THE EFFECTS OF NEUROMUSCULAR ELECTRICAL STIMULATION TRAINING ON ANABOLIC SIGNALING IN OLDER ADULTS

by

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DEDICATION

This is dedicated to my father, Larry Goldenstein, because I would not be here without his love, support, and guidance. His biggest dream was for me to get higher education and even though he is no longer here, his voice and dreams still resonate and continue to drive me to achieve more.

ACKNOWLEDGEMENTS

I would like to thank Dr. Mettler for introducing me to research and taking the time to teach, coach, and mentor. The last year and a half have been filled with new experiences and being exposed to new areas of research. Because of the opportunity she gave me, I am able to move onto the next step in a PhD program. I would also like to thank my thesis committee members, Dr. Kipp and Dr. McAllister. Thank you Dr. Kipp for taking the time to answer all of the questions that I had and for being very patient. Thank you, Dr. McAllister, for all the insight on my thesis. I appreciate the time that everyone on the committee has dedicated to helping my thesis be successful.

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Finally, I would like to thank my Dave for the love and support while I ventured down this path. This is the result of the support for the long hours and stressful nights when you supported me. This could not have been possible without so many people involved and I thank every single one for their part.

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LIST OF ABBREVIATIONS

Abbreviation	Description
ADL	activities of daily living
BIA	bioelectric impedance analysis
EMG	electromyography
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
MVC	maximum voluntary contraction
NMES	neuromuscular electrical stimulation
S6K1	p70-S6 kinase 1
VL	vastus lateralis
VMO	vastus medialis oblique
4E-BP1	eukaryotic initiation factor 4E binding protein 1
5RSTS	five repetition sit to stand

ABSTRACT

Neuromuscular electrical stimulation (NMES) generates involuntary muscle contraction and may be a safe and effective alternative for voluntary resistance training. However, further research needs to be done to fully understand the effects of NMES on muscle strength, self-efficacy for activities of daily living (ADL's), and on anabolic signaling. **PURPOSE:** The purpose of this study was to investigate the effects of a 12 session, 4-week neuromuscular electrical stimulation (NMES) intervention in healthy, older adults. The outcomes investigated were anabolic signaling, strength, and self-efficacy of daily activities. **METHODS:** Participants (n = 11; NMES = 8, Sham = 3) consisted of healthy, older adults (mean age: 71.7 ± 7.2 years). Participants performed maximal voluntary contractions (MVC) using the quadriceps on an isokinetic dynamometer, a 5RSTS test, and completed a survey about their self-efficacy for ADL's pre-post-Intervention. Day 1 and Day 12 of the intervention consisted of a muscle biopsy pre-NMES, 30 min post-NMES, and 120 min post-NMES. The participants were randomly placed in a treatment group (NMES) or Sham group. The participants were treated with exact same protocol except the Sham group did not receive stimulation. The NMES was administered 3 times a week for 4-weeks (12 sessions) at 60 Hz for 40 minutes on each leg. **RESULTS:** Phosphorylated S6K1($p = 0.020$) and phosphorylated mTOR ($p = 0.009$) had a significant main effect for time (S6K1 Day 1: Pre-NMES 0.65 ± 0.17, Post-30min 0.98 ± 0.17, Post-120min 1.01 ± 0.19; Post-Intervention: Day 12: Pre-NMES 0.63 ± 0.17, Post-30min 1.25 ± 0.17, Post-120min 0.89 ± 0.21) (mTOR Day 1:

Pre-NMES 0.46 ± 0.13, Post-30min 1.01 ± 0.13, Post-120min 0.92 ± 0.14; Day 12: Pre-NMES 0.49 ± 0.13, Post-30min 0.74 ± 0.14, Post-120min 0.48 ± 0.16) and the post hoc revealed Post-30 min was significantly upregulated when compared to Pre-NMES for both proteins (S6K1 $p = 0.017$, mTOR $p = 0.007$). There was no main effect or interaction for phosphorylated 4E-BP1, MVC, or 5RSTS. The intervention-by-group interaction for ADL Self-efficacy had a medium effect size ($\eta^2 = 0.197$).

CONCLUSION: The findings of this study suggest that a 4-week session of NMES upregulates signaling proteins of the mTORC1 pathway (p-mTOR and p-S6K1) 30 minutes after stimulation. Even though there was no significant difference in MVC or 5RST, there was a medium effect size for self-efficacy ADLs for this preliminary data set. Therefore, further research with more subjects is warranted in order to better understand the effects of this 4-week NMES intervention in older adults.

CHAPTER I

INTRODUCTION

The elderly population is living longer and is currently the fastest growing subpopulation in the growing world (Koopman & Van Loon, 2009). Therefore, it is imperative that the elderly population stays healthy because good health helps older adults remain more independent and maintain a more active lifestyle. However, as age progresses, muscle mass and strength decreases. This is known as sarcopenia (Wall et al. 2013; Koopman & Van Loon 2009) and is expected to affect ~200 million people by 2050 (Churchward-Venne et al. 2013). The European Working Group on Sarcopenia in Older People (EWGSOP) defined sarcopenia as a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life, and death (Offord & Witham 2017). Sarcopenia can have detrimental effects on older adults and can reduce strength, impair functional capacity, and can increase the risk of developing other diseases such as obesity and type II diabetes (Wall et al. 2013). The loss of muscle mass is associated with a decline in physical activity and activities of daily living (ADL's) and can increase the risk of falls and fractures, which can lead to an increased mortality risk (Churchward-Venne et al. 2013).

Sarcopenia may be caused by a multitude of factors. One example is a sedentary lifestyle, which includes reduced levels of physical activity due to bed rest or injury (Churchward-Venne et al. 2013; Offord and Witham 2017). A less than optimal diet with suboptimal protein intake is another possible factor (Churchward-Venne et al. 2013). As individuals age, there is a decrease in the size of muscle fibers and type II fibers (fast

twitch) are affected and transition to type I (slow twitch) (Offord and Witham 2017), which could lead to a decrease in strength. Reduced sensitivity to anabolic stimuli may be another cause (Dirks et al. 2017; Wall et al. 2012).

One proposed mechanism to combat sarcopenia in populations where the ability to perform physical activity is limited or not possible is neuromuscular electrical stimulation (NMES). NMES is a treatment that utilizes electrical current to evoke muscle contractions that mimic voluntary resistance exercise. The electrical current is conducted through electrodes that are placed on the skin that depolarize motor endplates and initiates a muscle contraction (Sillen et al. 2013).

Using electrical stimulation to involuntarily induce a muscle contraction was first reported around 350 years ago by Jan Swammerdam (Gondin et al. 2011); even though he was unable to explain the phenomenon at that time. In 1747, Jean Jallabert electrically stimulated the paralyzed upper limb of a patient who showed increased muscle function after a 3-month treatment period. Luigi Galvani accidently discovered that electrical current could induce a muscle contraction in a frog in 1791 (Gondin et al. 2011). The pioneer of electrotherapy, Guillaume Duchenne de Boulogne, stimulated the facial muscles with electrodes (Gondin et al. 2011). Electrical stimulation continued to advance and be utilized in treating war-related injuries during the first half of the nineteenth century to counteract muscle atrophy resulting from denervation (Gondin et al. 2011). However, electrical stimulation has not always been used in a clinical sense. In 1971, Yakov Kots, used stimulation in hopes that it would be more efficient than voluntary contractions to increase muscle strength in athletes. NMES alone did not produce better

results than voluntary resistance training but had increased benefits when used to complement voluntary resistance training to increase strength (Gondin et al. 2011).

NMES may mimic a voluntary contraction but there are differences between the two. According to the Henneman size principle, during voluntary contraction small fibers are recruited first, then larger fibers (Henneman & Olsen 1965). NMES has a different motor unit pattern and stimulates according to distance and orientation from the electrode (Barss et al. 2018). The superficial fibers will stimulate first and as the intensity increases, deeper fibers will activate (Jubeau et al. 2015; Neyroud et al. 2017; Barss et al. 2018). Voluntary contraction is asynchronous, recruiting fibers at different times varying depending on the time and intensity. Slow twitch fibers are recruited first, followed by fast twitch fibers as more force is needed (Henneman & Olsen 1965). NMES produces a synchronous contraction and stimulates slow and fast twitch fibers at the same time (Jubeau et al 2015). This may cause an increased metabolic demand which can lead to increased fatigue with NMES as compared to voluntary muscle contraction (Barrs et al. 2018; Jubeau et al 2015; Neyroud et al 2017).

NMES has been used as an alternative to exercise in clinical settings to strengthen and maintain muscle mass, treat individuals with osteoarthritis and those recovering from surgery (Dirks et al. 2014; Kern et al. 2014). NMES can increase strength when high frequency stimulation was applied to stroke patients (Doucet & Griffin 2013). Increased contralateral strength (Cattagni et al. 2018) was also demonstrated when the opposite leg was stimulated.

NMES has also been shown to work at the cellular level and increase anabolic signaling (mTORC1 pathway proteins such as mTOR and S6K1) in a single bout (Mettler

et al. 2017; Mettler et al. 2018; Wall et al. 2012) and with multiple sessions (Dirks et al, 2014). Dirks et al. (2014) demonstrated that NMES prevented the loss of muscle mass during a 5-day leg immobilization. Twenty-four healthy, young males had one leg immobilized and no significant muscle loss was detected with NMES. Wall et al. (2014) was the first study to show NMES stimulated anabolic signaling. Six elderly, diabetic men received a 60-min bout of unilateral NMES and showed an increase in anabolic signaling after one session (Wall et al. 2014). Dirks et al. (2016) continued the research for anabolic signaling and also concluded that NMES stimulated anabolic signaling after stimulating one leg of eighteen elderly men for a single session of 70 minutes. Increased anabolic signaling was also shown to increase in healthy older adults and stroke patients after a single NMES session (Mettler et al. 2017). In another study, Mettler et al. (2018) demonstrated that high-frequency stimulation was more effective at increasing the anabolic signaling when compared to low-frequency stimulation. Therefore, NMES can be beneficial in many ways.

NMES Protocols

However, other studies have shown no change in strength and muscle mass with repeated bouts of NMES treatment (Reidy et al. 2017). One possible reason for this may be the wide array of protocols with varying stimulation parameters and further studies need to be completed to define the most effective protocol. Some studies allow the patients to set the stimulation intensity to their tolerance, being advised to increase it to the maximum intensity they can tolerate that creates a contraction of the muscle (Kern et al. 2014; Vivodtzev et al. 2012; Reidy et al. 2017; Dirks et al. 2014; Dirks et al. 2015; Dirks et al. 2016; Wall et al. 2012) whereas others set a prescribed intensity of the

maximum voluntary contraction (MVC) (Cattagni et al. 2018; Mettler et al. 2017; Mettler et al. 2018). One issue with allowing the subject to set their own intensity is that maximal tolerance is very subjective and will vary greatly between individuals. Two subjects self-selected intensity could be very different and may not select a high enough intensity to achieve the expected results, such as increased anabolic signaling or increased strength.

Protocols also vary in pulse width, duty cycle, frequency, total treatment time, and duration of treatment. Dirks et al. (2014) had a 40-minute protocol, with 5 minutes of a warm-up phase, 30 minutes at 100 Hz, 400 µs, duty cycle of 5 s on and 10 s off, with a 5 min cool down phase. Whereas, Wall et al. (2018) administered the treatment for 60 minutes at 60 Hz, 500 µs, and a duty cycle of 3 s on and 3 s off. Dirks et al. (2014) demonstrated prevention of muscle mass loss and Wall et al. (2018) showed increase of anabolic signaling. Another study administered the treatment for 60 minutes at 200 µs, a duty cycle of 10 s on and 15 s off but adjusted the Hz (20 and 60Hz) for different groups (Mettler et al. 2018) and the same protocol was used in the next study by the researcher but only at 60 Hz (Mettler et al. 2017). In a different study, two separate frequencies (20 and 40 Hz) were used as well but performed with a different protocol, having a 20 Hz group that had a duty cycle of 10 s on and 10 s off for 40 minutes and the 40 Hz used a duty cycle of 5 s on and 5 s off (Doucet & Griffin 2013). All the previous studies demonstrated positive outcomes with NMES treatment but with so many differences in protocols throughout studies, it makes it hard to determine which is the optimal protocol.

Different frequencies can have an effect on the treatment and can vary from 20 Hz to 100 Hz. When the same intensity was applied with 20 Hz versus 60 Hz, the 20 Hz protocol maintained torque output better than the 60 Hz (Mettler et al. 2018). However,

when the two frequencies were compared and the anabolic signaling was analyzed, the 60 Hz groups showed significantly upregulated anabolic signaling when compared to the 20 Hz group (Mettler et al. 2018). Doucet and Griffin (2013) demonstrated that low frequency (20 Hz) improved endurance while high frequency (40 Hz) increased strength and motor activation in stroke patients. Long-term low frequency has been shown to modify fast twitch fibers to slow twitch fibers (Herzig et al. 2015), which could explain the increase in endurance for the low frequency group.

Functional Outcomes

A significant functional outcome that may result from NMES is an improvement in strength. NMES can be an alternative to traditional resistance training as a safer treatment for injured or older adults. There is conflicting evidence regarding whether or not NMES increases strength but as stated above, this could be a result of difference in protocol parameters across studies. Reidy et al. (2017) applied NMES to healthy older adults that were on bed rest for 5 days. The intervention was applied 3 times a day for 25 minutes each session, for a total of 12 sessions. The NMES group and the control group's strength decreased at the same rate and no strength was preserved with this treatment during 5 days of bed rest. However, a study performed on chronic obstructive pulmonary disease (COPD) found an 11% increase of strength over 6 weeks (Vivodtzev et al. 2012). With 9 weeks of a NMES intervention, a significant increase in strength of the quadricep was recorded in older adults (Kern et al. 2014). Cattagni et al. (2018) showed 4-5% increase of strength and increased EMG on the contralateral quadricep. Bax et al. (2005) found a significant increase in strength when the NMES was applied with knee flexed as when compared to stimulation with the knee extended but when NMES was applied at

≥50% or ≤30% of their MVC but there were no differences between the groups. In a review performed by Herzig et al. (2015), the researchers concluded that MVC increased anywhere from 7% to 62% in subjects receiving NMES.

Anabolic Signaling

The effectiveness of NMES needs to be examined further at the cellular level in older adults. The mammalian target of rapamycin complex 1 (mTORC1) is one of the major signaling pathways for muscle growth in response to resistance training stimuli. Akt stimulates the mTOR complex 1 (mTORC1) after several interactions. Muscle mass is regulated by mTORC1 and can be stimulated by many sources such as mechanical stress, nutrients, and hormones (Ogasawara et al. 2014). However, it can be suppressed by activation of AMP-activated protein kinase (AMPK) (Kazior et al. 2016). Two downstream proteins of the mTORC1 pathway are eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and ribosomal protein S6 kinase 1 (S6K1) (Schiaffino et al. 2013). Both 4E-BP1 and S6K1 are associated with cell growth and are activated by phosphorylation but S6K1 upregulates muscle growth whereas 4E-BP1 inhibits muscle growth when in the phosphorylated state (Lim et al. 2017).

One common mechanism to stimulate mTORC1 is through mechanical stress, such as voluntary contractions through resistance training. Studies have shown that mTORC1 signaling is increased with resistance training (Ogasawara et al. 2014). Male weight lifters perform 3 sets of 6 repetitions at 60% 1 RM which resulted in a 288% increase in mTOR, 809% increase in S6K1, and a 139% increase in 4E-BP1 3 hours after exercise when compared to the pre-exercise levels (Lim et al. 2017). Even though 4E-BP1 increased, it was a smaller increase when compared to mTOR and S6K1. Kazior et

al. (2016) showed in increase in total mTOR with combined voluntary resistance and endurance cycling after 7 weeks of training when compared to resistance training alone. Overall, it is demonstrated that mechanical stress is an effective way to stimulate the mTORC1 pathway.

Li et al. (2012) investigated the difference in mTOR signaling in young adults versus older adults. The researchers found that 12-weeks of resistance training did not affect phosphorylation of mTOR, 4E-BP1, or S6K1. However, it was reported that mTOR phosphorylation and 4E-BP1phosphorylation were lower overall in older adults when compared to younger adults by 41% and 30% after 12-weeks of training. S6K1 appeared not to be affected by age, as no significant difference was demonstrated between the two groups after training (Li et al. 2012). Reduced responsiveness of the mTORC1 pathway stimulation was also found in elderly subjects when compared to young subjects after resistance training in two other studies as well (Kumar et al. 2009; Fry et al. 2011). However, a later study demonstrated that by doubling the volume (from 3 to 6 sets), muscle protein synthesis was increased in the elderly and were equivalent to the young when measured over 4 hours post training (Churchward-Venne et al. 2013).

NMES has been used as an alternative to strengthen muscle in place of resistance training. However, little cellular level mechanistic evidence is available to justify that NMES stimulates muscle growth in the same way as voluntary muscle contractions. One investigation showed an increase in phosphorylated and total mTOR and S6K1 after stimulating the triceps surae muscle in rats (Ogasawara et al. 2014). Phosphorylated mTOR was highest immediately after exercise and continued to remain elevated compared to pre-training for 3 hours. S6K1 phosphorylation was also upregulated

immediately after exercise and then continued to be elevated for 3 hours (Ogasawara et al. 2014). Another study in mice reported that S6K1 was elevated 3 hours after stimulation (Witkowski et al. 2010). The first study to demonstrate that NMES increased mTORC1 pathway activation in humans was Wall et al. (2012). The researchers stimulated one leg of diabetic men for 60 minutes and took muscle biopsies at 2- and 4-hour time points after the stimulation from the stimulated leg and the control leg that did not receive stimulation. Muscle mTOR and S6K1 phosphorylation was elevated at both time points when compared to the control leg. Another study confirmed these findings, showing that mTOR and S6K1 were increased 4 hours after receiving NMES (Dirks et al. 2016).

The effects of frequency of NMES protocols on anabolic signaling was investigated by Mettler et al. (2018). Results showed an increase in phosphorylated mTOR and phosphorylated S6K1 with both low (20 Hz) and high (60 Hz) frequency groups following a single bout of NMES. However, anabolic response was higher in the high frequency groups. No significant change was demonstrated with 4E-BP1 phosphorylation in either condition. Studies have investigated the effects of NMES on neurological disease. One was in mice with an incomplete spinal cord injury. The mice were stimulated for 5 weeks, starting 48 hours post injury. The NMES group had higher values for p-S6K1 compared to the control group (Freitas et al. 2018). Another study on human subjects, investigated the effect on anabolic signaling in stroke patients when compared to healthy older adults (Mettler et al. 2017). A protocol of 60 Hz was used based on a previous study that showed upregulated anabolic signaling after a single bout of NMES in the hemiplegic leg of individuals with stroke as well as healthy older adults,

with no difference between groups. Both groups had an increase in phosphorylated mTOR and phosphorylated S6K1 and a decrease of 4E-BP1 after 60 minutes of NMES. Both of these studies show that the muscles can be directly stimulated via involuntary activation and to increase anabolic signaling regardless of a neurological deficit.

All of the results show promise for NMES as an effective alternative to voluntary exercise, which is an important need for older adults. The anabolic signaling pathway is stimulated by NMES and appears to mimic the effects of voluntary exercise. While NMES induces a muscle contraction differently from voluntary muscle contractions, NMES may activate the same cellular responses as a voluntary contraction which may translate to improvements in physical function. However, the full effect of NMES is not known and further research needs to be done to understand how NMES affects anabolic signaling in healthy, older skeletal muscle when NMES is applied repeatedly over a 4-week period.

Self-efficacy for ADL's

To our knowledge, no research has been done on the self-efficacy for activities of daily living following an NMES intervention. It is possible that if strength is increased, then the individual's self-efficacy to perform activities of daily living will increase. Self-efficacy is an individual's perception of their ability to engage in a behavior. In other words, the individual's self-confidence in completing a task or activity (Lox et al. 2010). An older adult may feel confident walking on a flat path from their living room to their bedroom but are less confident when they have to walk downstairs into their basement because they feel unstable going down the stairs. Self-efficacy is important because behavior is influenced through thought (Bandura, 1977). Therefore, if the individual does

not have a high belief that they can accomplish simple everyday tasks, they will have a harder time performing the tasks and may avoid performing the tasks all together. Self-efficacy theory describes several antecedents and consequences of self-efficacy. Two antecedents are physiological and affective states (Lox et al. 2010). An example of the physiological state would be when an older individual experiences fatigue when walking or getting out of a chair. People partially judge their anxiety or stress based on their state of physiological arousal and will more likely have success if low physiological arousal is experienced (Bandura 1977). An affective example is the emotional state, which could be positive or negative (Lox et al. 2010). If an elderly person previously fell while going down the stairs, a negative emotion would be associated with going down the stairs, which could reduce self-efficacy for that activity. However, if the individual increases strength (a physiological response), this could help them perform ADL's more efficiently. Being more efficient at ADL's, could lead to more positive emotions towards these activities and increase their self-efficacy.

One important consequence of self-efficacy is physical activity behavior (Lox et al. 2010). If an older adult does not believe that they can perform a simple task such as stooping or kneeling, it could affect their physical activity level (Mullen et al., 2012). Over 800 adults were first surveyed about their self-efficacy of walking, then were administered basic functional tests. The results showed that a higher self-efficacy of walking was positively associated with better function and fewer limitations (Mullen et al., 2012). A decreased physical activity level can have severe implications that can lead to disability, decreased quality of life, and other diseases.

Self-efficacy for ADL's is important for older adults to maintain a physically active lifestyle. Research is needed on whether NMES treatment can increase ADL self-efficacy. If NMES increases strength, then self-efficacy for daily activities may increase. Studies have shown changes in self-efficacy as a result of a resistance training intervention in older adults (Kekalainen et al. 2018; Neupert et al. 2009). In the present study, ADL self-efficacy was assessed pre- and post-intervention to determine if the treatment can improve this aspect of confidence.

Statement of Purpose

The purpose of this study was to investigate the effects of a 4-week neuromuscular electrical stimulation (NMES) intervention in healthy, older adults. The outcomes investigated were anabolic signaling, strength, physical function, and ADL self-efficacy.

Hypotheses

The study will address the following hypotheses:

1. In healthy older adults, anabolic signaling of the mTORC1 pathway will increase with a single bout of the NMES intervention and will remain elevated 30 minutes and 120 minutes after NMES treatment when compared to resting or pre-stimulation levels in the NMES treatment group. Anabolic signaling will be highest at 30 minutes post-intervention.
2. In healthy older adults, anabolic signaling will be upregulated after 4 weeks (treatment Day 12) of the NMES treatment in the NMES group when compared to

Day 1 of the NMES treatment at all three time points, (Pre-NMES, post- 30 minutes, and post- 120 minutes).

3. In healthy older adults, strength will be increased after the 4-week NMES intervention in the NMES treatment group. No change in strength will be observed in the Sham group.
4. In healthy older adults, the 5 repetition sit to stand (5RSTS) time will decrease after the 4-week NMES intervention in the NMES treatment group. No change will be observed in the Sham group.
5. Self-efficacy for ADL's will increase following the 4-week NMES intervention in the NMES treatment group. No change will be observed in the Sham group.

Operational Definitions

1. NMES Protocol: The protocol consists of 40 minutes each leg with a duty cycle of 10 seconds on and 15 seconds off at a frequency of 60 Hz and a pulse width of 200 microseconds. The intensity is set to 15% of the subjects MVC.
2. Older adults: Aged 60 and older
3. Anabolic signaling: Protein synthesis in skeletal muscle through the mTORC1 pathway
4. Pre-intervention: Timepoint that is at the beginning of the study prior to the start of the NMES intervention
5. Post-intervention: Timepoint that is at the end of the study after 12 NMES intervention sessions
6. Day 1: First day of the intervention
7. Day 12: Last day of the intervention

8. Biopsy timepoints:
    a. Pre-NMES: biopsy performed before the NMES treatment
    b. Post-30 minutes: biopsy performed 30 minutes after the NMES treatment
    c. Post-120 minutes: biopsy performed 120 minutes after the NMES treatment

Limitations and Delimitations

Limitations

1. This study was limited to healthy older adults and may not translate directly to other populations.
2. The protocol was specific to this study and may not apply to protocols with different parameters.
3. The 4-week protocol was specific to this study and longer training may result in different outcomes.
4. A small sample size was obtained and a larger population may provide increased statistical power to decrease Type II error.

Delimitations

1. This study was delimited to healthy adults 60 and older.
2. This study was delimited to the quadricep muscle.
3. This study was delimited to the 40 minutes NMES protocol on each leg at a frequency of 60 Hz.

Significance

Sarcopenia is a growing disease amongst older adults and can have a negative impact on their quality of life and increase mortality risk. Common treatments to combat this condition are through nutrition and exercise. However, frail, older adults may not be able to safely exercise and need an alternate means to stimulate muscular activity. The effects of NMES has been shown to mimic voluntary contractions and could be a safe and effective treatment when the ability for regular exercise is limited. Increases in strength have been shown to be a result of NMES which could improve an individual's quality of life by being able to perform ADL's more easily. As older adults are able to complete these everyday tasks more, the higher their self-efficacy will be, which could in turn increase their physical activity. This study encompasses a comprehensive approach and is looking at the effects of NMES on cellular, functional, and psychological areas to get a better understanding of the treatment. However, the most effective way to increase strength through NMES is unknown and further research still needs to be conducted. Further research also needs to be conducted to investigate the cellular changes with NMES treatment to fully understand how NMES affects muscle growth. Overall, NMES is a promising treatment to help combat sarcopenia and increase strength in older adults.

CHAPTER II

MANUSCRIPT

The elderly population is living longer and is currently the fastest growing subpopulation in the growing world (Koopman & Van Loon, 2009). Therefore, it is imperative that the elderly population stays healthy because good health helps older adults remain more independent and maintain a more active lifestyle. However, as age progresses, muscle mass and strength decreases. This is known as sarcopenia (Wall et al. 2013; Koopman & Van Loon 2009) and is expected to affect ~200 million people by 2050 (Churchward-Venne et al. 2013). The European Working Group on Sarcopenia in Older People (EWGSOP) defined sarcopenia as a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life, and death (Offord & Witham 2017). Sarcopenia can have detrimental effects on older adults and can reduce strength, impair functional capacity, and can increase the risk of developing other diseases such as obesity and type II diabetes (Wall et al. 2013). The loss of muscle mass is associated with a decline in physical activity and activities of daily living (ADL's) and can increase the risk of falls and fractures, which can lead to an increased mortality risk (Churchward-Venne et al. 2013).

Sarcopenia may be caused by a multitude of factors. One example is a sedentary lifestyle, which includes reduced levels of physical activity due to bed rest or injury (Churchward-Venne et al. 2013; Offord and Witham 2017). A less than optimal diet with suboptimal protein intake is another possible factor (Churchward-Venne et al. 2013). As individuals age, there is a decrease in the size of muscle fibers and type II fibers are

affected and transition to type I (Offord and Witham 2017), which could lead to a decrease in strength. Reduced sensitivity to anabolic stimuli may be another cause (Dirks et al. 2017; Wall et al. 2012).

One proposed mechanism to combat sarcopenia in populations where physical activity is limited or is not possible is neuromuscular electrical stimulation (NMES). NMES is a treatment that utilizes electrical current to evoke muscle contractions that mimic voluntary resistance exercise. The electrical current is conducted through electrodes that are placed on the skin that depolarize motor endplates and initiates a muscle contraction (Sillen et al. 2013).

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NMES has been used as an alternative to exercise in clinical settings to strengthen and maintain muscle mass, treat individuals with osteoarthritis and those recovering from surgery (Dirks et al. 2014; Kern et al. 2014). NMES can increase strength when high frequency stimulation was applied to stroke patients (Doucet & Griffin 2013). Increased contralateral strength (Cattagni et al. 2018) was also demonstrated when the opposite leg was stimulated.

NMES has also been shown to work at the cellular level and increase anabolic signaling (mTORC1 pathway proteins such as mTOR and S6K1) in a single bout (Mettler et al. 2017; Mettler et al. 2018; Wall et al. 2012) and with multiple sessions (Dirks et al, 2014). Dirks et al. (2014) demonstrated that NMES prevented the loss of muscle mass during a 5-day leg immobilization. Twenty-four healthy, young males had one leg immobilized and no significant muscle loss was detected with NMES. Wall et al. (2014) was the first study to show NMES stimulated anabolic signaling. Six elderly, diabetic men received a 60-min bout of unilateral NMES and showed an increase in anabolic signaling after one session (Wall et al. 2014). Dirks et al. (2016) continued the research for anabolic signaling and also concluded that NMES stimulated anabolic signaling after stimulating one leg of eighteen elderly men for a single session of 70 minutes. Increased anabolic signaling was also shown to increase in healthy older adults and stroke patients after a single NMES session (Mettler et al. 2017). In another study, Mettler et al. (2018) demonstrated that high-frequency stimulation was more effective at increasing the anabolic signaling when compared to low-frequency stimulation. Therefore, NMES can be beneficial in many ways.

Functional Outcomes

A significant functional outcome that may result from NMES is an improvement in strength. NMES can be an alternative to traditional resistance training as a safer treatment for injured or older adults. There is conflicting evidence regarding whether or not NMES increases strength but as stated above, this could be a result of difference in protocol parameters across studies. Reidy et al. (2017) applied NMES to healthy older adults that were on bed rest for 5 days. The intervention was applied 3 times a day for 25 minutes each session, for a total of 12 sessions. The NMES group and the control group's strength decreased at the same rate and no strength was preserved with this treatment during 5 days of bed rest. However, a study performed on chronic obstructive pulmonary disease (COPD) found an 11% increase of strength over 6 weeks (Vivodtzev et al. 2012). With 9 weeks of a NMES intervention, a significant increase in strength of the quadricep was recorded in older adults (Kern et al. 2014). Cattagni et al. (2018) showed 4-5% increase of strength and increased EMG on the contralateral quadricep. Bax et al. (2005) found a significant increase in strength when the NMES was applied with knee flexed as when compared to stimulation with the knee extended but when NMES was applied at ≥50% or ≤30% of their MVC but there were no differences between the groups. In a review performed by Herzig et al. (2015), the researchers concluded that MVC increased anywhere from 7% to 62% in subjects receiving NMES.

Anabolic Signaling

The effectiveness of NMES needs to be examined further at the cellular level in older adults. The mammalian target of rapamycin complex 1 (mTORC1) is one of the major signaling pathways for muscle growth in response to resistance training stimuli.

Akt stimulates the mTOR complex 1 (mTORC1) after several interactions. Muscle mass is regulated by mTORC1 and can be stimulated by many sources such as mechanical stress, nutrients, and hormones (Ogasawara et al. 2014). However, it can be suppressed by activation of AMP-activated protein kinase (AMPK) (Kazior et al. 2016). Two downstream proteins of the mTORC1 pathway are eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and ribosomal protein S6 kinase 1 (S6K1) (Schiaffino et al. 2013). Both 4E-BP1 and S6K1 are associated with cell growth and are activated by phosphorylation but S6K1 upregulates muscle growth whereas 4E-BP1 inhibits muscle growth when in the phosphorylated state (Lim et al. 2017).

One common mechanism to stimulate mTORC1 is through mechanical stress, such as voluntary contractions through resistance training. Studies have shown that mTORC1 signaling is increased with resistance training (Ogasawara et al. 2014). Male weight lifters perform 3 sets of 6 repetitions at 60% 1 RM which resulted in a 288% increase in mTOR, 809% increase in S6K1, and a 139% increase in 4E-BP1 3 hours after exercise when compared to the pre-exercise levels (Lim et al. 2017). Kazior et al. (2016) showed in increase in total mTOR with combined voluntary resistance and endurance cycling after 7 weeks of training when compared to resistance training alone. Overall, it is demonstrated that mechanical stress is an effective way to stimulate the mTORC1 pathway.

Li et al. (2012) investigated the difference in mTOR signaling in young adults versus older adults. The researchers found that 12-weeks of resistance training did not affect phosphorylation of mTOR, 4E-BP1, or S6K1. However, it was reported that mTOR phosphorylation and 4E-BP1phosphorylation were lower overall in older adults

when compared to younger adults by 41% and 30% after 12-weeks of training. S6K1 appeared not to be affected by age, as no significant difference was demonstrated between the two groups after training (Li et al. 2012). Reduced responsiveness of the mTORC1 pathway stimulation was also found in elderly subjects when compared to young subjects after resistance training in two other studies as well (Kumar et al. 2009; Fry et al. 2011). However, a later study demonstrated that by doubling the volume (from 3 to 6 sets), muscle protein synthesis was increased in the elderly and were equivalent to the young when measured over 4 hours post training (Churchward-Venne et al. 2013).

NMES has been used as an alternative to strengthen muscle in place of resistance training. However, little cellular level mechanistic evidence is available to justify that NMES stimulates muscle growth in the same way as voluntary muscle contractions. One investigation showed an increase in phosphorylated and total mTOR and S6K1 after stimulating the triceps surae muscle in rats (Ogasawara et al. 2014). Phosphorylated mTOR was highest immediately after exercise and continued to remain elevated compared to pre-training for 3 hours. S6K1 phosphorylation was also upregulated immediately after exercise and then continued to be elevated for 3 hours (Ogasawara et al. 2014). Another study in mice reported that S6K1 was elevated 3 hours after stimulation (Witkowski et al. 2010). The first study to demonstrate that NMES increased mTORC1 pathway activation in humans was Wall et al. (2012). The researchers stimulated one leg of diabetic men for 60 minutes and took muscle biopsies at 2- and 4-hour time points after the stimulation from the stimulated leg and the control leg that did not receive stimulation. Muscle mTOR and S6K1 phosphorylation was elevated at both time points when compared to the control leg. Another study confirmed these findings,

showing that mTOR and S6K1 were increased 4 hours after receiving NMES (Dirks et al. 2016).

The effects of frequency of NMES protocols on anabolic signaling was investigated by Mettler et al. (2018). Results showed an increase in phosphorylated mTOR and phosphorylated S6K1 with both low (20 Hz) and high (60 Hz) frequency groups following a single bout of NMES. However, anabolic response was higher in the high frequency groups. No significant change was demonstrated with 4E-BP1 phosphorylation in either condition. Studies have investigated the effects of NMES on neurological disease. One was in mice with an incomplete spinal cord injury. The mice were stimulated for 5 weeks, starting 48 hours post injury. The NMES group had higher values for p-S6K1 compared to the control group (Freitas et al. 2018). Another study on human subjects, investigated the effect on anabolic signaling in stroke patients when compared to healthy older adults (Mettler et al. 2017). A protocol of 60 Hz was used based on a previous study that showed upregulated anabolic signaling after a single bout of NMES in the hemiplegic leg of individuals with stroke as well as healthy older adults, with no difference between groups. Both groups had an increase in p-mTOR and p-S6K1 and a decrease of 4E-BP1 after 60 minutes of NMES. Both of these studies show that the muscles can be directly stimulated via involuntary activation and to increase anabolic signaling regardless of a neurological deficit.

All of the results show promise for NMES as an effective alternative to voluntary exercise, which is an important need for older adults. The anabolic signaling pathway is stimulated by NMES and appears to mimic the effects of voluntary exercise. While NMES induces a muscle contraction differently from voluntary muscle contractions,

NMES may activate the same cellular responses as a voluntary contraction which may translate to improvements in physical function. However, the full effect of NMES is not known and further research needs to be done to understand how NMES affects anabolic signaling in healthy, older skeletal muscle when NMES is applied repeatedly over a 4-week period.

Self-efficacy for ADL's

To our knowledge, no research has been done on the self-efficacy for activities of daily living following an NMES intervention. It is possible that if strength is increased, then the individual's self-efficacy to perform activities of daily living will increase. Self-efficacy is an individual's perception of their ability to engage in a behavior. In other words, the individual's self-confidence in completing a task or activity (Lox et al. 2010). An older adult may feel confident walking on a flat path from their living room to their bedroom but are less confident when they have to walk downstairs into their basement because they feel unstable going down the stairs. Self-efficacy is important because behavior is influenced through thought (Bandura, 1977). Therefore, if the individual does not have a high belief that they can accomplish simple everyday tasks, they will have a harder time performing the tasks and may avoid performing the tasks all together. Self-efficacy theory describes several antecedents and consequences of self-efficacy. Two antecedents are physiological and affective states (Lox et al. 2010). An example of the physiological state would be when an older individual experiences fatigue when walking or getting out of a chair. People partially judge their anxiety or stress based on their state of physiological arousal and will more likely have success if low physiological arousal is experienced (Bandura 1977). An affective example is the emotional state, which could be

positive or negative (Lox et al. 2010). If an elderly person previously fell while going down the stairs, a negative emotion would be associated with going down the stairs, which could reduce self-efficacy for that activity. However, if the individual increases strength (a physiological response), this could help them perform ADL's more efficiently. Being more efficient at ADL's, could lead to more positive emotions towards these activities and increase their self-efficacy.

One important consequence of self-efficacy is physical activity behavior (Lox et al. 2010). If an older adult does not believe that they can perform a simple task such as stooping or kneeling, it could affect their physical activity level (Mullen et al., 2012). Over 800 adults were first surveyed about their self-efficacy of walking, then were administered basic functional tests. The results showed that a higher self-efficacy of walking was positively associated with better function and fewer limitations (Mullen et al., 2012). A decreased physical activity level can have severe implications that can lead to disability, decreased quality of life, and other diseases.

Self-efficacy for ADL's is important for older adults to maintain a physically active lifestyle. Research is needed on whether NMES treatment can increase ADL self-efficacy. If NMES increases strength, then self-efficacy for daily activities may increase. Studies have shown changes in self-efficacy as a result of a resistance training intervention in older adults (Kekalainen et al. 2018; Neupert et al. 2009). ADL self-efficacy was assessed pre- and post-intervention to determine if the treatment can improve this aspect of confidence.

The purpose of this study was to investigate the effects of a 4-week NMES intervention in healthy, older adults. The effects investigated were anabolic signaling,

strength, physical function, and ADL self-efficacy. We hypothesized that in healthy older adults: 1) anabolic signaling of the mTORC1 pathway will increase with a single bout of the NMES intervention and will remain elevated 30 minutes and 120 minutes after NMES treatment when compared to resting or pre-stimulation levels in the NMES treatment group. Anabolic signaling will be highest at 30 minutes post-intervention; 2) anabolic signaling will be upregulated after 4 weeks (treatment Day 12) of the NMES treatment in the NMES group when compared to Day 1 of the NMES treatment at all three time points, (Pre-NMES, post-30 minutes, and post-120 minutes); 3) strength will be increased after the 4-week NMES intervention in the NMES treatment group. No change in strength will be observed in the Sham group; 4) the 5 repetition sit to stand (5RSTS) time will decrease after the 4-week NMES intervention in the NMES treatment group. No change will be observed in the Sham group; 5) Self-efficacy for ADL's will increase following the 4-week NMES intervention in the NMES treatment group. No change will be observed in the Sham group.

Methods

Participants

Eleven older adults [71.7 ± 2.2 yr. of age; male (n = 4), female (n = 7); NMES (n = 8), Sham (n = 3)] participated in the study. The participants were Caucasian (n = 10) and Hispanic (n = 1). Inclusion criteria consisted of age 60 and older, relatively healthy, and a medical clearance form signed by their physician. Exclusion criteria consisted of: lower body resistance training or therapy on the lower limbs in the last 2 months. Contraindications for the electrical stimulation (swollen or inflamed areas, open wounds, pain in the lower limb, implanted pacemaker, or implanted surgical devices), knee injury or current pain, neuromuscular disease, taking insulin, and history of seizures. Subjects

were recruited through email, newspaper ads, and flyers. The telephone health screening form (see Appendix A) was used to determine eligibility for the study. The study received Texas State University IRB approval.

Data Collection

Day 1 Pre- and Post-Intervention Testing

Participants were emailed and then called to review pre-testing instructions the week prior to testing. Subjects were advised to refrain from strenuous activities and exercise for 48 hours prior to the testing sessions and to avoid caffeine and tobacco products on the day of testing. Subjects were also instructed to wear shorts and to shave the anterior aspect of their thigh to allow the electromyography (EMG) electrodes to maintain the best contact possible with the skin.

The first day of testing started in the Neuromuscular Physiology Lab. A member of the research team reviewed the informed consent in detail with each subject. The subject was given the opportunity to ask questions, then signed the informed consent. Height and weight were obtained with the stadiometer (Health-O-Meter Professional 500KL, Alsip, IL) and recorded. Then, body fat percent was measured with the handheld bio-electric impendence device (BIA) (Omron, Lake Forest, IL) using the height and weight that was obtained.

Strength Testing Protocol. Strength testing was performed on an isokinetic dynamometer (Biodex Systems 4 Pro, Shirley, NY). The subject was seated in the Biodex with hips at 85° and the tested leg was secured at a 60° knee flexion to perform an isometric knee extension. The chest, waist, thigh, and lower leg straps were secured to reduce movement during testing. EMG electrodes (Delsys Trigno Wireless System,

Natick, MA) were positioned on the distal belly of the vastus lateralis (VL) and the distal vastus medialis oblique (VMO) in line with the muscle fiber pennation angle. To ensure the same electrode placement was used for Pre- and Post-Intervention testing, a template was made where all three electrodes were marked as well as anatomical landmarks such as the patella, femoral condyles, and anterior superior iliac spine. The Biodex chair settings were also recorded and the same chair settings were used for the NMES intervention and post-testing.

Subjects began with familiarization and the starting leg was randomized using a randomizer software (Urbaniak & Plous, 2018). For familiarization, each subject performed submaximal contractions at 6 different intensities for 4-5s. Then, 3 isometric knee extension maximal voluntary contractions (MVCs) were completed for 3-5s, to ensure each subject understood how to perform a maximal contraction. After a 10-minute rest, three, 4-s isometric knee extension MVCs were performed with 6s rest between contractions. Subjects were given verbal encouragement during each MVC. Once completed, the strength testing protocol was repeated on the opposite limb. Torque was recorded and measured using LabChart software (Version 8, ADInstruments, Colorado Springs, CO) using the PowerLab 16/35 (ADInstruments, Colorado Springs, CO) for data acquisition.

Day 2 Pre- and Post-Intervention Testing: Survey and Physical Function Assessment

Subjects were advised to refrain from strenuous activities and exercise 48 hours prior to the testing sessions. A physical function assessment and a survey were performed during Day 2 at least 1 week after Day 1 testing. The survey consisted of the Activities-specific Balance Confidence (ABC) scale (Powell and Myers, 1995), which was used to

assess self-efficacy for ADLs (a basic series of activities a person completes every day) and asks the individual about their belief in performing 16 daily activities (how confident are you to walk up and down the stairs, sweep the floor, etc.) (see Appendix D). Participants rated their ability to complete the given task on an 11-point scale from 0% (no confidence) to 100% (completely confident). The subjects were informed that all of their answers are completely confidential and that the survey would consist of questions about their beliefs about themselves and their abilities to perform physical activities. The 16 survey items (0 = 0%, 10 = 100%) were averaged for a mean ADL self-efficacy score. The ABC scale has been found to be valid and reliable with high internal consistency (Powell and Myers, 1995).

Once the survey was completed, the subjects performed the five-repetition sit-to stand test (5RSTS). The subjects sat in an armless chair and were instructed to sit up straight with their arms folded over their chest. On the command "Go," the subject stood up with full hip and knee extension then lowered themselves onto the chair until they touched the chair and this was repeated 5 times. The performance was timed and stopped when the subject touched the chair on the fifth repetition. After each set, a 30 second rest was given, and 3 sets were completed. The shortest time was used for analysis.

Post-Day 1 and Day 2 testing were repeated after completion of the intervention. Post-Day 1 was 48 hours after the 12^{th} session of the intervention, with Post-Day 2 testing being administered 48 hours after the Day 1 testing.

Intervention

The intervention began at least two days after Day 2 testing was completed. Subjects were randomly assigned (Urbaniak & Plous 2018) to the neuromuscular

electrical stimulation (NMES) (milli-current) group or the Sham (micro-current) group. The Sham group followed the same protocol as the milli-current group, but did not receive any stimulation. The Sham group was informed that they were receiving micro-current stimulation and that most individuals do not experience physical sensation during this treatment. After completion of the study, Sham subject were debriefed and given the opportunity to receive 4-weeks of the NMES (milli-current) treatment.

Both groups (NMES and Sham) received the intervention 3 times a week for 4 weeks for a total of 12 sessions. Four 3 x 5-inch electrodes (ValuTrode Neurostimulation Electrodes, Fall Brook, CA) were placed on the quadriceps at proximal and distal aspects of the vastus lateralis (VL) and the vastus medialis (VM).

The NMES protocol duty cycle consisted of 10 seconds of stimulation with 15 seconds of rest, repeating for a total of 40 minutes (96 cycles). The stimulation was set at 60 hertz (Hz) with a pulse width of 200 μs (Digitimer DS7A, Garden City, England). The intensity of the stimulation was determined by the subject's MVC (obtained on Day 1 testing) and was set at 15% MVC. The first leg stimulated was randomly selected (Urbaniak & Plous, 2018). The stimulation began at 0 milliamps (mA) and would be gradually increased each contraction until the 15% MVC target torque was achieved, then the 40-minute intervention started. Every 5 minutes, the stimulation intensity was increased if the torque fell below the desired target. After the 40 minutes was completed on one leg, the intervention was applied to the other leg. On Day 7 of the intervention, MVCs were tested again. The target torque was readjusted at that point to 15% of their new MVC. LabChart (Version 8, ADinstruments, Colorado Springs CO) software and the

PowerLab (ADinstruments, Colorado Springs, CO) was used to administer the stimulation and record torque.

Muscle Biopsy

Muscle biopsies were taken from the VL on the first (Day 1) and last day (Day 12) of the intervention (Figure 1). Biopsies were obtained only for the NMES group (n = 6). The preparation instructions given to the subjects prior to the biopsy study were to discontinue any aspirin and fish oil for 7 days prior to the biopsy (if the subject took these supplements), to hold any medications or supplements until the completion of the study that day, to avoid strenuous activity 48 hours prior to the biopsy study, to shave their thighs, and to avoid caffeine and tobacco the day of the study. The subjects were given a nutritional supplement (Ensure Plus, Abbott, Abbott, IL) which consisted of 28% fat, 56% carbohydrate, and 15% protein, to have as their evening meal the night prior to each biopsy study and was to be consumed between the hours of 1800-2000. The amount was determined by the Harris Benedict Equation (Harris and Benedict, 1919).

Harris Benedict Equations for pre-biopsy evening meal:

Male: BMR = 66.473 + (913.7516 * kg) + (5.0033 * cm) - (6.755 * age),

Recommended Daily Intake = BMR x 1.55

Recommended Daily Intake/3= amount of Ensure in (ml)

Female: BMR = 655.0955 + (9.5634 * kg) + (1.8496 * cm) - (4.6756 * age)

Recommended Daily Intake = BMR x 1.55

(Recommended Daily Intake/3) = amount of Ensure (ml)

Subjects arrived at the Neuromuscular Physiology Laboratory in the morning and were fasted since the evening before. Once at the lab, the subject was instructed to lay

down with minimal movement for 2 hours. The first muscle biopsy (pre-NMES biopsy) was obtained from the VL prior to the application of the intervention. The biopsy site was cleaned with betadine and 1% lidocaine was injected at the biopsy site, then a small incision was made. The muscle biopsy (Pre-NMES) was performed with a 5mm Bergstrom biopsy needle as previously described (Wolfe, 1992). Once the tissue sample was obtained, blood, adipose and connective tissue were removed and the muscle tissue was immediately frozen in liquid nitrogen. The tissue sample was then placed in a cryovial and stored at -80°C to be processed at a later date. The second muscle biopsy was taken from the same incision 30 minutes after the intervention was completed (Post-30 min biopsy). The subject remained laying down until the final muscle biopsy was obtained. The third biopsy (Post-120min biopsy) was obtained with a new incision 2 hours after the NMES was completed. A call was made to each participant 48 hours post-biopsy to follow-up on the incision site. The following questions were asked: if there is any discoloration or bruising around the biopsy site, if there is any redness, swelling, or warmth around the biopsy site or lower leg/foot, if there is any fluid discharge around the biopsy incision, and if they have any other concerns.

Data Analysis

Western Blots

The muscle tissue was processed to measure cell signaling for the following anabolic signaling proteins: total mTOR, phosphorylated mTOR, total S6K1, phosphorylated S6K1, total 4E-BP1, and phosphorylated 4E-BP1. The homogenate was centrifuged at 6000 rpm at 4°C for 10 min and then the supernatant was removed. Then the supernatant was used to determine the protein concentration by using a Bradford

assay (SmartSpec Plus Spectrophotometer, Bio-Rad, Hercules, CA). Then, the supernatant was mixed with 2X sample buffer in a 1:1 ratio and then the solution was boiled. A single well was loaded with 50 µg of protein from each sample and samples were loaded in duplicate into either a 7.5 or 12% gel (Criterion TGX Stain-Free, Bio-Rad, Hercules, CA), depending on the protein that was analyzed. One gel consisted of all samples from 1 subject (Intervention Day 1 (Pre-NMES, Post-30min, Post-120 min) and Intervention Day 12 (Pre-NMES, Post-30min, Post-120 min)). A known standard (phosphorylated p70 MCF7 control cell extract; Cell Signaling Technology, Beverly, MA) was also loaded for control purposes. A molecular weight marker (Precision Plus Protein All Blue Prestained Protein, Bio-Rad, Hercules, CA) was also added to a lane. The proteins were separated via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Criterion; Bio-Rad, Hercules, CA) at 150V for 60 minutes. Next, the proteins were transferred (Criterion Blotter, Bio-Rad, Hercules, CA) from the gel to a polyvinylidene difluoride membrane (Immun-Blot polyvinylidene difluoride, Bio-Rad, Hercules, CA) at 50V for 60 minutes. Once transferred, the membrane was blocked in either 5% nonfat dairy milk or 5% bovine serum albumin (p-mTOR only) solution for 60 minutes. The primary antibody was then applied, and the membrane was covered and incubated overnight at 4ºC.

Then a secondary antibody (donkey anti-rabbit IgG horseradish peroxidase-conjugated (1:12,500)) (Santa Cruz Biotechnology, Dallas, TX) was applied and rocked for 60 minutes at room temperature. A chemiluminescence agent (Clarity Western ECL Substrate, Bio-Rad, Hercules, CA) was applied to the membrane and imaged (Chemidoc Imaging System, Bio-Rad, Hercules, CA). The membrane was then stripped (Restore

Western Blot Stripping Buffer, Thermo Scientific, Rockford, IL) and the next primary antibody was applied and rocked overnight at 4ºC. This process was repeated for each primary antibody. The primary antibody dilutions used were: phospho-p70 S6K1 (1:250, Thr^{389}), total p70 S6K1 (1:1000, Thr^{389}), phospho-mTOR (1:500, Ser^{2448}), total mTOR (1:1333, Ser^{2448}), phospho-4E-BP1 (1:1000; $Thr^{37/46}$), and total 4E-BP1 (1:1000; $Thr^{37/46}$, Cell Signaling Technology, Beverly, MA).

Band density was analyzed with Quantity One 1-D analysis software (Version 4.6.6, BioRad). Each protein is expressed in a ratio of phosphorylated to total protein content.

Muscle Strength and EMG Analysis

The maximum torque was determined by measuring the highest point of each of the three contractions and the highest MVC was used for analysis. The leg that was not biopsied on intervention Day 12 was used for analysis of MVC and EMG (for Pre- and Post-Intervention). This was done to avoid the potential influence the biopsy procedure may have had on the Post-Intervention MVC data. For the Sham group, the leg used for MVC and EMG analysis was randomly selected. The root mean square (RMS) EMG and median power frequency EMG were measured from the VL and VMO during the MVC. EMG were measured for 1 second during peak MVC (0.5s was measured on both sides of the peak torque generated during the MVC). MVC and EMG data were measured for Pre-Intervention and Post-Intervention and MVC was also measured on Day 7 of the intervention.

Statistical Analysis

Statistical analyses were performed using SPSS software (Version 25, IBM SPSS, Chicago, IL). Definitions for the terminology used for statistical analysis are defined here. Pre-intervention: Data (timepoint) that are obtained at the beginning of the study prior to the start of the NMES intervention. Post-intervention: Data (timepoint) that are obtained at the end of the study after 12 NMES intervention sessions. Day 1: First day of the NMES intervention. Day 12: Last day of the NMES intervention. There are 3 biopsy timepoints. Pre-NMES: biopsy performed before the NMES treatment was applied. Post-30 min: biopsy performed 30 minutes after completion of the NMES treatment. Post-120 min: biopsy performed 120 minutes after completion of the NMES treatment. There were two groups (NMES, Sham). The NMES group received the 4-week stimulation. The Sham group went through the same protocol the NMES group did but did not receive any stimulation.

A General Linear Model with repeated measures analysis of variance (ANOVA) using the Generalized Linear Model command with a normal distribution and an identity link function (Version 25, IBM SPSS) were used to test statistical significance of the anabolic signaling data. The within-subjects factors Time (Pre-NMES, Post-30min, Post-120 min) and Day (Day 1, Day 12) were used to test changes in the anabolic signaling response (phosphorylated mTOR, total mTOR, phosphorylated S6K1, total S6K1, phosphorylated 4E-BP1, total 4E-BP1). Pre-NMES was the biopsy taken before the NMES, Post-30min biopsy was taken 30 minutes after NMES, and Post-120min biopsy was performed 120 minutes after the NMES. MVC, 5RSTS, EMG (RMS and median frequency), and ADL Self-efficacy were analyzed with a 2 x 2 repeated measures

ANOVA with Intervention (Pre-Intervention, Post-Intervention) as the within subject factor and Group (NMES, Sham) as the between subjects' factor. An unpaired t-test was used to test for Pre-Post Intervention differences amongst different subject demographics (age, height) and a repeated measures ANOVA was used to test body mass, BMI, and body fat percentage. Data are reported as mean ± SE with significance set at $p < 0.05$.

Results

Participant Characteristics.

There were no differences between groups or pre-post intervention for age, height, body mass, BMI, and body fat percentage ($p < 0.05$) (Table 1).

Strength and EMG Analysis.

The group x intervention interaction was not significant ($p = 0.521$) and there was no significant main effect for MVC for intervention (pre-, post-) ($p = 0.229$) or group ($p = 0.934$) (Figure 4). EMG for VL RMS and VM RMS showed no significant difference for group x intervention interaction ($p = 0.524$, $p = 0.798$), intervention (pre-, post-) ($p = 0.558$, $p = 0.962$), or group ($p = 0.553$, $p = 0.363$) for VL and VM, respectively. The RMS for VM NMES group (pre-, 0.10 ± 0.23 and post-, 0.10 ± 0.02) and the Sham group (pre-, 0.06 ± 0.04 and post-, 0.07 ± 0.03) had minimal changes between the timepoints, but the RMS for VL NMES group (pre-, 0.10 ± 0.21 and post- 0.25 ± 0.13) and Sham group (pre-, 0.09 ± 0.04 and post-, 0.88 ± 0.20) showed an increased trend. The EMG median frequency for VL and VM were not significant either for group x intervention interaction ($p = 0.193$, $p = 0.748$), intervention (pre-, post-) ($p = 0.328$, $p = 0.986$), or group ($p = 0.756$, $p = 0.283$) for VL and VM, respectively.

Functional Testing.

For 5RSTS there was no significant effect for group x intervention interaction (p = 0.903), intervention (p = 0.133), or group (p = 0.150) (Figure 5).

ADL Self-Efficacy.

The 16-question scale was found to be highly reliable (Cronbach's Alpha = 0.959 at pre- and 0.959 at post-intervention) with a relatively high overall mean (full sample) (pre = 92.66 ± 4.04, post = 93.17 ± 3.99). No significant effect was found for the interaction F(1, 9) = 0.433, p = 0.527, η^2 = 0.046, intervention (pre-, post-intervention) F(1, 9) = 3.416, p = 0.098, η^2 = 0.275, or for group (NMES and Sham) F(1, 9) = 0.092, p = 0.769, η^2 = 0.01. Overall, the scale mean was high with low variability, therefore lack of significant changes may be due to a ceiling effect. Upon investigating individual items, four questions had noticeably lower means and more variability than the rest. Questions 6 (stand on a chair and reach for something), 14 (step on or off an escalator while holding onto the railing), 15 (step on or off an escalator while holding parcels and cannot hold onto the railing), and 16 (walk on icy sidewalks) had the lowest means from the full sample (pre = 82.73, 90.91, 86.24, and 72.73 and post = 83.64, 92.73, 86.36, and 75.45) amongst the questions. These activities were interpreted as more relevant to investigate for improvement for this sample because subjects were initially less confident in their ability to do these activities. Those items were averaged to form a shortened ADL self-efficacy scale, which showed good reliability (Cronbach's alpha = 0.918 for pre- and 0.897 for post-intervention), This shortened scale from the full sample had a lower mean (pre = 83.15 ± 7.53, post = 84.54 ± 7.31) than the full scale. No main effect was found for intervention (pre-intervention, post-intervention), F (1, 9) = 0.459, p = 0.515, η^2 = 0.049

or the interaction F (1, 9) = 2.211, $p = 0.171$, $\eta^2 = 0.197$. No significant main effect was found for group (Sham, NMES), F (1, 9) = 0.459, $p = 0.515$, $\eta^2 = 0.049$. However, the interaction effect does have a medium effect size ($\eta^2 = 0.197$). There was a trend that the NMES group increased in ADL self-efficacy (using the shortened scale) (85.90 to 88.13) whereas the sham group did not (75.83 to 75.00).

Anabolic Signaling.

The anabolic signaling data are expressed as a ratio of phosphorylated protein content to total protein content, which are shown in Figures 6, 7, and 8. Phosphorylated S6K1 had a significant main effect for time (Pre-NMES, Post-30 min, Post-120 min) ($p = 0.020$) (Figure 7). The post hoc results revealed that Post-30 min was significantly upregulated when compared to pre-NMES ($p = 0.017$). For phosphorylated S6K1, the interaction Day x Time interaction ($p = 0.520$) or the main effect for Day (Day 1, Day 12) ($p = 0.774$) were not significant. The phosphorylated mTOR interaction, Day x Time interaction, was not significant ($p = 0.227$). However, there was a significant main effect was for phosphorylated mTOR Day (Day 1, Day 12) ($p = 0.041$) and Time (Pre-NMES, Post-30 min, Post-120 min) ($p = 0.009$) (Figure 6). The Bonferroni post hoc showed that Day 1 phosphorylation of mTOR was significantly greater when compared to Day 12 ($p = 0.041$) and Post-30 min was significantly upregulated compared to pre-NMES ($p = 0.007$). Phosphorylated 4E-BP1 did not have a significant difference for Day x Time interaction ($p = 0.923$) or for main effect for Day (Day 1, Day 12) ($p = 0.353$), Time (Pre-NMES, Post-30 min, Post-120 min) ($p = 0.310$) (Figure 8).

Discussion

The purpose of this study was to examine the effects of a 4-week NMES intervention on anabolic signaling, strength, EMG, physical function, and self-efficacy ADL's. There is little research on NMES and anabolic signaling response, with few to no studies examining a 4-week treatment. This preliminary 4-week NMES intervention study determined that anabolic signaling was significantly increased with NMES. At 30-min after the NMES treatment, phosphorylated mTOR and phosphorylated S6K1 were significantly upregulated compared to the resting state (Pre-NMES). Only phosphorylated mTOR was upregulated on the first day of the NMES treatment when compared to the last (12^{th}) treatment day. However, because of low statistical power, with more subjects this could change. Even though MVC and 5RSTS showed no significant performance increase, self-efficacy did have a moderate effect size for improvement for four ADLs. This means that the subjects showed a trend towards improvement regarding how they felt about performing ADLs.

Anabolic Signaling and NMES

Both phosphorylated mTOR and phosphorylated S6K1 showed an increase in at the Post-30min mark when compared to Pre-NMES, whereas phosphorylated 4E-BP1 did not change. An increase in anabolic response (upregulated for p-mTOR and p-S6K1) may result in muscle growth response (Ogasawra et al. 2014). Lack of an increase in phosphorylated 4E-BP1 was not a negative finding due to that fact that phosphorylated 4E-BP1 is inhibitory, and would reduce muscle protein synthesis (Luciano et al. 2016). These findings are consistent with previous research. An increase in anabolic response for phosphorylated mTOR and phosphorylated S6K1 with no increase of phosphorylated

4E-BP1 was also found 30-mins after a single bout of NMES in older, healthy adults and individuals with stroke (Mettler et al. 2017). These findings were confirmed again when a single bout of NMES was performed on young, healthy adults (Mettler et al. 2018). Both studies used 60 Hz as the stimulation frequency (Mettler et al. 2017; Mettler et al. 2018), which was also used in the current study. In another study, the upregulated anabolic signaling was also found after a single bout of NMES at 60 Hz in older, diabetic men. Although not significant, data showed a trend towards increased anabolic response in the same signaling proteins (Wall et al. 2012) and was further confirmed with an upregulation of phosphorylated mTOR and S6K1 after a 70-minute single session of NMES set at 100 Hz (Dirks et al. 2016). Additionally, when 22 patients with COPD received NMES treatment on their quadriceps and calves for 6 weeks at 50 Hz, there was also a significant increase in phosphorylated S6K1 and no changes in phosphorylated 4E-BP1 in the quadricep muscle tissue (Vivodtzev et al. 2012). This study performed NMES for 35 min on the quadricep then 25 min on the calf, 5 times a day for 6 weeks and trained both limbs simultaneously (Vivodtzev et al. 2012).

However, Dirks et al. (2015) found that phosphorylated mTOR was increased after a 7-day intervention in comatose patients but phosphorylated S6K1 was decreased. This study did not find an increase in phosphorylated S6k1 but a short 7-day study may not be long enough to create an increase in phosphorylated S6K1. In another study healthy subjects were placed on bed rest and received stimulation for 5 days with protein supplementation, phosphorylated S6K1 increased and phosphorylated 4E-BP1 decreased (Reidy et al. 2017). The preliminary data here also showed an increase in phosphorylated S6K1 but did not show a significant decrease in 4E-BP1. The subject population and

treatment time could have an effect on the results whereas the present study used healthy, older adults for a 4-week intervention while Dirks et al. (2015) and Reidy et al. (2017) used inactive subjects (comatose patients and bed rest) for short (5-7 days) studies. Comatose patients could potentially have other medical issues that would conflict with muscle growth and both populations were immobilized, which leads to muscle wasting (Dirks et al. 2015).

Other studies looked at the effects of gene expression related to muscle growth with NMES in older adults and found that NMES increased gene expression of genes involved in upregulation of the anabolic process. The same studies investigated catabolic gene expression as well and found catabolic gene expression was either decreased or inhibited with NMES (Dirks et al. 2014; Reidy et al., 2017; Kern et al. 2014). The Reidy et al. (2017) study was performed on bed rest subjects, who were more subject to muscle atrophy due to inactivity whereas the subjects in the present study were active. These data show that NMES can create an anabolic response in the muscle in the mTORC1 pathway and regulating genes.

In three inactivity protocols, cross sectional area (CSA) of quadricep muscle was analyzed with anabolic signaling. NMES slowed the decrease of muscle atrophy when compared to the control group (Dirks et al. 2014; Dirks et al. 2015; Reidy et al. 2017). Even though two studies had a decrease in CSA with NMES, an increase with gene expression related to anabolic signaling was found after 5 days of either bedrest or immobilization (Dirks et al. 2014; Reidy et al. 2017). The third study found that CSA was maintained and an increase in phosphorylated mTOR was also detected after 7 days in comatose subjects (Dirks et al. 2015). By maintaining or slowing down the decrease in

the CSA of the quadricep muscle with inactivity, NMES may be an effective treatment to maintain lean muscle mass.

In the present study, when Day 1 and Day 12 anabolic responses were compared, only phosphorylated mTOR had Day 12 significantly lower when compared to Day 1. One possible explanation for the present finding could be an adaptation to the NMES, meaning a 4-week intervention is enough for the muscles to adapt and no longer see a positive increase with training, which may cause a lower amount of phosphorylated mTOR. A study with older women showed a plateau in resistance training at 3 weeks (Signorile et al. 2005). However, another study had untrained older adults that executed a 22-week resistance training program with a non-periodization, block periodization, and an undulating periodization all had significant results in peak torque and peak power with no difference in between groups (Conlong et al. 2017).

A study performed on rats demonstrated a decrease in phosphorylation of a downstream target of mTOR, S6K1, after 12 and 18 bouts of resistance training when compared to the control group and 1 bout of exercise. However, phosphorylation of 4E-BP1 remained unchanged. After 12 days of detraining, phosphorylated levels of S6K1 showed an increase after a single session of resistance training (Ogasawara et al 2013). The bouts of exercise were performed every other day until the desired amount of session were met. The subjects in the current study trained similarly receiving NMES 3 times a week, but had at least one time per week where the subjects had two days in between sessions. After 10 weeks of resistance training, phosphorylated S6K1 was reduced in trained subjects as opposed to untrained subjects (Wilkinson et al. 2008). This was found again after a single resistance training session, where power lifters did not have an

increase in phosphorylated S6K1 but untrained subjects did (Coffey et al. 2006). It is possible that the current subjects acclimated to the training quickly, possibly causing the anabolic signaling to become desensitized to the NMES. Further research is needed to determine if training adaptations with NMES also plateau or if progressive increases in the NMES protocol might be needed to continue adaptations for longer. There was no change for phosphorylated S6K1 or phosphorylated 4E-BP1 between Day 1 and Day 12.

The effects of NMES have been similar to findings in voluntary resistance training studies. An increase in phosphorylated mTOR was seen after a single bout of exercise in elite weight lifters 3 hours after resistance training when compared to the resting state (Lim et al., 2017). Phosphorylated mTOR was also upregulated after a 12-week resistance training program in rats when compared to the control group. All rats were sacrificed 48 hours after the last training session, to include the strength resistance training, hypertrophy resistance training groups, and control groups (Luciano et al, 2017). In another study on rats, phosphorylated S6K1 was elevated 24 hours after 1 bout of resistance training via maximum isometric contraction (Ogasawara et al. 2013). Li et al. (2012) demonstrated that even though older adults have overall less phosphorylated mTOR and phosphorylated 4E-BP1 than their younger counterparts, there was an increase in phosphorylated mTOR and phosphorylated 4E-BP1 with a single 12-week resistance training program when compared to the pre-training state. These findings of increased phosphorylated mTOR corresponds with our results. However, the increase in phosphorylated 4E-BP1 and no change in phosphorylated S6K1 contrasted with our findings.

Another study on older men also showed lower levels of phosphorylated S6K1 and 4E-BP1 when compared to younger subjects but phosphorylation was not affected by age and peaked 1-2 hours post exercise (Kumar et al. 2009). Fry et al. (2011) performed muscle biopsies at resting, then at 3, 6, and 24 hours after a single session of resistance training on older adults. No increase of phosphorylated mTOR, S6K1, or 4E-BP1 at any of the timepoints when compared to resting (Fry et al. 2011). This study may have waited too long to perform an initial biopsy and missed the upregulation if it peaked in less than 3 hours. In contrast, a study performed on rat showed an increase in phosphorylated mTOR and phosphorylated S6K1 at 1, 3, and 6 hours after a single session of exercise which consisted of a maximum isometric contraction via percutaneous electrical stimulation for 5 sets of 3 x 10 seconds (Ogasawara et al. 2013) whereas we only saw an increase at 30 min after the NMES intervention, not 120 min. It is possible that NMES does not affect the mTORC1 pathway as long as voluntary resistance training does, but further studies need to be conducted in humans to confirm.

Strength and EMG

No significant changes were found for quadriceps strength as measured by MVC or EMG (RMS and median power frequency) for Group or pre- post- 4-week NMES intervention. Reidy et al. (2017) found no increase of strength with NMES that was applied for 40 minutes, three times a day for 5 days on healthy, older bedrest patients. Muscle atrophy occurs with disuse of a limb, leading to a decrease in strength. However, NMES did not preserve strength when the NMES group was compared to the control group. (Reidy et al. 2017). This contradicts the increase in MVC observed in other NMES training studies (Gondin et al. 2011; Kern et al. 2014; Vivodtzev et al. 2012).

However, these protocols took place over 6-9 weeks and used a stimulation frequency of 50-75 Hz whereas the present study was only a 4-week intervention using 60 Hz. Possibly the intervention used in the present study was not long enough to see the increase in strength and an additional 4 more weeks may have been beneficial.

Additionally, in the present study, the MVC performance should not have been affected by position because the intervention was performed in the same position the MVC was performed (knee at 60°). However, some protocols have performed the NMES and strength testing in different joint positions. In one study, the knee was slightly bent at 30° during NMES and performed the strength testing at 60°and found no increase in MVC (Reidy et al. 2017). Another study performed NMES with the subjects in a sitting position but did not describe how the strength testing was performed, but reported an increase in strength (Vivodtzev et al. 2012). A third study found an increase in strength and performed the NMES with the knee fully extended and tested at 60° knee flexion (Kern et al. 2014). In one study, subjects performed NMES-induced contractions at a knee flexion of 60° produced higher torque than NMES-induced contractions administered at a 15° knee flexion angle (Bremner et al. 2015). It was also found that performing NMES in a knee flexed position resulted in larger isokinetic strength as opposed to an extended position (Fahay et al. 1985). A small sample size caused low statistical power, and power could increase with a larger sample size. MVC showed a small trend toward improvement and with a larger sample size, the comparison may become significant.

No difference was found in the EMG analysis pre-post intervention, which corresponds to results found in two resistance training studies. One study was with older

trained women that participated in an 8-week intervention (Filho et al. 2017) and another one with untrained older adults that participated in a 22-week intervention (Conlon et al. 2017) and neither found an increase in muscle activation.

Physical Function Assessment

The 5RSTS was tested as a means of assessing a basic physical function that is performed as a daily task. The 5RSTS has also been used as a test of lower body strength (Bohannon, 1997) since getting up out of a chair only takes a few seconds. No change was observed in the NMES or SHAM group with the 5RSTS pre- post- the 4-week intervention. This mirrors the findings of no increases in strength with the MVC also observed in the present study. It is plausible that an increase in strength would be associated with a decrease in time for the 5RSTS. Getting out of a chair requires strength and power and the more strength an individual has, the easier it should be for them to get up from a chair. In a previous study, there was a significant improvement in performance with the 5RSTS as well as with MVC in older healthy adults after 9 weeks of NMES training (Kern et al. 2014). One major difference was that Kern et al. (2014) had 16 subjects and a longer 9-week intervention period. Two studies with an 8-week and 12-week resistance training program in older adults had increased performance for 5RSTS at the end of the intervention when compared to the beginning (Razob et al. 2018; Ramirez-Campillo et al. 2018). It would be expected that NMES would produce the same results as resistance training, however, these protocols were also longer than the present study. Another study found an increase in strength as well as increased walking distance in COPD patients (Vivodtzev et al. 2012) whereas another one did not have an increase in strength in subjects on a 5 day bed rest and found no changes in the 6-minute walk test,

timed up and go, and gait speed (Reidy et al. 2017). These studies have subjects that are not healthy or are immobilized, both which would have an effect on the results. A deconditioned sample such as COPD subjects, have a larger area to increase their functional outcomes than subjects. Subjects that are inactive are subject to muscle atrophy, which may have a larger decrease in performance with functional outcomes, especially the more muscle atrophy that occurs. More subjects in the present study would increase the statistical power and may result in a significant improvement.

ADL Self-efficacy

The results showed no significant change in the subjects' perception of their self-efficacy with ADLs. One reason the results may not have been significant was because there was a high scale mean with low variability which could have led to a ceiling effect. Since the individuals had a high perception of their self-efficacy for ADL's, there was less room for improvement. After closer examination, it was determined that there were four questions with the most variability and lower pre-intervention means compared to the rest of the questions; therefore, those questions were analyzed separately. A medium effect size was shown for the intervention-by-group interaction with the shortened scale. This shows a trend towards an increase in self-efficacy towards those four ADL's on the shortened scale in the NMES group but not the Sham group. This is important because the more confidence an older adult has in an activity, the more likely they will perform it. This boost in confidence can increase the quality of life for an older adult which may lead to increased physical activity. It has been shown that greater physical activity is associated with higher levels of self-efficacy, increased functional performance, and few functional limitations (Mullen et al. 2012).

To our knowledge, no previous studies have been performed on self-efficacy for ADLs with NMES training. However, some studies have shown that resistance training interventions led to increased self-efficacy (Katula et al. 2008; Kekalainen et al. 2018; Hart et al. 2019). Older adults completed a 12-week resistance training study and had an increase in their self-efficacy of strength and satisfaction of physical function when compared to the control group at the end of the study (Katula et al. 2008). A meta-analysis revealed that resistance training increased many quality of life domains in older adults, such as physical functioning, physical role function, mental health, and vitality (Hart et al. 2019). Therefore, it would be expected that the NMES intervention would have the same impact as resistance training on self-efficacy. A larger sample size would be very beneficial to get a true understanding of the effects of NMES on older adults' self-efficacy.

Limitations

This study had some limitations. The sample size was small, which could have resulted in lack of statistical significance due to lower statistical power. This is preliminary data and as more data are collected, the additional finding may improve significantly. Another limitation was the inability to obtain a muscle biopsy at all 6 timepoints for two subjects. However, with human research, it can often not be avoided. Our subjects were required to be relatively healthy but there was one subject who met all the inclusion criteria but was more deconditioned than the others, which created a large SE for the Sham group. A larger sample size in the future will hopefully have a greater range of abilities. Since our study sample was relatively healthy, those subjects could have had less room for improvement than a more deconditioned sample. Using less active

subjects may show a greater change pre-post NMES intervention for all the variables tested in this study.

Another limitation is the possible placebo effect. Subjects that were in the Sham group did not receive any treatment but subjects were informed they were receiving treatment. The Sham group showed a trend towards increased performance in the 5RST and MVC, albeit not significant. Because the Sham group subjects believed they were receiving a treatment, it may have had a positive effect on them. This could be a possible explanation on why the Sham group showed a trend toward improvement in 5RST and MVC with no stimulation. Another reason may be after spending so much time with the research assistants, subjects were more invested and wanted to please the research team by exerting more effort, plus were more comfortable at the end of the study compared to the beginning. Pre-Intervention testing started the first day the subjects visited the lab for the study; whereas, Post-Intervention testing was done after they had been there 14 times for testing and the intervention. This study did a familiarization with the strength protocol prior to the testing however, it was on the same day. A separate day for familiarization may have been beneficial so the subjects might be more comfortable on the second day.

Conclusion

In conclusion, the findings of this study suggest that a 4-week session of NMES upregulates anabolic signaling proteins of the mTORC1 pathway (phosphorylated mTOR and phosphorylated S6K1) 30 minutes after stimulation. Phosphorylated mTOR also showed a decrease in Day 12 when compared to Day 1. There were no significant differences in strength or 5RSTS. However, there was a medium effect size for the shortened self-efficacy ADL scale. As this was only preliminary data, further research

with more subjects needs to be conducted to better understand the effects of a 4-week intervention of NMES. However, the results are promising and could have positive clinical implications for older adults such as being able to perform ADLs with more confidence, increased strength, and an increased quality of life.

References

1. Bandura A. Self-efficacy: Toward a unifying theory of behavioral change. *Psychological Review,* 84(2):191-215, 1977.
2. Barss TS, Ainsley EN, Claveria-Gonzalez FC, Luu MJ, Miller DM, Wiest MJW, and Collins DF. Utilizing physiological principles of motor unit recruitment to reduce fatigability of electrically-evoked contractions: A narrative review. *Archives of Physical Medicine and Rehabilitation,* 99:779-791, 2018.
3. Bax L, Staes F, and Verhagen A. Does neuromuscular electrical stimulation strengthen the quadriceps femoris? *Sports Med,* 35(3):191-212, 2005.
4. Bohannon R. Reference values for the five-repetition sit-to-stand test: a descriptive meta-analysis of data from elders. *Percept Mot Skills,* 103(1):215-222, 2006.
5. Bremner, CB, Holcomb WR, and Brown CD. Knee joint influences neuromuscular electrical stimulation-induced torque. *Athletic Training and Sports Health Care,* 7(4): 165-173, 2015.
6. Cattagni T, Lepers R, and Maffiuletti NA. Effects of neuromuscular electrical stimulation contralateral quadriceps function. *Journal of Electromyography and Kinesiology,* 38:111-118, 2018.
7. Churchward-Venne TA, Breen L, and Phillips SM. Alterations in human muscle protein metabolisms with aging: Protein and exercise as countermeasures to offset sarcopenia. *Biofactors,* 40(2):199-205, 2013.
8. Coffey VG, Zhong Z, Shield A, Canny BJ, Chibalin AV, Zierath JR, Hawley JA. Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans. *FASEB J,* 20:190-192, 2006.

9. Conlon JA, Newton RU, Tufano JJ, Penailillo LE, Banyard HG, Hopper AJ, Ridge AJ, and Haff GG. The efficacy of periodized resistance training on neuromuscular adaptations for older adults. *Eur J Appl Physiol,* 117:1181-1194, 2017.
10. Dirks ML, Wall BT, Snijders T, Ottenbros CLP, Verdijk, and Van Loon LJC. Neuromuscular electrical stimulation prevents muscle disuse atrophy during leg immobilization in humans. *Acta Physiol,* 210:628-641, 2014.
11. Dirks ML, Hansen D, Van Assche A, Dendale P, and Van Loon LJC. Neuromuscular electrical stimulation prevents muscle wasting in critically ill comatose patients. *Clinical Science,* 128:357-365, 2015.
12. Dirks ML, Groen BBL, Franssen R, Van Kranenburg J, and Van Loon LJC. Neuromuscular electrical stimulation prior to presleep protein feeding stimulates the use of protein-derived amino acids for overnight muscle protein synthesis. *J Appl Physiol*, 122:20-27, 2017.
13. Dirks ML, Wall BT, Kramer IR, Zorenc AH, Goessens JPB, Gijsen AP, and Van Loon LJC. A single session of neuromuscular stimulation does not augment postprandial muscle protein accretion. *Am J Physiol Endocrinol Metab,* 311:E278-E285, 2016.
14. Doucet BM and Griffin L. High-versus low frequency stimulation effects on fine motor control in chronic hemiplegia: A pilot study. *Top Stroke Rehabil,* 20(4):299-307, 2013.

15. Fahey TD, Harvey M, Schroeder RV, Ferguson, F. Influence of sex differences and knee joint position on electrical stimulation-modulated strength increase. *Medicine and Science in Sports and Exercise,* 17(1):144-147, 1985.
16. Filho JC, Gurjao AL, Ceccato M, Prado AK, Gallo LH, and Gobbi S. Chronic effects of different rest intervals between sets on dynamic and isometric muscle strength and muscle activity in trained older women. *American Journal of Physical Medicine and Rehabilitation,* 96(9):627-633, 2017.
17. Freitas GR, Santo CC, Machado-Pereira MM, Bobinski F, Santos ARS, and Ilha J. Early cyclical neuromuscular electrical stimulation improves strength and trophism by Akt pathway and signaling in partially paralyzed biceps muscle after spinal cord injury in rats. *Physical Therapy,* 98(3):172-181, 2018.
18. Fry CS, Drummond MJ, Glenn EL, Dickinson JM, Gundermann DM. Aging impairs contraction-induced human skeletal muscle mTORC1 signaling and protein synthesis. *Skeletal Muscle,* 1, 11, 2011.
19. Gondin J, Brocca L, Bellinzona E, D'Antona G, Maffiuletti NA, Miotti D, Pellegrino MA, and Bottinelli R. Neuromuscular electrical stimulation training induces atypical adaptions of the human skeletal muscle phenotype: a functional and proteomic analysis. *J Appl Physiol,* 110:433-450, 2011.
20. Gondin J, Cozzone PJ, and Bendahan D. Is high-frequency neuromuscular electrical
stimulation a suitable tool for muscle performance improvement in both healthy humans
and athletes? *Eur J Appl Physiol,* 111:2473-2487, 2011.

21. Harris JA, and Benedict FG. A biometric study of basal metabolism in man. *J Chem Inf Model,* 53:284, 1919.
22. Hart PD and Buck DJ. The effect of resistance training on health-related quality of life in older adults: Systematic review and meta-analysis. *Health Promotion Perspectives,* 9(1):1-12, 2019.
23. Henneman E and Olsen CB. Relations between structure and function in the design of skeletal muscle. *J Neurophys,* 28:581-598, 1965.
24. Herzig D, Maffiuletti NA, and Eser P. The application of neuromuscular electrical stimulation training in non-neurologic patient populations: A narrative review. *PMR,* 7:1167-1178, 2015.
25. Jubeau M, Le Fur Y, Duhamel G, Wegrzyk J, Confort-Gouny S, Vilmen C, Cozzone PJ, Mattei JP, Bendahan D, and Gondin J. Localized metabolic changes and T2 changes induced by voluntary and evoked contractions. *Medicine & Science in Sports & Exercise*, 921-930, 2014.
26. Katula JA, Rejeski WJ, and Marsh AP. Enhancing quality of life in older adults: A comparison of muscular strength and power training. *Health and Quality of Life Outcomes,* 6:45, 2008.
27. Kekalainen T, Kokko K, Sipila S, and Walker S. Effects of a 9-month resistance training intervention on quality of life, sense of coherence, and depressive symptoms in older adults: randomized controlled trial. *Qual Life Res,* 27:455-465, 2018.

28. Kern H, Barberi L, Lofler S, Sbardella S, Burggraf S, Fruhmann H, Carraro U, Mosole S, Sarabon N, Vogelauer, Mayr W, Krenn M, Cvecka J, Romanello V, Pietrangelo L, Protasi F, Sandri M, Zampieri, and Musara A. Electrical stimulation counteracts muscle decline in seniors. *Frontiers in Aging NeuroScience,* 6(189), 2014.
29. Kekalainen T, Kokko K, Tammelin T, Sipila S, and Walker S. Motivational characteristics and resistance training in older adults: A randomized controlled trial and 1-year follow-up. *Scan J Med Sci Sports,* 28, 2416-2426, 2018.
30. Koopman R and Van Loon LJC. Aging, exercise, and muscle protein metabolism. *J Appl Physiol,* 106, 2040-2048, 2009.
31. Kumar V, Shelby A, Rankin D, Patel R, Atherton P. Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol,* 587, 211-217, 2009.
32. Lim CH, Luu TS, Phoung LQ, Jeong TS, and Kim CK. Satellite cell activation and mTOR signaling pathway response to resistance and combined exercise in elite weight lifters. *Eur J Appl Physiol,* 117:2355-2363, 2017.
33. Lox CL, Martin Ginis KA, and Petruzzello SJ. The Psychology of Exercise: Integrating Theory and Practice, 3rd edition. Holcomb Hathaway: Scottsdale Arizona, 2010.
34. Mettler JA, Bennett SM, Doucet BM, and Magee DM. Neuromuscular electrical stimulation and anabolic signaling in patients with stroke. *Journal of Stroke and Cerebrovascular Diseases,* 26(12):2954-2963, 2017.

35. Mettler JA, Magee DM, and Doucet BM. High-frequency neuromuscular electrical stimulation increases anabolic signaling. *Medicine & Science in Sports & Science,* 50(8):1540-1548, 2018.
36. Mettler JA, Magee DM, and Doucet BM. Low-frequency electrical stimulation with variable intensity preserves torque. *Journal of Electromyography and Kinesiology,* 42:49-56, 2018.
37. Mullen SP, McAuley E, Satariano WA, Kealey M, and Prohaska TR. Physical activity and functional limitations in older adults: the influence of self-efficacy and functional performance. *Journals of Gerontology,* 67(3):354-361, 2012.
38. Nuepert SD, Lachman ME, and Whitbourne SB. Exercise self-efficacy and control beliefs: Effects on exercise behavior after an exercise intervention for older adults. *Journal of Aging and Physical Activity,* 16, 1-16, 2009.
39. Neyroud D, Samararatne J, Kayser B, and Place N. Neuromuscular fatigue after repeated jumping with concomitant electrical stimulation. *International Journal of Sports Physiology and Performance,* 12:1335-1340, 2017.
40. Offord NJ and Witham MD. The emergence of sarcopenia as an important entity in older people. *Clinical Medicine,* 17(4):363-366, 2017.
41. Ogaswara R, Kobayashi K, Tsutaki A, Lee K, Abe T, Fujita S, Nakazato K, and Ishii N. mTOR signaling response to resistance exercise is altered by chronic resistance training and detraining in skeletal muscle. *J Appl Physiol,* 114:934-940, 2013.

42. Ogasawara R, Sato K, Matsutani K, Nakazato K, and Fujita S. The order of concurrent endurance and resistance exercise modifies mTOR signaling and protein synthesis in rat skeletal muscle. *Am J Physiol Endocrinol Metab,* 306:E1155-1162, 2014.
43. Powell LE and Myers AM. The activities-specific balance confidence (ABC) scale. *Journal of Gerontology: Medical Sciences,* 50A(1):M28-M34, 1995.
44. Ramirez-Campillo R, Alvarez C, Garcia-Hermos A, Celis-Morales C, Ramirez-Velez R, Gentil P, and Izquierdo M. High-speed resistance training in elderly women: Effects of cluster training sets on functional performance and quality of life. *Experimental Gerontology,* 110:216-222, 2018.
45. Razaob, NA, Rahman NN, Tajuddin AE, and Nordin NA. Outcomes of chair-based exercise with progressive resistance training on physical performances among older adults: A preliminary study. *Jurnal Sains Kesihatan Malalysia*, 165-170, 2018.
46. Reidy PT, McKenzie AL, Brunker P, Nelson DS, Barrows KM, Supiano M, LaStayo PC, and Drummond MJ. Neuromuscular electrical stimulation combined with protein ingestion preserves thigh muscle mass but not muscle function in healthy older adults during 5 days of bed rest. *Rejuvenation Research,* 20(6):449-461, 2017.
47. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, and Sandri M. Mechanisms regulating skeletal muscle growth and atrophy. *FEBS Journal,* 280:4294-4314, 2013.

48. Signorile JF, Carmel MP, Lai S, and Roos BA. Early plateaus of power and torque gains during high- and low-speed resistance training of older women. *J Appl Physiol*, 98:1213-1220, 2005.
49. Sillen MJH, Franseen FME, Gosker HR, Wouters EFM, and Spruit MA. Metabolic and structural changes in lower-limb skeletal muscle following neuromuscular stimulation: A systematic review. *PLOS One*, 8(9), 2013.
50. Urbaniak GC and Plous S. Research Randomizer (Version 4.0) [Computer software]. 2013. Retrieved from http://randomizer.org/
51. Vivodtzev I, Debigare R, Gagon P, Mainguy V, Saey D, Dube A, Pare ME, Belanger M, and Maltais F. Functional and muscular effects of neuromuscular electrical stimulation in patients with severe COPD. *Chest Journal*, 141(3):716-725, 2012.
52. Wall BT, Dirks ML, Verdijk LB, Snijders T, Hansen D, Vranckx P, Burd NA, Dendale P, and Van Loon LJC. Neuromuscular electrical stimulation increases protein synthesis in elderly type 2 diabetic men. *Am J Physiol Endocrinol Metab*, 303:E614-E623, 2012.
53. Wilkinson, SB, Phillips SM, Atherton PJ, Patel R, Yarasheski KE, Tarnospolsky MA, Rennie MJ. Differential effects of resistance training and endurance exercise in the fed state on signaling molecule phosphorylation and protein synthesis in human muscle. *J Physiol*, 586:3701-3717, 2008.

54. Witkowski S, Lovering RM, and Spangenburg EE. High-frequency electrically stimulated skeletal muscle contractions increase p70s6k phosphorylation independent of unknown IGF-1 sensitive signaling pathways. *FEBS,* 584:2891-2895, 2010.

55. Wolfe R. Protein synthesis and breakdown. *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis.* New York: Wiley-Liss; 377-417, 1992.

CHAPTER III

SUMMARY AND RECOMMENDATIONS FOR FUTURE RESEARCH

Summary

The purpose of this study was to investigate the effects of a 4-week neuromuscular electrical stimulation (NMES) intervention in healthy, older adults. The outcomes investigated were anabolic signaling of the mTORC1 pathway, strength, physical function outcome, and ADL self-efficacy. Little research has been done on the effects of NMES on anabolic signaling, and even less has been done on a 4-week treatment. Furthermore, no studies were found that have been conducted on the effects of NMES on self-efficacy.

This study consisted of 11 individuals that were randomly selected into the NMES treatment group or the Sham group. Pre- and post-testing was conducted prior to the intervention and started two days after the intervention ended. The first day of testing consisted of strength testing of the lower limbs with MVCs and EMG to measure muscle activity. On the second day of testing, a survey of self-efficacy for ADLs and the 5RSTS was administered. The intervention was 3 times a week for 4-weeks for 40 minutes on each leg. The Sham group was told they were receiving treatment and performed everything the same as the NMES group, except no stimulation was delivered. Biopsies were obtained on the first and last day of the intervention and were taken prior to NMES, 30 minutes post NMES, and 120 minutes post NMES.

Phosphorylated S6K1 and phosphorylated mTOR had a significant main effect for time (Pre-NMES, Post-30 min, Post-120 min) and the post hoc revealed Post-30 min was significantly upregulated when compared to Pre-NMES for both proteins. Phosphorylated

m-TOR also had a significant main effect for Day (Day 1, Day 12). There was no main effect for phosphorylated 4E-BP1, MVC, 5RSTS. Self-efficacy ADLs interaction had a medium effect size.

In conclusion, the findings of this study suggest that a 4-week session of NMES upregulates anabolic signaling proteins of the mTORC1 pathway (phosphorylated mTOR and phosphorylated S6K1) 30 minutes after stimulation. However, it is unclear if anabolic signaling improved with multiple training session when Day 12 was compared to Day 1 of the intervention. As this was only preliminary data, further research with more subjects needs to be conducted to better understand the effects of a 4-week intervention of NMES.

Recommendations for Future Research

Further research needs to be conducted on NMES to fully understand the effects of NMES on anabolic signaling in older adults. This study was conducted over 4-weeks but a longer intervention would be beneficial to learn about the long-term effects of NMES. Also, only three proteins were investigated. Evaluating other muscle proteins should be performed to investigate how NMES effects the muscle at a cellular level. Furthermore, NMES has shown promise as an alternative to resistance training. NMES should be researched on subjects that are not able to safely exercise, such as sarcopenic populations, to see if NMES would give that population the same benefits.

TABLES

TABLE 1. Participant characteristics.

Variable (n = 11)	NMES Group (n = 8)		Sham Group (n = 3)	
	Pre-	Post-	Pre-	Post-
Age, years	71 ± 1.5		73.7 ± 6.3	
Height, cm	164.0 ± 1.8		165.7 ± 1.6	
Body mass, kg	68.6 ± 3.8	68.7 ± 3.6	78.8 ± 11.0	77.8 ± 9.9
BMI, kg·m^{-2}	25.4 ± 1.1	25.5 ± 1.1	29.0 ± 4.7	28.3 ± 4.3
Body fat, %	34.6 ± 2.3	35.3 ± 2.3	42.1 ± 3.9	41.2 ± 4.0

Data are presented as mean and SE of all participants per group pre and post intervention.

FIGURES

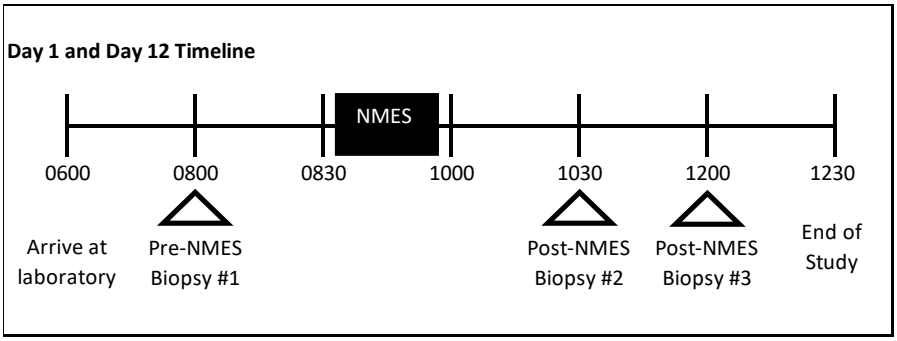

Figure 1. Muscle Biopsy Study Timeline Schematic (Intervention Day 1 and Day 12).

Figure 2. Experimental setup: Biodex isokinetic dynamometer with five securing straps and stimulating electrode placement for NMES application. A consent was signed by the subject allowing use and publication of the photographs.

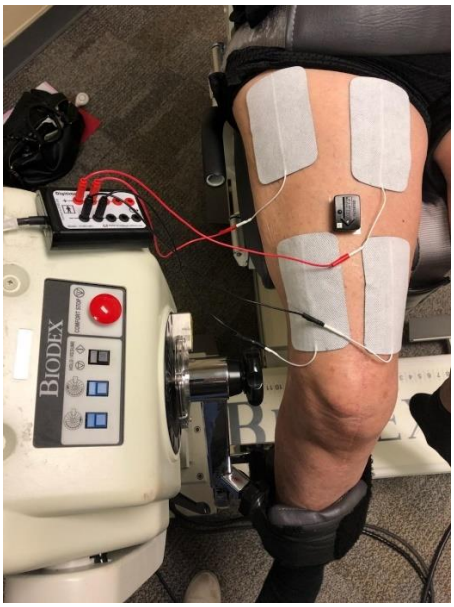

Figure 3. Stimulating electrode placement.

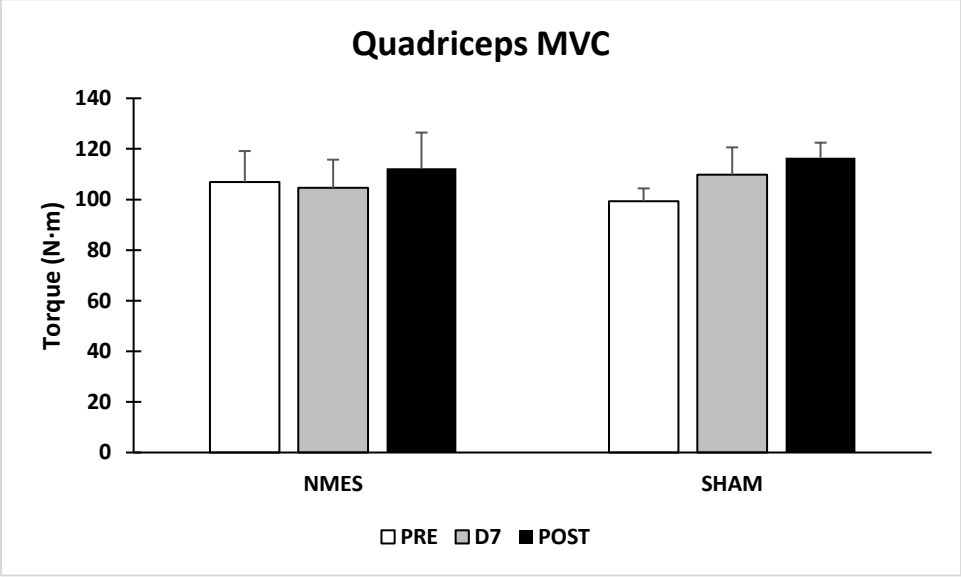

Figure 4. Quadriceps MVC: Strength was measured by MVC and was reported Pre-Intervention, Day 7 of the intervention, and Post-Intervention. NMES = 8; Sham = 3. Data are presented as mean ± SE. No significant changes between groups or time-point of the intervention. Significance is set at $p \leq 0.05$.

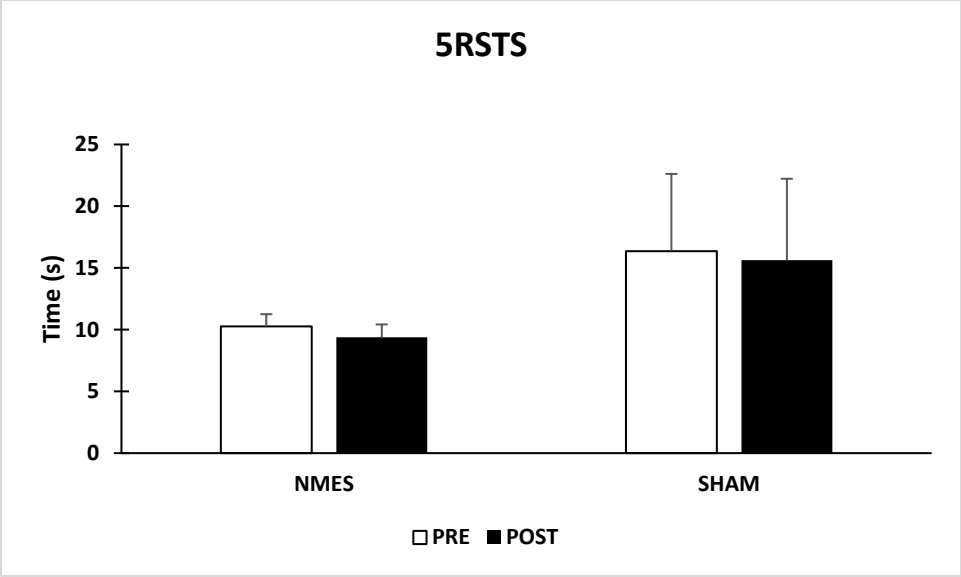

Figure 5. 5RSTS: A physical function assessment was performed using the 5RSTS Pre-Intervention and Post-Intervention for NMES and Sham groups. No significant changes between groups or time-point of the intervention. Values are presented as means ± SE. Significance is set at $p \leq 0.05$.

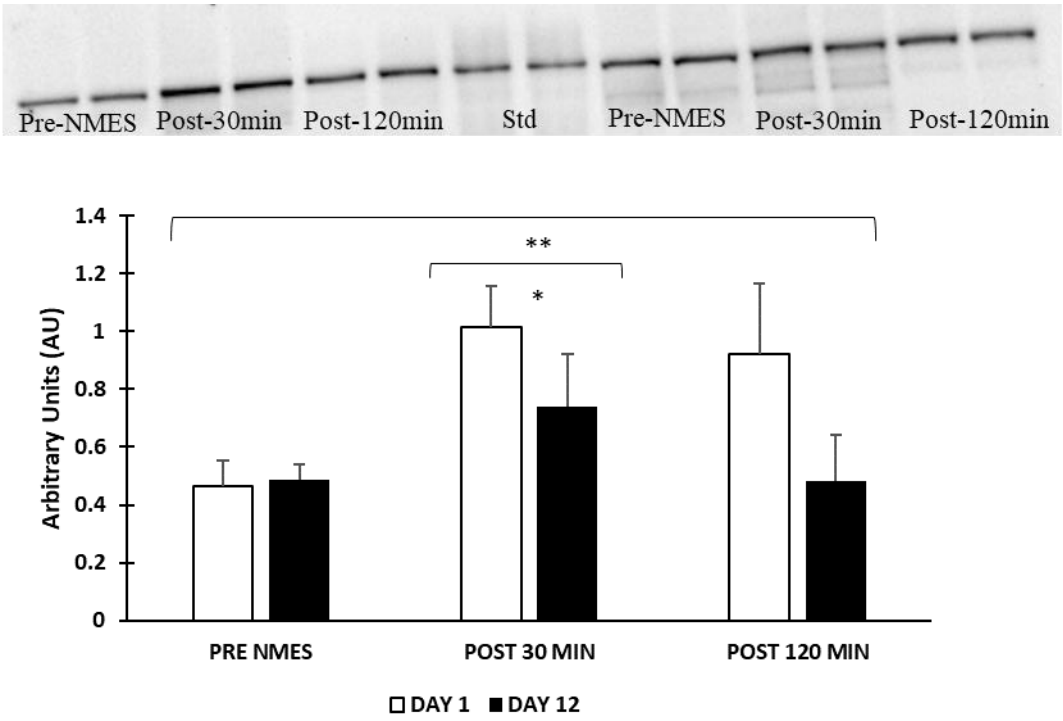

Figure 6. Anabolic signaling: phosphorylated mTOR; Skeletal muscle protein expression of p-mTOR Day 1 (pre-NMES, Post-30min, and Post-120min) and Day 12 (Pre-NMES, Post-30min, and Post-120min). Phosphorylation is expressed as phosphorylated:total protein content. NMES group only (n = 6). * with bracket indicates main effect for time, significantly greater than pre-NMES. ** with bracket indicates main effect for Day, Day 1 significantly greater than Day 12. Std = Standard. Values are presented as means ± SE. Significance is set at $p \leq 0.05$.

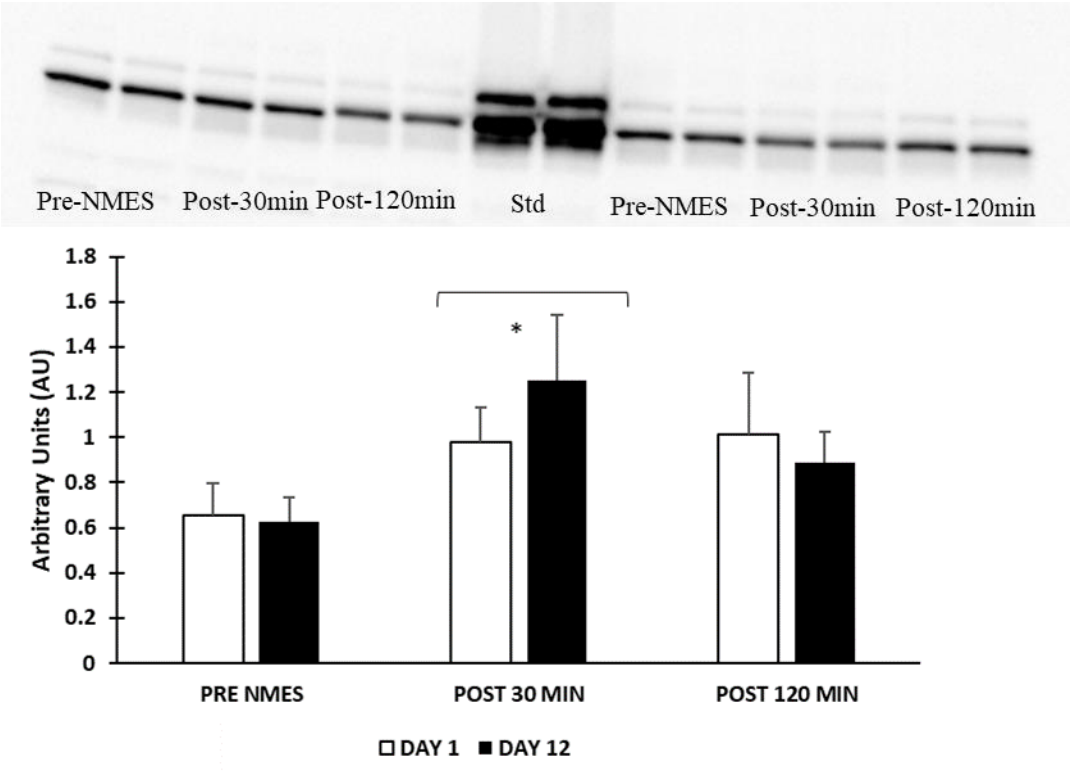

Figure 7. Anabolic signaling: phosphorylated S6K1; Skeletal muscle protein expression of p-S6K1 Day 1 (Pre-NMES, Post-30min, and Post-120min) and Day 12 (Pre-NMES, Post-30min, and Post-120min). Phosphorylation is expressed as phosphorylated:total S6K1. NMES group only (n=6). * with bracket indicates main effect for time, significantly greater than Pre-NMES. Std = Standard. Values are presented as means ± SE. Significance is set at $p \leq 0.05$.

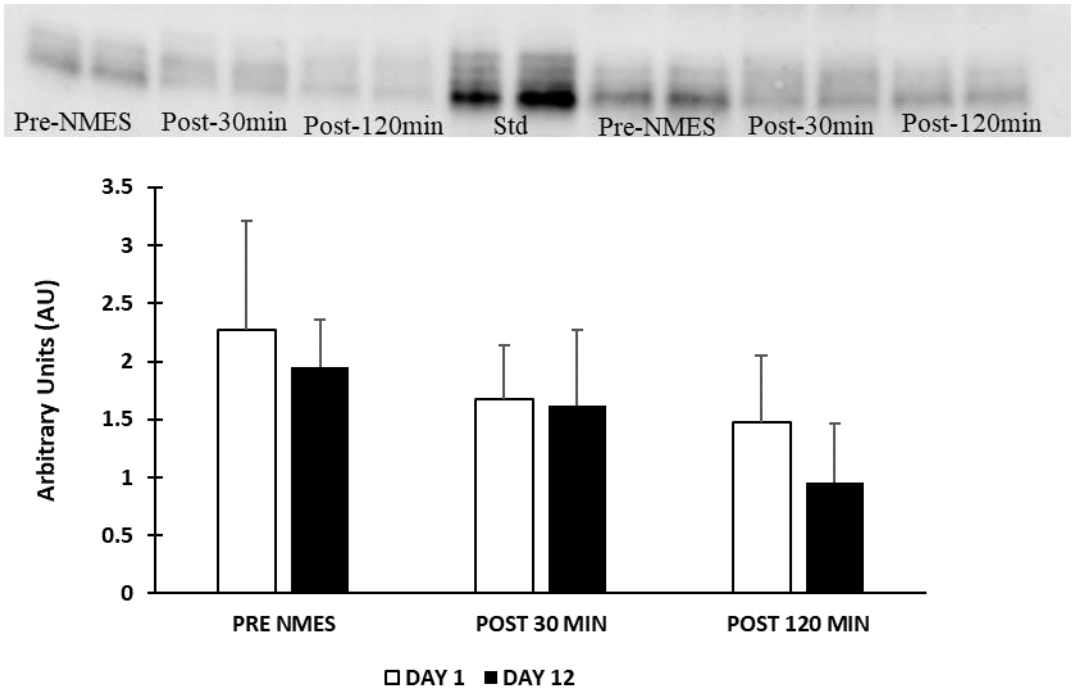

Figure 8. Anabolic signaling: phosphorylated 4E-BP1; Skeletal muscle protein expression of p-4E-BP1 Day 1 (Pre-NMES, Post-30min, and Post-120min) and Day 12 (Pre-NMES, Post-30min, and Post-120min). Phosphorylation is expressed as phosphorylated:total 4E-BP1. NMES group only (n=6). There no significant differences. Std = Standard. Values are presented as means ± SE. Significance is set at $p \leq 0.05$.

APPENDIX SECTION

Appendix A: Screening Forms

TELEPHONE HEALTH HISTORY SCREEN INFORMATION

Full Name:	**Date:**
DOB:	**Age:**
Address:	
Primary Phone:	**Alternate Phone:**
Email:	

Emergency Contact	**Name:**
Relationship:	**Phone Number:**

Height:	**Weight:**	**BMI:**

Yes	No	
		Do you exercise?
		What type of exercise? walking, resistance training, exercise class, etc.
		How often?
		Do you participate in any therapies (Physical Therapy, Occupational Therapy)?
		What area of the body and for what condition?
		Do you have any knee or hip problems (pain, swelling)?
		Explain
		Have you lost or gained any weight in the last 3 months?
		How much?
		Do you smoke?
		Have you ever smoked?
		How long and how many packs/day?
		Do you have a pacemaker?

		Have you had any surgeries?
		What for? When?
		Do you have any surgical hardware implants?
		Where? What for?
		Do you take insulin to control diabetes?

Do you have any other medical conditions?

	Yes	No
Diabetes		
Clotting disorders		
Autoimmune conditions		
Stroke		
Seizures		
Other neurological conditions		
Kidney or liver disease		
High blood pressure		
Heart Disease		
Thyroid condition		
Cancer in the last 5 years		
Varicose Veins		
Swollen or Infected areas		
Current injury to legs		
Other medical conditions not already addressed		

If yes, describe other medical conditions.

Yes	No	
		Do you have any allergies?
		List:
		Are you allergic to silver?
		Are you allergic to betadine/shell fish?
		Are you allergic to Lidocaine? (any reactions to numbing agents during dental or medical procedures?
		Have you had or do you have any allergies to Dermabond (medical glue)?
		Are you taking Warfarin (Coumadin) or any other anticoagulants including Aspirin?
		Are you taking any supplements, vitamins, or herbals?

		List:		
		Are you taking any prescription medications?		
Medication			**Dose**	**For**

Have you previously (currently) participated in a research study? When?

If you don't qualify for this study, would you be interested in participating in other studies as they become available? Yes No

Will you be unable to make a session (trips, work, etc.) for the next 8 weeks?

Appendix B: Testing Instructions

Five Repetition Sit to Stand Test

1. Equipment
    a. Chair (43-45cm) with arms
        i. Should not be against a wall or on a mat
        ii. Use the same chair for the entire study
    b. Timer
2. Testing
    a. Participant will sit with straight with arms folded over their chest
    b. Instructions: **When I say "GO," stand completely up and sit down 5 times as quickly and safely as you can. Make sure you fully extend when you stand and you do not have to touch the back of the chair when you sit. Ready, 3, 2, 1, GO.**
    c. Complete 3 trials with 30 seconds rest.
3. Score
    a. Record to the nearest 10^{th} second when the subject sits on the 5^{th} repetition.

Inability to rise from a chair five times less than 13.6 seconds is associated with increased disability and morbidity (Guralnick 2000)

Optimal cutoff time in predicting recurrent fallers is 15 seconds (Buatois et al. 2008)

Times exceeding the following can be considered to have worse than average performance (Bohannon, 2006).

- 60-69yr 11.4s
- 70-79yr 12.6s
- 80-89yr 14.8s

OPA Biodex Practice Protocol

Biodex Setting: Fatigue Practice

Set up Monitor
Pair Electrodes

Quadriceps
Submax
Your hands should be holding onto the handles. Make sure to stay seated and only use the thigh muscles on the front of your thigh. On my command, you will kick your leg out. You will kick your leg out to match the line on the screen and try to hold it as steady as you can until I tell you to stop. The line will change for different efforts.

20 N-m 2x hold for 5s
40 N-m 2x hold for 5s
50 N-m 2x hold for 3s

MVC
For maximum contractions, you will kick out as hard as you can until I say stop. Kick out as fast and as forcefully as possible then hold. Remember to stay seated and just use your thigh to kick out. I will count down from 3 and tell you to go. Ready, 3, 2, 1, GO.

MVC 2x hold for 3s

60 N-m 2x hold for 3s
25 N-m 2x hold for 5s

Start Timer

Hamstrings
Submax
Your hands should be holding onto the handles. Make sure to stay seated and only use the thigh muscles on the back of your thigh. On my command, you will pull your leg towards you. You will pull your leg to match the line on the screen and try to hold it as steady as you can until I tell you to stop. The line will change for different efforts. I will count down from 3 and tell you to go. Ready, 3, 2, 1, GO.

10 N-m 2x hold for 5s
20 N-m 2x hold for 3s
25 N-m 2x hold for 3s

MVC
For maximum contractions, you will pull back as hard as you can until I say stop. Pull back as fast and as forcefully as possible then hold. Remember to stay seated and just use your thigh to pull back. I will count down from 3 and tell you to go. Ready, 3, 2, 1, GO.

MVC 2x hold for 3s

Save As: OPA Subject # MVC and Fatigue Task Practice Date

Appendix C: Biopsy Flow Sheet

Date:	
Subject	**OPA**
Study Day	**NMES Day 1/12**

Biopsy	Time
1	
2	
3	

Real Time	Goal Time	Study Time	Sample Label	Procedures	Volume (ml)	BDNF (tiger top)	Green Top (glucose)	Notes/comments
	7:00 AM			subject arrives to lab				
	8:00 AM	pre	Pre	Pre- Blood Draw	**ml**	7.5 mL	10 mL	
	9:00 AM		**M1**	**Pre-Biopsy 1**				
	9:30 AM		**2 leg x 40-min each NMES Intervention**			start time	_____	
	11:00 AM		**2 leg x 40-min each NMES Intervention**			end time	_____	
	11:05 AM	NMES + 5 min	Post	Post- Blood Draw	**ml**	7.5 mL	10 mL	
	11:30 AM	NMES+30 min	**M2**	**Post-Biopsy 2**				
	1:00 PM	NMES+2 hrs	**M3**	**Post-Biopsy 3**				

Appendix D: Survey for ADL self-efficacy

For each of the following, please indicate your level of confidence in doing the activity without
losing your balance or becoming unsteady. Circle your response (0%=no confidence, 100%=completely confident).

If you do not currently do the activity in question, try and imagine how confident you would be if you had to do the activity. If you normally use a walking aid to do the activity or hold onto someone, rate your confidence as if you were using these supports. If you have any questions about answering any of these items, please ask us!

How confident are you that you will not lose your balance or become unsteady when you:	**Not Confident**					**Somewhat Confident**					**Completely Confident**
1. Walk around the house?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
2. Walk up or down stairs?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
3. Bend over and pick up a slipper from the front of a closet floor?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
4. Reach for a small can off a shelf at eye level?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
5. Stand on your tiptoes and reach for something above your head?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
6. Stand on a chair and reach for something?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%

7. Sweep the floor?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
8. Walk outside the house to a car parked in the driveway?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
9. Get into or out of a car?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
10. Walk across a parking lot to the mall?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
11. Walk up or down a ramp?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
12. Walk in a crowded mall where people rapidly walk past you?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
13. Are bumped into by people as you walk through the mall?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
14. Step onto or off an escalator while you are holding onto a railing?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
15. Step onto or off an escalator while holding onto parcels such that you cannot hold onto the railing?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
16. Walk outside on icy sidewalks?	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%

Appendix E: Intervention Form

NMES DATA SHEET

Subject ID _____ Training Day_____ Date_____

Administrator_____

R / L Leg	MVC			15% MVC Target Torque					
Time	0 (Min)	5	10	15	20	25	30	35	40
Beg Torque									
End Torque									
mA									

R / L Leg	MVC			15% MVC Target Torque					
Time	0 (Min)	5	10	15	20	25	30	35	40
Beg Torque									
End Torque									
mA									

Administer pain survey

Adminster perception survey after final pain survey

REFERENCES

1. Bandura A. Self-efficacy: Toward a unifying theory of behavioral change. *Psychological Review,* 84(2):191-215, 1977.
2. Barss TS, Ainsley EN, Claveria-Gonzalez FC, Luu MJ, Miller DM, Wiest MJW, and Collins DF. Utilizing physiological principles of motor unit recruitment to reduce fatigability of electrically-evoked contractions: A narrative review. *Archives of Physical Medicine and Rehabilitation,* 99:779-791, 2018.
3. Bax L, Staes F, and Verhagen A. Does neuromuscular electrical stimulation strengthen the quadriceps femoris? *Sports Med,* 35(3):191-212, 2005.
4. Bohannon R. Reference values for the five-repetition sit-to-stand test: a descriptive meta-analysis of data from elders. *Percept Mot Skills,* 103(1):215-222, 2006.
5. Bremner, CB, Holcomb WR, and Brown CD. Knee joint influences neuromuscular electrical stimulation-induced torque. *Athletic Training and Sports Health Care,* 7(4): 165-173, 2015.
6. Buatois S, and Benetos A. Five time sit to stand test is a predictor of recurrent falls in healthy community-living subjects aged 65 and older. *Journal of American Geriatrics Society,* 56(8):1575-1577, 2008.
7. Cattagni T, Lepers R, and Maffiuletti NA. Effects of neuromuscular electrical stimulation contralateral quadriceps function. *Journal of Electromyography and Kinesiology,* 38:111-118, 2018.

8. Churchward-Venne TA, Breen L, and Phillips SM. Alterations in human muscle protein metabolisms with aging: Protein and exercise as countermeasures to offset sarcopenia. *Biofactors*, 40(2):199-205, 2013.
9. Coffey VG, Zhong Z, Shield A, Canny BJ, Chibalin AV, Zierath JR, Hawley JA. Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans. *FASEB J*, 20:190-192, 2006.
10. Conlon JA, Newton RU, Tufano JJ, Penailillo LE, Banyard HG, Hopper AJ, Ridge AJ, and Haff GG. The efficacy of periodized resistance training on neuromuscular adaptations for older adults. *Eur J Appl Physiol*, 117:1181-1194, 2017.
11. Dirks ML, Wall BT, Snijders T, Ottenbros CLP, Verdijk, and Van Loon LJC. Neuromuscular electrical stimulation prevents muscle disuse atrophy during leg immobilization in humans. *Acta Physiol*, 210:628-641, 2014.
12. Dirks ML, Hansen D, Van Assche A, Dendale P, and Van Loon LJC. Neuromuscular electrical stimulation prevents muscle wasting in critically ill comatose patients. *Clinical Science*, 128:357-365, 2015.
13. Dirks ML, Groen BBL, Franssen R, Van Kranenburg J, and Van Loon LJC. Neuromuscular electrical stimulation prior to presleep protein feeding stimulates the use of protein-derived amino acids for overnight muscle protein synthesis. *J Appl Physiol*, 122:20-27, 2017.

14. Dirks ML, Wall BT, Kramer IR, Zorenc AH, Goessens JPB, Gijsen AP, and Van Loon LJC. A single session of neuromuscular stimulation does not augment postprandial muscle protein accretion. *Am J Physiol Endocrinol Metab,* 311:E278-E285, 2016.
15. Doucet BM and Griffin L. High-versus low frequency stimulation effects on fine motor control in chronic hemiplegia: A pilot study. *Top Stroke Rehabil,* 20(4):299-307, 2013.
16. Fahey TD, Harvey M, Schroeder RV, Ferguson, F. Influence of sex differences and knee joint position on electrical stimulation-modulated strength increase. *Medicine and Science in Sports and Exercise,* 17(1):144-147, 1985.
17. Filho JC, Gurjao AL, Ceccato M, Prado AK, Gallo LH, and Gobbi S. Chronic effects of different rest intervals between sets on dynamic and isometric muscle strength and muscle activity in trained older women. *American Journal of Physical Medicine and Rehabilitation,* 96(9):627-633, 2017.
18. Freitas GR, Santo CC, Machado-Pereira MM, Bobinski F, Santos ARS, and Ilha J. Early cyclical neuromuscular electrical stimulation improves strength and trophism by Akt pathway and signaling in partially paralyzed biceps muscle after spinal cord injury in rats. *Physical Therapy,* 98(3):172-181, 2018.
19. Fry CS, Drummond MJ, Glenn EL, Dickinson JM, Gundermann DM. Aging impairs contraction-induced human skeletal muscle mTORC1 signaling and protein synthesis. *Skeletal Muscle,* 1, 11, 2011.

20. Gondin J, Brocca L, Bellinzona E, D'Antona G, Maffiuletti NA, Miotti D, Pellegrino MA, and Bottinelli R. Neuromuscular electrical stimulation training induces atypical adaptions of the human skeletal muscle phenotype: a functional and proteomic analysis. *J Appl Physiol*, 110:433-450, 2011).
21. Gondin J, Cozzone PJ, and Bendahan D. Is high-frequency neuromuscular electrical
stimulation a suitable tool for muscle performance improvement in both healthy humans
and athletes? *Eur J Appl Physiol,* 111:2473-2487, 2011.
22. Harris JA, and Benedict FG. A biometric study of basal metabolism in man. *J Chem Inf Model.* 53:284, 1919.
23. Hart PD and Buck DJ. The effect of resistance training on health-related quality of life in older adults: Systematic review and meta-analysis. *Health Promotion Perspectives*, 9(1):1-12, 2019.
24. Henneman E and Olsen CB. Relations between structure and function in the design of skeletal muscle. *J Neurophys*, 28:581-598, 1965.
25. Herzig D, Maffiuletti NA, and Eser P. The application of neuromuscular electrical stimulation training in non-neurologic patient populations: A narrative review. *PMR*, 7:1167-1178, 2015.
26. Jubeau M, Le Fur Y, Duhamel G, Wegrzyk J, Confort-Gouny S, Vilmen C, Cozzone PJ, Mattei JP, Bendahan D, and Gondin J. Localized metabolic changes and T2 changes induced by voluntary and evoked contractions. *Medicine & Science in Sports & Exercise*, 921-930, 2014.

27. Katula JA, Rejeski WJ, and Marsh AP. Enhancing quality of life in older adults: A comparison of muscular strength and power training. *Health and Quality of Life Outcomes,* 6:45, 2008.
28. Kekalainen T, Kokko K, Sipila S, and Walker S. Effects of a 9-month resistance training intervention on quality of life, sense of coherence, and depressive symptoms in older adults: randomized controlled trial. *Qual Life Res,* 27:455-465, 2018.
29. Kern H, Barberi L, Lofler S, Sbardella S, Burggraf S, Fruhmann H, Carraro U, Mosole S, Sarabon N, Vogelauer, Mayr W, Krenn M, Cvecka J, Romanello V, Pietrangelo L, Protasi F, Sandri M, Zampieri, and Musara A. Electrical stimulation counteracts muscle decline in seniors. *Frontiers in Aging NeuroScience,* 6(189), 2014.
30. Kekalainen T, Kokko K, Tammelin T, Sipila S, and Walker S. Motivational characteristics and resistance training in older adults: A randomized controlled trial and 1-year follow-up. *Scan J Med Sci Sports,* 28, 2416-2426, 2018.
31. Koopman R and Van Loon LJC. Aging, exercise, and muscle protein metabolism. *J Appl Physiol,* 106, 2040-2048, 2009.
32. Kumar V, Shelby A, Rankin D, Patel R, Atherton P. Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol,* 587, 211-217, 2009.
33. Lim CH, Luu TS, Phoung LQ, Jeong TS, and Kim CK. Satellite cell activation and mTOR signaling pathway response to resistance and combined exercise in elite weight lifters. *Eur J Appl Physiol,* 117:2355-2363, 2017.

34. Lox CL, Martin Ginis KA, and Petruzzello SJ. The Psychology of Exercise: Integrating Theory and Practice, 3rd edition. Holcomb Hathaway: Scottsdale Arizona, 2010.
35. Mengyao L, Verdijk LB, Sakamoto K, Ely B, Van Loon LJC, and Musi N. Reduced AMPK-ACC and mTOR signaling in muscle from older men, and effect of resistance exercise. *Mechanisms of Ageing and Development,* 133:655-664, 2012.
36. Mettler JA, Bennett SM, Doucet BM, and Magee DM. Neuromuscular electrical stimulation and anabolic signaling in patients with stroke. *Journal of Stroke and Cerebrovascular Diseases,* 26(12):2954-2963, 2017.
37. Mettler JA, Magee DM, and Doucet BM. High-frequency neuromuscular electrical stimulation increases anabolic signaling. *Medicine & Science in Sports & Science,* 50(8):1540-1548, 2018.
38. Mettler JA, Magee DM, and Doucet BM. Low-frequency electrical stimulation with variable intensity preserves torque. *Journal of Electromyography and Kinesiology,* 42:49-56, 2018.
39. Mullen SP, McAuley E, Satariano WA, Kealey M, and Prohaska TR. Physical activity and functional limitations in older adults: the influence of self-efficacy and functional performance. *Journals of Gerontology,* 67(3):354-361, 2012.
40. Nuepert SD, Lachman ME, and Whitbourne SB. Exercise self-efficacy and control beliefs: Effects on exercise behavior after an exercise intervention for older adults. *Journal of Aging and Physical Activity,* 16, 1-16, 2009.

41. Neyroud D, Samararatne J, Kayser B, and Place N. Neuromuscular fatigue after repeated jumping with concomitant electrical stimulation. *International Journal of Sports Physiology and Performance,* 12:1335-1340, 2017.
42. Offord NJ and Witham MD. The emergence of sarcopenia as an important entity in older people. *Clinical Medicine,* 17(4):363-366, 2017.
43. Ogaswara R, Kobayashi K, Tsutaki A, Lee K, Abe T, Fujita S, Nakazato K, and Ishii N. mTOR signaling response to resistance exercise is altered by chronic resistance training and detraining in skeletal muscle. *J Appl Physiol,* 114:934-940, 2013.
44. Ogasawara R, Sato K, Matsutani K, Nakazato K, and Fujita S. The order of concurrent endurance and resistance exercise modifies mTOR signaling and protein synthesis in rat skeletal muscle. *Am J Physiol Endocrinol Metab,* 306:E1155-1162, 2014.
45. Paddon-Jones D, Sheffield-Moore, M, Urban R, Aarsland A, Wolfe R, Ferrando A. The catabolic effects of prolonged inactivity and acute hypercortisolemia are offset by dietary supplementation. *J Clin Endocrinol Metab*, 90:1453-1459, 2005.
46. Powell LE and Myers AM. The activities-specific balance confidence (ABC) scale. *Journal of Gerontology: Medical Sciences,* 50A(1):M28-M34, 1995.
47. Ramirez-Campillo R, Alvarez C, Garcia-Hermos A, Celis-Morales C, Ramirez-Velez R, Gentil P, and Izquierdo M. High-speed resistance training in elderly women: Effects of cluster training sets on functional performance and quality of life. *Experimental Gerontology,* 110:216-222, 2018.

48. Razaob, NA, Rahman NN, Tajuddin AE, and Nordin NA. Outcomes of chair-based exercise with progressive resistance training on physical performances among older adults: A preliminary study. *Jurnal Sains Kesihatan Malalysia*, 165-170, 2018.
49. Reidy PT, McKenzie AL, Brunker P, Nelson DS, Barrows KM, Supiano M, LaStayo PC, and Drummond MJ. Neuromuscular electrical stimulation combined with protein ingestion preserves thigh muscle mass but not muscle function in healthy older adults during 5 days of bed rest. *Rejuvenation Research*, 20(6):449-461, 2017.
50. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, and Sandri M. Mechanisms regulating skeletal muscle growth and atrophy. *FEBS Journal*, 280:4294-4314, 2013.
51. Signorile JF, Carmel MP, Lai S, and Roos BA. Early plateaus of power and torque gains during high- and low-speed resistance training of older women. *J Appl Physiol*, 98:1213-1220, 2005.
52. Sillen MJH, Franseen FME, Gosker HR, Wouters EFM, and Spruit MA. Metabolic and structural changes in lower-limb skeletal muscle following neuromuscular stimulation: A systematic review. *PLOS One*, 8(9), 2013.
53. Urbaniak GC and Plous S. Research Randomizer (Version 4.0) [Computer software]. 2013. Retrieved from http://randomizer.org/

54. Vivodtzev I, Debigare R, Gagon P, Mainguy V, Saey D, Dube A, Pare ME, Belanger M, and Maltais F. Functional and muscular effects of neuromuscular electrical stimulation in patients with severe COPD. *Chest Journal,* 141(3):716-725, 2012.

55. Wall BT, Dirks ML, Verdijk LB, Snijders T, Hansen D, Vranckx P, Burd NA, Dendale P, and Van Loon LJC. Neuromuscular electrical stimulation increases protein synthesis in elderly type 2 diabetic men. *Am J Physiol Endocrinol Metab,* 303:E614-E623, 2012.

56. Wilkinson, SB, Phillips SM, Atherton PJ, Patel R, Yarasheski KE, Tarnospolsky MA, Rennie MJ. Differential effects of resistance training and endurance exercise in the fed state on signaling molecule phosphorylation and protein synthesis in human muscle. *J Physiol,* 586:3701-3717, 2008.

57. Witkowski S, Lovering RM, and Spangenburg EE. High-frequency electrically stimulated skeletal muscle contractions increase p70s6k phosphorylation independent of unknown IGF-1 sensitive signaling pathways. *FEBS,* 584:2891-2895, 2010.

58. Wolfe R. Protein synthesis and breakdown. *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis.* New York: Wiley-Liss; 377-417, 1992.

59. Zuzanna K, Willis SJ, Moberg M, Apro W, Calbet JAL, Holmberg HC, and Blomstrand E. Endurance exercise enhances the effect of strength training on muscle fiber size and protein expression of Akt and mTOR. *PLOS One,* 11(2), 2016.