3: _____mA
Stimulus intensity used with c-NMES contraction #4: _____mA
Stimulus intensity used with c-NMES contraction #5: _____mA
Stimulus intensity used with c-NMES contraction #6: _____mA
Stimulus intensity used with c-NMES contraction #7: _____mA
Stimulus intensity used with c-NMES contraction #8: _____mA
Stimulus intensity used with c-NMES contraction #9: _____mA
Stimulus intensity used with c-NMES contraction #10: _____mA
Stimulus intensity used with m-NMES contraction #1: _____mA
Stimulus intensity used with m-NMES contraction #2: _____mA
Stimulus intensity used with m-NMES contraction #3: _____mA
Stimulus intensity used with m-NMES contraction #4: _____mA
Stimulus intensity used with m-NMES contraction #5: _____mA
Stimulus intensity used with m-NMES contraction #6: _____mA
Stimulus intensity used with m-NMES contraction #7: _____mA
Stimulus intensity used with m-NMES contraction #8: _____mA
Stimulus intensity used with m-NMES contraction #9: _____mA
Stimulus intensity used with m-NMES contraction #10: _____mA

Chair (Front/Back): _____

Seat-Back (Front/Back): _____

Dynamometer Height (Up/Down): _____

Participant #: Diss2016-_____

PARTICIPANT DATA COLLECTION SHEET-DAY 2

Participant #: Diss2016-_____

Order of NMES (opposite used on day 1):

c-NMES/m-NMES OR m-NMES/c-NMES

Day 2 Pre-test MVIC (peak torque) @ 60° _____Nm

Day 2 Pre-test MVICs (peak torque by repetition):

1)_____Nm **2)**_____Nm **3)**_____Nm **4)**_____Nm **5)**_____Nm **6)**_____Nm

% difference in MVIC Acceptable (3 consecutive within 10%)? Yes_____ No_____

1 vs. 2_____%, **1 vs. 3**_____%, **2 vs. 3**_____%, **2 vs. 4**_____%, **3 vs. 4**_____%,

3 vs. 5_____%, **4 vs. 5** _____%, **4 vs. 6**_____%, **5 vs. 6**_____%

Pre-test MVIC deemed maximal:

1)_____**2)**_____**3)**_____ **4)**____**5)**____**6)**____

NMES-induced peak torque with c-NMES: _____Nm Repetition _____

NMES-induced peak torque with m-NMES: _____Nm Repetition _____

c-NMES %MVIC: _____/_____=_____%

m-NMES %MVIC: _____/_____=_____%

m-NMES & c-NMES ≥30% MVIC?

YES NO

Stimulus intensity used with c-NMES contraction #1: _____mA
Stimulus intensity used with c-NMES contraction #2: _____mA
Stimulus intensity used with c-NMES contraction #3: _____mA
Stimulus intensity used with c-NMES contraction #4: _____mA
Stimulus intensity used with c-NMES contraction #5: _____mA
Stimulus intensity used with c-NMES contraction #6: _____mA
Stimulus intensity used with c-NMES contraction #7: _____mA
Stimulus intensity used with c-NMES contraction #8: _____mA
Stimulus intensity used with c-NMES contraction #9: _____mA
Stimulus intensity used with c-NMES contraction #10: _____mA
Stimulus intensity used with m-NMES contraction #1: _____mA
Stimulus intensity used with m-NMES contraction #2: _____mA
Stimulus intensity used with m-NMES contraction #3: _____mA
Stimulus intensity used with m-NMES contraction #4: _____mA
Stimulus intensity used with m-NMES contraction #5: _____mA
Stimulus intensity used with m-NMES contraction #6: _____mA
Stimulus intensity used with m-NMES contraction #7: _____mA
Stimulus intensity used with m-NMES contraction #8: _____mA
Stimulus intensity used with m-NMES contraction #9: _____mA
Stimulus intensity used with m-NMES contraction #10: _____mA

Participant #: Diss2016-_____

PARTICIPANT DATA COLLECTION SHEET-DAY 3

Participant #: Diss2016-_____

Randomly assigned order for day 3 and 4: _____
1= c-NMES, m-NMES **2**= m-NMES, c-NMES

Day 3 Pre-test MVIC (peak torque) @ 60° _____Nm

Day 3 Pre-test MVICs (peak torque by repetition):

1)_____Nm **2)**_____Nm **3)**_____Nm **4)**_____Nm **5)**_____Nm **6)**_____Nm

% difference in MVIC Acceptable (3 consecutive within 10%)? Yes_____ No_____

1 vs. 2_____%, **1 vs. 3**_____%, **2 vs. 3**_____%, **2 vs. 4**_____%, **3 vs. 4**_____%,

3 vs. 5_____%, **4 vs. 5** _____%, **4 vs. 6**_____%, **5 vs. 6**_____%

Pre-test MVIC deemed maximal:

1)____**2)**_____**3)**_____ **4)**____**5)**____**6)**____

Repetition Repeated because submaximal:

Day 3- 30% MVIC=_____Nm

Day 3 stimulus intensity to achieve ≥30% MVIC with c-NMES OR m-NMES: _____mA

Day 3 Post-test MVIC (peak torque) @ 60° _____Nm

Post-test MVIC deemed maximal:

1)_____**2)**_____

Day 3 NMES-induced torque contraction #1: _____Nm

Day 3 NMES-induced torque contraction #2: _____Nm

Day 3 NMES-induced torque contraction #3: _____Nm

Day 3 NMES-induced torque contraction #4: _____Nm

Day 3 NMES-induced torque contraction #5: _____Nm

Day 3 NMES-induced torque contraction #6: _____Nm

Day 3 NMES-induced torque contraction #7: _____Nm

Day 3 NMES-induced torque contraction #8: _____Nm

Day 3 NMES-induced torque contraction #9: _____Nm

Day 3 NMES-induced torque contraction #10: _____Nm

Day 3 NMES-induced torque contraction #11: _____Nm

Day 3 NMES-induced torque contraction #12: _____Nm

Day 3 NMES-induced torque contraction #13: _____Nm

Day 3 NMES-induced torque contraction #14: _____Nm

Day 3 NMES-induced torque contraction #15: _____Nm

Day 3 NMES-induced torque contraction #16: _____Nm

Day 3 NMES-induced torque contraction #17: _____Nm

Day 3 NMES-induced torque contraction #18: _____Nm

Participant #: Diss2016-_____

PARTICIPANT DATA COLLECTION SHEET-DAY 4

Participant #: Diss2016-_____

Randomly assigned order for day 3 and 4: _____
1= c-NMES, m-NMES **2**= m-NMES, c-NMES

Day 4 Pre-test MVIC (peak torque) @ 60° _____Nm

Day 4 Pre-test MVICs (peak torque by repetition):

1)_____Nm **2)**_____Nm **3)**_____Nm **4)**_____Nm **5)**_____Nm **6)**_____Nm

% difference in MVIC Acceptable (3 consecutive within 10%)? Yes_____ No_____

1 vs. 2_____%, **1 vs. 3**_____%, **2 vs. 3**_____%, **2 vs. 4**_____%, **3 vs. 4**_____%,

3 vs. 5_____%, **4 vs. 5** _____%, **4 vs. 6**_____%, **5 vs. 6**_____%

Pre-test MVIC deemed maximal:

1)_____**2)**_____**3)**_____ **4)**____**5)**____**6)**____

Repetition Repeated because submaximal:

Day 4- 30% MVIC=_____Nm

Day 4 stimulus intensity to achieve ≥30% MVIC with c-NMES OR m-NMES: _____mA

Day 4 Post-test MVIC (peak torque) @ 60° _____Nm

Post-test MVIC deemed maximal:

1)_____**2)**_____

Day 4 NMES-induced torque contraction #1: _____Nm

Day 4 NMES-induced torque contraction #2: _____Nm

Day 4 NMES-induced torque contraction #3: _____Nm

Day 4 NMES-induced torque contraction #4: _____Nm

Day 4 NMES-induced torque contraction #5: _____Nm

Day 4 NMES-induced torque contraction #6: _____Nm

Day 4 NMES-induced torque contraction #7: _____Nm

Day 4 NMES-induced torque contraction #8: _____Nm

Day 4 NMES-induced torque contraction #9: _____Nm

Day 4 NMES-induced torque contraction #10: _____Nm

Day 4 NMES-induced torque contraction #11: _____Nm

Day 4 NMES-induced torque contraction #12: _____Nm

Day 4 NMES-induced torque contraction #13: _____Nm

Day 4 NMES-induced torque contraction #14: _____Nm

Day 4 NMES-induced torque contraction #15: _____Nm

Day 4 NMES-induced torque contraction #16: _____Nm

Day 4 NMES-induced torque contraction #17: _____Nm

Day 4 NMES-induced torque contraction #18: _____Nm

Participant #: Diss2016-_____

PARTICIPANT DATA COLLECTION SHEET-DAY 5

Participant #: Diss2016-_____

Randomly assigned order for day 5 and 6: _____
1= c-NMES, m-NMES **2**= m-NMES, c-NMES

Day 5 Pre-test MVIC (peak torque) @ 60° _____Nm

Day 5 Pre-test MVICs (peak torque by repetition):

1)_____Nm **2)**_____Nm **3)**_____Nm **4)**_____Nm **5)**_____Nm **6)**_____Nm

% difference in MVIC Acceptable (3 consecutive within 10%)? Yes_____ No_____

1 vs. 2_____%, **1 vs. 3**_____%, **2 vs. 3**_____%, **2 vs. 4**_____%, **3 vs. 4**_____%,

3 vs. 5_____%, **4 vs. 5** _____%, **4 vs. 6**_____%, **5 vs. 6**_____%

Pre-test MVIC deemed maximal:

1)_____**2)**_____**3)**_____ **4)**____**5)**____**6)**____

Repetition Repeated because submaximal:

Day 5 maximum comfortable stimulus intensity with c-NMES OR m-NMES: _____mA

Day 5 Post-test MVIC (peak torque) @ 60° _____Nm

Post-test MVIC deemed maximal:

1)_____**2)**_____

Day 5 NMES-induced torque contraction #1: _____Nm

Day 5 NMES-induced torque contraction #2: _____Nm

Day 5 NMES-induced torque contraction #3: _____Nm

Day 5 NMES-induced torque contraction #4: _____Nm

Day 5 NMES-induced torque contraction #5: _____Nm

Day 5 NMES-induced torque contraction #6: _____Nm

Day 5 NMES-induced torque contraction #7: _____Nm

Day 5 NMES-induced torque contraction #8: _____Nm

Day 5 NMES-induced torque contraction #9: _____Nm

Day 5 NMES-induced torque contraction #10: _____Nm

Day 5 NMES-induced torque contraction #11: _____Nm

Day 5 NMES-induced torque contraction #12: _____Nm

Day 5 NMES-induced torque contraction #13: _____Nm

Day 5 NMES-induced torque contraction #14: _____Nm

Day 5 NMES-induced torque contraction #15: _____Nm

Day 5 NMES-induced torque contraction #16: _____Nm

Day 5 NMES-induced torque contraction #17: _____Nm

Day 5 NMES-induced torque contraction #18: _____Nm

Participant #: Diss2016-_____

PARTICIPANT DATA COLLECTION SHEET-DAY 6

Participant #: Diss2016-_____

Randomly assigned order for day 5 and 6: _____
1= c-NMES, m-NMES **2**= m-NMES, c-NMES

Day 6 Pre-test MVIC (peak torque) @ 60° _____Nm

Day 6 Pre-test MVICs (peak torque by repetition):

1)_____Nm **2)**_____Nm **3)**_____Nm **4)**_____Nm **5)**_____Nm **6)**_____Nm

% difference in MVIC Acceptable (3 consecutive within 10%)? Yes_____ No_____

1 vs. 2_____%, **1 vs. 3**_____%, **2 vs. 3**_____%, **2 vs. 4**_____%, **3 vs. 4**_____%,

3 vs. 5_____%, **4 vs. 5** _____%, **4 vs. 6**_____%, **5 vs. 6**_____%

Pre-test MVIC deemed maximal:

1)____**2)**_____**3)**_____ **4)**____**5)**____**6)**____

Repetition Repeated because submaximal:

Day 6 maximum comfortable stimulus intensity with c-NMES OR m-NMES: _____mA

Day 6 Post-test MVIC (peak torque) @ 60° _____Nm

Post-test MVIC deemed maximal:

1)_____**2)**_____

Day 6 NMES-induced torque contraction #1: _____Nm

Day 6 NMES-induced torque contraction #2: _____Nm

Day 6 NMES-induced torque contraction #3: _____Nm

Day 6 NMES-induced torque contraction #4: _____Nm

Day 6 NMES-induced torque contraction #5: _____Nm

Day 6 NMES-induced torque contraction #6: _____Nm

Day 6 NMES-induced torque contraction #7: _____Nm

Day 6 NMES-induced torque contraction #8: _____Nm

Day 6 NMES-induced torque contraction #9: _____Nm

Day 6 NMES-induced torque contraction #10: _____Nm

Day 6 NMES-induced torque contraction #11: _____Nm

Day 6 NMES-induced torque contraction #12: _____Nm

Day 6 NMES-induced torque contraction #13: _____Nm

Day 6 NMES-induced torque contraction #14: _____Nm

Day 6 NMES-induced torque contraction #15: _____Nm

Day 6 NMES-induced torque contraction #16: _____Nm

Day 6 NMES-induced torque contraction #17: _____Nm

Day 6 NMES-induced torque contraction #18: _____Nm

Participant #: Diss2016-_____

APPENDIX G – Period Effects Tables

Table A1.

Manuscript 1 Cross-over Difference by Sequence

		Percent Decline in MVIC			**Percent Decline in NMES-induced Torque†**		
Sequence	Participant	m-NMES	c-NMES	Δ	m-NMES	c-NMES	Δ
c-NMES/m-NMES	3	2.95	-4.17	7.11	-2.47	-24.65	22.18
	5	-2.92	-1.29	-1.63	-38.18	-29.82	-8.35
	9	-2.53	4.08	-6.61	-33.41	-41.12	7.71
	12	-0.88	6.04	-6.92	-13.94	-14.94	1.00
	16	-3.29	-13.21	9.93	-19.35	-24.76	5.41
	22	-1.45	-5.87	4.42	-21.18	-20.59	-0.59
	24	3.44	-4.37	7.81	-33.35	-28.65	-4.71
m-NMES/c-NMES	2	-7.07	-9.69	2.62	-30.53	-18.82	-11.71
	6	-6.63	-25.38	18.75	-30.59	-13.29	-17.29
	11	-7.24	-6.95	-2.90	-37.06	-10.82	-26.24
	15	-9.35	-5.14	-4.21	-27.65	-33.35	5.71
	23	-5.97	-7.09	1.12	-24.82	-20.88	-3.94
	26	-10.27	-10.98	0.71	-38.76	-32.00	-6.76
	28	-1.40	5.30*	-3.90	-0.94	-27.47	26.53

Table A1 (continued).

		Percent Decline in TTI†			T-TTI (Nm*s)		
Sequence	Participant	m-NMES	c-NMES	Δ	m-NMES	c-NMES	Δ
c-NMES/m-NMES	3	-5.88	-26.06	20.18	11578.27	14979.87	-3401.60
	5	-40.88	-32.12	-8.76	5917.35	3899.24	2018.12
	9	-41.18	-44.71	3.53	7506.35	5437.45	2068.90
	12	-16.65	-23.53	6.88	10584.24	10587.69	-3.45
	16	-23.29	-29.12	5.82	6605.26	7677.51	-1072.25
	22	-38.65	-24.00	-14.65	7428.78	7704.10	-275.32
	24	-36.24	-31.18	-5.06	5656.01	8055.78	-2399.78
m-NMES/c-NMES	2	-34.65	-21.00	-13.65	5667.40	7220.67	-1553.27
	6	-34.88	-17.41	-17.47	4569.03	17579.07	-13010.03
	11	-38.59	-12.65	-25.94	4235.81	6211.09	-1975.27
	15	-30.59	-35.71	5.12	6747.12	6152.91	594.21
	23	-26.59	-21.71	-4.88	5349.50	7107.29	-1757.79
	26	-40.82	-38.12	-2.71	9264.45	11450.03	-2185.58
	28	-2.0	-30.41	28.41	7669.28	7885.37	-216.09

Table A1 (continued).

		Self-reported Discomfort† (mm)		
Sequence	Participant	m-NMES	c-NMES	Δ
c-NMES/m-NMES	3	8.17	16.61	-8.44
	5	41.78	36.39	5.39
	9	23.61	37.50	-13.89
	12	1.50	3.67	-2.17
	16	18.78	4.22	14.56
	22	47.33	37.17	10.17
	24	45.28	51.83	-6.56
m-NMES/c-NMES	2	27.33	22.11	5.22
	6	13.94	52.61	-38.67
	11	5.22	5.50	-0.28
	15	69.67	24.00	45.67
	23	64.17	68.00	-3.83
	26	79.67	11.39	68.28
	28	22.78	35.17	-12.39

Note. †Values are each participant's mean over the 17-18 levels of time. Δ = Cross-over difference (m-NMES – c-NMES)

Table A2.

Manuscript 2 Cross-over Difference by Sequence

		Percent Decline in MVIC			**Percent Decline in NMES-induced Torque†**		
Sequence	Participant	m-NMES	c-NMES	Δ	m-NMES	c-NMES	Δ
c-NMES/m-NMES	2	-13.93	-7.70	-6.23	-23.59	-16.12	-7.47
	5	5.51	2.19	3.33	-23.65	-17.18	-6.47
	12	-0.67	3.72	-4.39	-22.59	-24.76	2.18
	15	-6.93	-6.66	-0.28	-15.94	-32.88	16.94
	22	-9.87	-11.15	1.29	-28.29	-20.29	-8.00
	26	-6.50	-12.76	6.26	-25.59	-36.00	10.41
m-NMES/c-NMES	6	-6.17	-23.61	17.44	-28.24	-12.06	-16.18
	7	-1.33	-6.82	5.49	-15.94	-8.88	-7.06
	9	1.55	-5.72	7.27	-23.12	-29.24	6.12
	16	0.17	-8.30	8.47	-31.35	-21.71	-9.65
	23	-1.79	7.17	-8.96	-10.06	-13.12	3.06

Table A2 (continued).

		Percent Decline in TTI†			T-TTI (Nm*s)		
Sequence	Participant	m-NMES	c-NMES	Δ	m-NMES	c-NMES	Δ
c-NMES/m-NMES	2	-25.18	-16.24	-8.94	6568.59	10095.24	-3526.66
	5	-24.76	-15.59	-9.18	14693.47	14697.32	-3.85
	12	-27.18	-28.06	0.88	21400.74	12571.83	8828.91
	15	-17.41	-35.65	18.24	15847.70	7796.81	8050.89
	22	-29.47	-24.35	-5.12	13703.15	13483.10	220.05
	26	-32.47	-40.35	7.88	6950.39	9296.67	-2346.29
m-NMES/c-NMES	6	-32.12	-16.12	-16.00	7606.23	16041.03	-834.80
	7	-16.71	-7.59	-9.12	12682.56	7658.52	5024.04
	9	-28.47	-36.53	8.06	6076.51	8873.89	-2797.38
	16	-31.94	-21.71	-10.24	11689.01	16803.87	-5114.86
	23	-13.12	-14.12	1.00	5850.50	4422.51	2877.99

Table A2 (continued).

Sequence	Participant	Self-reported Discomfort† (mm)		
		m-NMES	c-NMES	Δ
c-NMES/m-NMES	2	48.61	49.22	-0.61
	5	77.61	70.00	7.61
	12	2.78	2.78	0.00
	15	71.11	60.89	10.22
	22	65.72	66.33	-0.61
	26	72.22	16.44	55.78
m-NMES/c-NMES	6	23.83	41.72	-17.89
	7	44.28	39.39	4.89
	9	14.06	31.22	-17.17
	16	24.39	6.00	18.39
	23	79.83	57.11	22.72

Table A2 (continued).

Sequence	Participant	Maximum Comfortable Stimulus Intensity (mA)			Initial Normalized NMES-induced Torque (Nm)		
		m-NMES	c-NMES	Δ	m-NMES	c-NMES	Δ
c-NMES/m-NMES	1	79.20	62.00	17.20	1.49	0.92	0.57
	2	57.60	69.00	-11.40	1.15	1.39	-0.24
	5	68.00	73.50	-5.50	1.69	1.39	0.30
	11	76.00	51.50	24.50	1.55	0.84	0.71
	12	79.20	55.50	23.70	2.27	1.23	1.04
	15	79.20	55.00	24.20	1.88	1.00	0.89
	17	79.20	80.00	-0.80	2.40	2.34	0.05
	22	79.20	73.50	5.70	1.82	1.41	0.41
	24	54.40	54.50	-0.10	0.77	1.38	-0.61
	26	79.20	71.50	7.70	0.72	0.93	-0.21
m-NMES/c-NMES	3	66.40	55.50	10.90	1.95	1.65	0.31
	6	79.20	84.50	-5.30	0.97	1.45	-0.49
	7	57.60	38.50	19.10	1.46	0.70	0.76
	9	64.00	55.00	9.00	0.82	1.12	-0.31
	13	66.40	62.50	3.90	2.66	2.17	0.50
	14	52.00	52.00	0.00	0.69	0.97	-0.28
	16	79.20	76.00	3.20	1.46	1.61	-0.15
	18	66.40	68.00	-1.60	1.40	1.22	0.18
	23	76.80	59.50	17.30	0.59	0.43	0.15

Table A2 (continued).

		Maximum Comfortable Stimulus Intensity (mA)			Initial Normalized NMES-induced Torque (Nm)		
Sequence	Participant	m-NMES	c-NMES	Δ	m-NMES	c-NMES	Δ
m-NMES/c-NMES	25	72.00	67.00	5.00	1.18	1.05	0.12
	28	40.80	47.50	-6.70	1.06	1.66	-0.60

Note. †Values are each participant's mean over the 17 or 18 levels of time. Δ = Cross-over difference (m-NMES – c-NMES)

APPENDIX H – Simple Effects Analysis Syntax

GLM VisualAnalScore_mNMESMAX_1 VisualAnalScore_mNMESMAX_2
VisualAnalScore_mNMESMAX_3 VisualAnalScore_mNMESMAX_4
VisualAnalScore_mNMESMAX_5 VisualAnalScore_mNMESMAX_6
VisualAnalScore_mNMESMAX_7 VisualAnalScore_mNMESMAX_8
VisualAnalScore_mNMESMAX_9 VisualAnalScore_mNMESMAX_10
VisualAnalScore_mNMESMAX_11 VisualAnalScore_mNMESMAX_12
VisualAnalScore_mNMESMAX_13 VisualAnalScore_mNMESMAX_14
VisualAnalScore_mNMESMAX_15 VisualAnalScore_mNMESMAX_16
VisualAnalScore_mNMESMAX_17 VisualAnalScore_mNMESMAX_18
VisualAnalScore_cNMESMAX_1 VisualAnalScore_cNMESMAX_2
VisualAnalScore_cNMESMAX_3 VisualAnalScore_cNMESMAX_4
VisualAnalScore_cNMESMAX_5 VisualAnalScore_cNMESMAX_6
VisualAnalScore_cNMESMAX_7 VisualAnalScore_cNMESMAX_8
VisualAnalScore_cNMESMAX_9 VisualAnalScore_cNMESMAX_10
VisualAnalScore_cNMESMAX_11 VisualAnalScore_cNMESMAX_12
VisualAnalScore_cNMESMAX_13 VisualAnalScore_cNMESMAX_14
VisualAnalScore_cNMESMAX_15 VisualAnalScore_cNMESMAX_16
VisualAnalScore_cNMESMAX_17 VisualAnalScore_cNMESMAX_18
/WSFACTOR= Condition 2 Time 18
/EMMEANS= TABLES(Condition*Time) COMPARE(Condition)ADJ(BONFERRONI)

APPENDIX I – Photo Release

PHOTO PERMISSION RELEASE

I hereby give my permission to Cody Bremner to use any photo(s) taken of myself during his research entitled "A comparison of multipath and conventional neuromuscular electrical stimulation". The photo(s) will be used for research purposes and for the presentation of the research, which may include presentations (e.g., poster, oral) and publications in print and online.

Name of Participant: Jonathan Pierce

Signature: Jonathan Pierce Date: 8/1/14

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