NEUROMUSCULAR ELECTRICAL STIMULATION USE IN TRANSTIBIAL AMPUTATIONS

by

Sara Peterson

BA, Metropolitan State University, 1998

MBA, Baker College, 2007

Submitted to the Graduate Faculty of

The School of Health and Rehabilitation Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2018

UNIVERSITY OF PITTSBURGH

SCHOOL OF HEALTH AND REHABILITATION SCIENCES

This dissertation was presented

by

Sara Peterson

It was defended on

May 22, 2018

and approved by

Hongwu Wang, PhD, Assistant Professor, Department of Rehabilitation Sciences, University of Oklahoma Health Sciences Center

Mary Ann Miknevich, MD, Assistant Clinical Professor of Physical Medicine & Rehabilitation, University of Pittsburgh

Sara R. Piva, PhD, Professor, Department of Physical Therapy

Rory Cooper, PhD, Professor, Chair, Department of Rehabilitation, Science and Technology

Alicia Koontz, PhD, Dissertation Advisor, Associate Professor, Department of Rehabilitation, Science and Technology

NEUROMUSCULAR ELECTRICAL STIMULATION USE IN TRANSTIBIAL AMPUTATIONS

Sara Peterson, PhD

University of Pittsburgh, 2018

Individuals with amputations experience an invasive surgery compromising the residual limb resulting in detriments such as a decrease in muscle strength, muscle atrophy, pain and gait impairments. Neuromuscular Electrical Stimulation (NMES) has been shown to improve the strength and volume of the quadriceps muscles in individuals who have experienced a total knee arthroplasty, osteoarthritis of the knee, anterior cruciate ligament repair and in individuals who suffer from a chronic illness. NMES has also been shown to decrease pain in conditions such as knee osteoarthritis, chronic back pain, and total knee arthroplasty. We proposed a novel idea to apply NMES to individuals with amputation to see if we can achieve the same results. The purpose of this study was to demonstrate the efficacy of NMES for individuals with a transtibial amputation. We aimed to demonstrate that when compared to a control group, individuals who received three months of NMES intervention will show greater knee extension strength, increased volume of the residual limb, decreased chronic and phantom pain and improved gait relative to the baseline measures. We also evaluated the feasibility of this intervention and determined effect sizes to power a larger study. Twenty unilateral transtibial participants who were greater than one-year post amputation were recruited and randomized into two groups. The study consisted of a baseline visit and four follow up visits. Changes in outcomes were examined between baseline and 12 weeks using a Mann-Whitney U test and box and scatterplots. Fifteen subjects completed the study. The data showed no statistical significant changes in all the outcomes except for a significant decrease in residual limb pain at 12 weeks ($p=0.03$). Clinically

significant changes in various outcome measures were found for some individual participants. Although the study findings were inconclusive participants felt that their strength and residual limb shape improved with the NMES intervention and that the NMES was very easy to use. This pilot study demonstrated feasibility and excellent compliance with an at-home NMES intervention. A future study involving refined measurement techniques and testing with a larger cohort of transtibial amputees is warranted.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to thank my dissertation chair and advisor, Alicia Koontz, PhD, ATP for her never-ending patience, support and guidance. I am grateful that I had the opportunity to study under such a leader in the field of Rehabilitation Science. You are a wonderful advisor, mentor, educator and friend.

I would like to thank the members of my dissertation committee for always being available and helping me through this dissertation process. Thank you to Hongwu Wang, PhD, Sara Piva, PhD, Rory Cooper, PhD and a special thanks to Mary Ann Miknevich, MD for referring subjects to this study.

I would also like to thank my colleagues, Bob Maguire and Zack Anzalone for their support and encouragement throughout the dissertation process. I would like to thank the HERL staff who assisted with equipment, fabricating the custom plates for the Biodex and for the administrative support. I would like to thank my MSPO students for their assistance, OPERF and SHRS for funding the project. I would also like to thank my family and friends for their support and empowerment.

1.0 INTRODUCTION

Functional NMES involves low-level electrical stimulation to specific nerves to activate the musculature to improve muscle strength, circulation and function [1]. Use of functional NMES has been researched among persons with spinal cord injuries, multiple sclerosis, and cerebral vascular accidents [2-4]. However, to date, no randomized control trials have investigated the use of NMES above and below the knee in one treatment session to increase muscle strength and volume of the residual limb and treat post-amputation symptoms such as edema and pain for individuals with amputation. Thus, this study determined the efficacy of NMES use with persons with transtibial amputations. The overall goal of the research is to determine if 12 weeks of at-home NMES results in benefits to persons with unilateral, transtibial amputations (TTA) who are more than one-year status post their amputation surgery. Understanding the effects of NMES on the amputee's residual limb will provide valuable insight on strengthening, shaping, improving gait and decreasing pain in the residual limb.

1.1 SPECIFIC AIMS AND HYPOTHESES

Aim 1:

To determine the efficacy of a NMES home based training program on knee extension strength, residual limb volume, pain and gait.

Hypothesis 1 (H1): STRENGTH

In comparison to a control group, NMES training will show greater increases in isometric and isokinetic knee extension strength post training when compared to baseline (pre-training) in the residual limb as measured using a Biodex 4 system.

Rationale: Lack of strength in the residual limb leads to multiple detriments, such as loss of balance, slower gait and impaired activities of daily living for persons with transtibial amputations. [5-8]. Since NMES has shown benefits to individuals with a range of diagnoses to improve limb function and muscle strength [9-17] there is reasonable evidence to apply NMES to an amputee population.

Hypothesis 2 (H2): VOLUME

In comparison to a control group, NMES training will show greater increases in volume of the residual limb after (post) training when compared to baseline (pre-training) as measured using a hand held three-dimensional motion-tracking laser scanner CAD/CAM system and tape measure.

Rationale: As has been shown in other studies in other populations, NMES has been shown to increase muscle strength [9, 10] and if applied over time it can increase muscle mass, as seen through a computed tomography (CT) scan or magnetic resonance imaging (MRI) [13, 18]. Therefore, it's possible that with an increase in muscle mass and strength we will find increases in residual limb volumes and circumferences.

Hypothesis 3 (H3): PAIN

In comparison to a control group, NMES training will show greater decreases in chronic residual limb pain, phantom limb pain and phantom sensation after (post) training when compared to baseline (pre-training) as measured by a pain questionnaire designed for amputees measured using a numeric rating scale.

Rationale: Ninety-five percent of amputees surveyed reported experiencing one or more types of amputation-related pain in the previous month [19]. Persistent pain following amputation affects the quality of life, hinders rehabilitation and the use of a prosthesis for a reported 60-85% of amputees [19-21]. Studies have seen improvement with the use of TENS for pain therefore it is possible that a side effect of using the NMES will be a reduction of pain in the residual limb [22, 23].

Hypothesis 4 (H4): GAIT PARAMETERS

In comparison to a control group, NMES training will show greater increases in velocity and step length and percentage in stance time on the amputated limb after (post) training when compared to baseline (pre-training) as measured using the GAITrite® system.

Rationale: As shown in previous studies, NMES has been shown to increase muscle strength [13-15, 24-26] therefore it is possible that with increased strength (H1) and an improved

health of the residual limb (H2 and H3) we will find that step length on the amputated side, velocity of both limbs and percentage in stance time on the amputated side increases. Neder et al, in 2002 found significant increases in the 6-minute walk test distance after NMES treatment in patients which chronic obstructive pulmonary disease [27]. Previous studies also showed that NMES improved cadence, walking velocity, stance time of the involved limb, overall improved gait pattern and lower limb function in the total knee arthroplasty, knee osteoarthritis population and people who had knee anterior cruciate ligament repair [9, 28, 29].

1.2 BACKGROUND AND SIGNIFICANCE

1.2.1 Residual Limb Anatomy and Function

Bones and muscles

The lower limb is comprised of three main bones, the femur, tibia and fibula (patella and 26 bones in the foot). The muscles of the proximal lower limb aid in rotation, abduction, adduction, extension and flexion of the hip and knee [30]. These muscles are typically all intact during a transtibial amputation but may suffer weakness and or atrophy due to the surgery [30]. The muscles of the distal lower limb are comprised of ankle stabilizers and foot intrinsic muscles. In the anterior compartment (Figure 1) of the distal lower limb contains the anterior tibialis, extensor digitorum longus, and peroneus tertius. The lateral compartment contains the peroneus

longus and peroneus brevis muscles. The posterior compartment (Figure 1) contains the soleus, and more superficial, the gastrocnemius, medial and lateral head [31]. The soleus and gastrocnemius muscles attach onto the posterior calcaneus through the Achilles tendon. A transtibial amputation occurs below the knee and above the ankle. The foot is ablated along with the foot intrinsic muscles. Due to the foot being ablated, the remaining muscles have lost their insertion point.

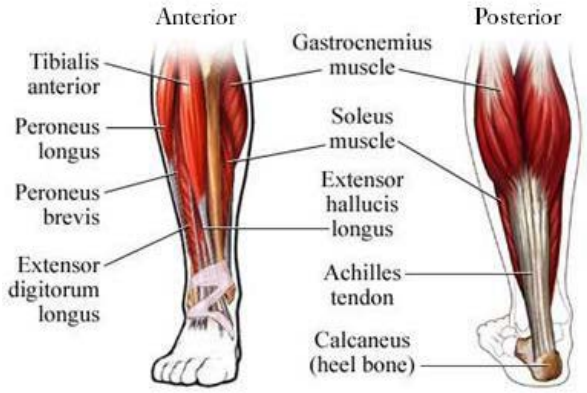


Figure 1 Muscles in the anterior and posterior compartments of the leg

(Trainharder.com, 2007-2010)

Arteries and nerves

Each compartment of the lower limb is innervated by various nerves and receives blood supply through various arteries. The anterior compartment muscles are innervated by the deep fibular nerve and supplied by the anterior tibial artery [32]. The lateral compartment muscles are innervated by the superficial fibular nerve and supplied by the fibular artery [32]. The posterior compartment muscles are innervated by the tibial nerve (Figure 2) and supplied by the posterior

tibial artery [32]. Therefore, an amputation is not a simple procedure because it entails stretching, transecting the nerves that will allow them to retract into the soft tissue to avoid a symptomatic neuroma [33]. Other avenues include a traction neurectomy, cauterization, ligation, capping or end loop anastomosis [33].

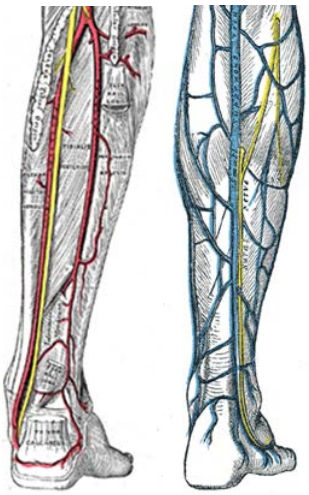


Figure 2 Lower leg schema, arteries depicted in red, veins depicted in blue, nerves depicted in yellow. (Gray's Anatomy, 1977)

1.2.1.1 What happens during an amputation

The goal of amputation is to remove unhealthy tissue and create a residual limb that is less painful and more useful prior to amputation. Comparing a transfemoral amputation to a transtibial amputation, the knee is preserved, therefore allowing for extension and flexion of the knee which may increase proprioception and reduce energy expenditure [31]. There are many decisions a surgeon has to make while performing the surgery. The surgeon decides on the length of the amputation through a Doppler reading [34]. Surgeons also use transcutaneous oxygen tension monitoring and appearance of the viable tissue to determine residual limb length [35].

The tibia and fibula are cut perpendicular to the long axis of the bone. The tibia is beveled with a rasp to increase comfort while weight bearing in a prosthesis [31]. A bone bridge between the tibia and fibula, called the Ertl procedure, is sometimes used during a transtibial amputation to create stability, increased weight bearing tolerance and a more functional gait [36]. The Ertl procedure is not typically used on persons with vascular disease or infection due to healing complications [36]. The anterior and lateral muscle compartments are divided and often a myodesis, suturing muscle and fascia to the bone or periosteum, or a myoplasty, suturing the agonist muscle to the antagonist muscle across the end of the bone, may be performed. An amputation involves many techniques to preserve health to the bone, nerves, arteries and veins.

Once the residual limb is healed, restoring the quality of life for amputees is vital. In normal gait, the muscles in the anterior compartment provide dorsiflexion and eversion of the foot, the muscles in the lateral compartment provide eversion of the foot and the muscles in the posterior compartment provide plantarflexion and inversion of the foot. Since these muscles have lost their insertion point, it is a challenge for prosthetic clinicians to replicate and replace the function of the amputee's anatomical limb with a prosthetic limb.

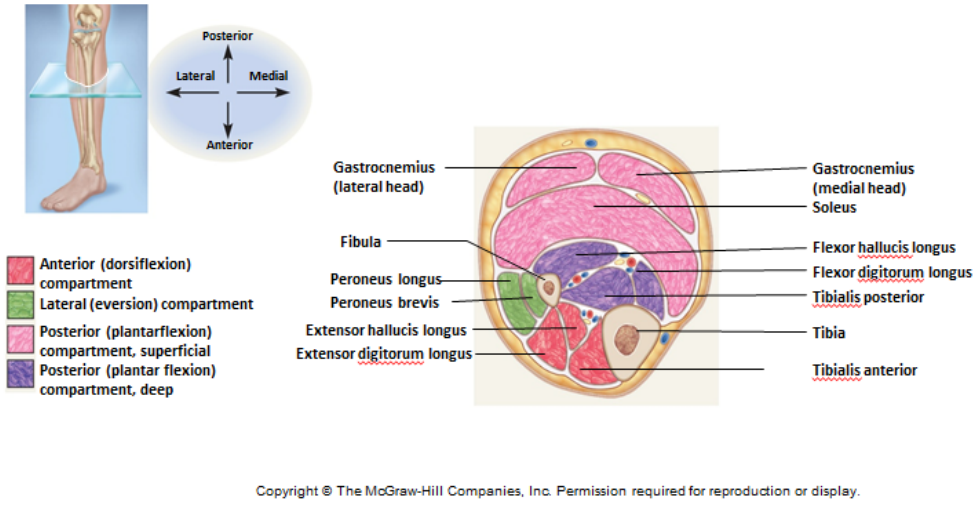


Figure 3 Cross-section of lower leg below the knee

1.2.2 Problems with living with an amputation

Once the amputee receives a prosthesis, he or she may encounter complications with the fit and function of the prosthesis and the residual limb. Besides chronic residual limb pain and phantom pain there are additional problems an amputee faces, muscle atrophy, residual limb volume fluctuation, decreased strength, improper socket fit and abnormal gait. These complications impact the quality of life for amputees.

Clinical prosthetists experience first-hand the difficulty for transtibial amputees to exercise the involved limb and keep the residual limb healthy. This population may experience many deficits, we defined four potential deficits that we researched to see how we can improve the quality of life for persons with transtibial amputations. In future studies, results may also be applied to a more generalized population in the rehabilitation science realm. With the use of

NMES, we explored if it was feasible to increase a transtibial amputee's involved limb strength. Secondly, if we could stabilize the transtibial amputee's limb volume, it may provide for a better fitting prosthetic socket. Thirdly, we wanted to explore the possibility to reduce residual limb pain, phantom limb pain and phantom sensation of the involved limb. Last, if we could reduce pain the amputee encounters and improve limb strength and maintain an optimal fitting prosthesis, it may improve gait parameters of the transtibial amputee. Therefore, we explored and tested three common gait parameters.

1.2.2.1 Strength

Transtibial amputees experience decreased strength in the residual limb below the knee due to the amputation across the muscle and the missing insertion site [37]. Studies have shown a relation between decreased strength and muscle atrophy in persons with amputations [38]. Remström et al., in 1983, found decreased isometric and isokinetic knee extension and flexion strength, through the use of an isokinetic dynamometer, in the amputated limb compared to the sound limb. His findings showed that the values in isometric and isokinetic knee extension and flexion strength in the amputated residual limb were significantly correlated to shorter step length, slower walking speed and smaller circumference of the thigh [6]. Furthermore, the results of the study generalized that individuals with a transtibial amputation who have a better preserved thigh muscle strength have a good walking capacity [6]. Lack of strength of the quadriceps and hamstrings in the residual limb leads to multiple detriments, such as loss of balance, slower gait and impaired activities of daily living for transtibial amputees [5, 7, 8]. Literature has shown that resistance and strength training can strengthen the thigh muscles to

improve these detriments for individuals with transtibial amputations [39, 40]. However, there is little evidence indicating strengthening techniques for the muscles below the knee for individuals with transtibial amputations.

One study has shown that training the residual limb muscles below the knee, while monitoring the contractions with a biofeedback device similar to those used to detect muscle activity in upper limb myoelectrics was successful with increasing limb volume, self-reported improved limb strength, better prosthetic suspension and an increase in velocity during gait [5]. Muscle activity was measured biweekly at the anterior tibialis, and the medial and lateral heads of the gastrocnemius muscles through the biofeedback device. Participants showed an increase in muscle activities over an eight week period through marked increases in the level of biofeedback signals [5]. This study showed that individuals with a transtibial amputation have muscle activation of the anterior tibialis and gastrocnemius muscle groups. Instead of having the participant contract their muscles, we hope to have the NMES treatment activate the muscles in the same manner and produce a similar or better outcome.

1.2.2.2 Residual Limb Size Volume

Limb amputation results in significant changes in body structures and functions. There is the physical loss of a body part as well as the closely related effects of the underlying disease, comorbidities, and concurrent injuries [41]. When the limb is amputated at the transtibial level, the muscles above the knee stay intact and the function of the knee joint is rarely affected [42] .

After an amputation, atrophy at the residual limb is dependent upon accurately suturing the cut muscle in a fixed position to maintain functional movement [37]. Muscles that are not

fixed accurately tend to degenerate or atrophy and are replaced by adipose tissue [37, 43]. The amount of atrophy also tends to increase the more proximal the amputation [43]. This is proven to be related to the distance the severed axons need to reach effector muscles [44]. Motor nerve impairment caused by amputation contributes to muscle atrophy [30]. Changes in muscle activation patterns also lead to atrophy. The hamstrings replace the gastroc/soleus muscles as the main muscles for propulsion [37]. Therefore, the transtibial amputee must adapt to a new muscular state: gait symmetry is altered, energy expenditure for walking is higher and resistance and gait training is needed in order to achieve optimal balance control [37].

Lack of exercise or movement of the muscle also contributes to muscle atrophy. This combination, results in overall weakness of the upper leg, even though muscles are intact. There is literature that supports effective treatments to reverse atrophy at the quadriceps muscles above the knee [30, 39, 40].

Known therapy exercises have assisted with not only improving atrophy of quadriceps and hamstrings but also the back and trunk for individuals with amputation [39, 40]. Likewise, studies have shown that NMES treatment can strengthen and improve atrophy of the quadriceps muscle of the affected limb for persons with knee osteoarthritis [9, 10]. If NMES can improve the affected intact limb strength, it may improve the amputated limb strength as well. Therefore, NMES treatment may be a viable treatment to improve atrophy in the quadriceps of transtibial amputees.

Muscle atrophy occurs below the knee in the transtibial residual limb due to the loss of the muscle insertion at the ankle and foot [31]. After amputation, muscle recruitment strategies and joint stabilization change [45-47] and studies have compared the difference between smaller

thigh circumference and weakness of the residual limb compared to the non-amputated side [7, 48, 49].

Kegel et al. reported that transtibial amputees show volume increases when given isometric contraction exercises to “dorsiflex” and “plantarflex” the foot. This study also revealed an increase in cross-sectional area at the gastrocnemius muscle, improved suspension, (e.g., means in which a prosthesis is attached to the residual limb), and an increase in velocity during gait [5]. There are no other documented modalities to reverse atrophy of the amputated limb below the knee.

Residual limb volume fluctuation

Residual limb volume fluctuation, the increase and decrease of volume, is a concern for many amputees and affects daily prosthetic socket fit, in turn, affecting quality of life [31]. Residual limb volume fluctuation is primarily caused by a person’s activity, diet, health status and medications [50]. In a prior study, Sanders reports that a person’s behavior toward diet and health status is related to volume fluctuations [51]. Removing or adding prosthetic socks throughout the day is the most common method to accommodate the residual limb for volume changes [52]. Studies show that differences in suspension types also effects residual limb volume [39, 53-59]. When comparing a transtibial amputee’s activity level, Sanders et al., found that transtibial amputees lose more residual limb volume during standing compared to walking or sitting [50]. This is a problem for the amputee because it affects the way the prosthetic socket fits. The amputee may need to add or remove prosthetic socks throughout the day. Morbidities such as diabetes, deep vein thrombosis, cellulitis, kidney (renal) complications, and congestive heart conditions all increase the chance of residual limb volume fluctuation [60, 61]. There are

over 900 medications that can contribute to edema [62]. Various types of medications can cause limb volume fluctuation that are used to treat high blood pressure, high cholesterol, inflammation, depression, estrogen levels and diabetes [62-66]. Literature has shown that transcutaneous electrical stimulation (TENS) has improved circulation in older people with chronic lower leg ulcers [67]. NMES applied to abled bodied people reduced foot and ankle volume while standing motionless for 30 minutes [68]. Low intensity NMES has been shown to increase blood flow to the buttocks in abled bodied individuals and individuals with a spinal cord injury while sitting [69]. These findings prove that TENS and NMES improves blood flow, decreases venous stasis, increases lymph flow and decreases volume. These findings may provide the same benefits for the transtibial amputee population. Therefore, this study hopes to show that NMES will stabilize volume fluctuation through improved circulation and better prosthetic socket fit.

Socket fit /residual limb volume problems

The bones and muscles in an intact, fully functioning limb transmit forces to the ground creating a natural ground reaction force during gait [70]. One of the goals to a successful prosthetic fitting is to emulate a normal ground reaction force on the amputated side. The prosthetic socket acts as an interface between the residual limb and prosthesis with the main goal of transmitting forces from the residual limb to the ground replicating the intact limb [31]. A correctly fitting socket is essential and must provide total contact with the limb in pressure tolerant areas to maximize surface load bearing otherwise discomfort will occur [30]. Many amputees face issues of improper socket fit. Atrophy and or edema below the knee may create a poor fitting socket, whereas, atrophy of the thigh muscles affects gait and function of the prosthesis [30]. Atrophy of

the muscles below the knee may cause the limb to slip further into the socket creating undue pressures on pressure sensitive areas, i.e., fibular head [71]. Atrophy of the muscles below the knee may also cause the “bell clapping” effect where the distal end of the limb has atrophied and can move in the socket anteriorly and posteriorly [72]. Another concern is pistoning of the socket [54]. If the socket is too loose the residual limb may have a tendency to move proximal and distal during gait causing friction and or instability [50]. Edema or swelling of the limb may prevent the residual limb to rest (sit) adequately in the socket, therefore the socket contours do not match the residual limb anatomy causing pressures and eventually skin breakdown [73]. Edema may also cause the prosthetic limb to fit too tight in the socket creating vascular problems and verrucous hyperplasia [30]. The importance of a proper fitting prosthetic socket is the most pertinent factor to determining successful prosthetic use [74]. Therefore, if we can create hypertrophy of the residual limb muscles, this may create a better fitting socket for individuals with transtibial amputation.

1.2.2.3 Pain

A national survey administered through the Amputee Coalition of America revealed that 95% of amputees surveyed reported experiencing one or more types of amputation-related pain in the previous month [19]. Persistent pain following amputation affects the quality of life, hinders rehabilitation and the use of a prosthesis for a reported 60-85% of amputees [19-21]. In the amputee population, there are two types of pain, chronic pain in the residual limb, which is referred to as residual limb pain (RLP) and phantom limb pain (PLP). Chronic residual limb pain is defined as discomfort that lasts for more than three months and is constant or frequent [75].

Constant pain is defined as pain that is always present but may vary in intensity [76] and frequent pain is defined as experiencing pain three to six times a week [76]. PLP is described as pain in the limb that has been amputated. Phantom limb pain is usually intermittent and is primarily localized to a specific area.

Although the limb is missing, the nerve endings at the amputation site continue to send pain signals to the brain making the brain think the limb is still there. Sometimes, the brain memory of pain is retained and is interpreted as pain, regardless of signals from injured nerves. This is interpreted as phantom pain. PLP often evolves from multiple origins that can incorporate cortical, spinal, and peripheral locations and therefore can be difficult to treat effectively [77].

Ephraim et.al reported that phantom limb pain is prevalent in 82.2% of the individuals who received on amputation within the past two years and 83.8% in individuals who received an amputation five to nine years prior [19]. The percentages for residual limb pain are lower; 76.1% and 72% respectively. Nikolajsen reported in a literature review, that 85% of American Veterans surveyed in 1982 by Sherman and Sherman reported phantom pain with an average mean number of years since amputation of 28.6$\pm$11.2 with only 12.1% respondents reporting a significant decrease in phantom pain over time with the remaining reporting no or some increase [21, 78].

Sherman and Sherman suggested 78% of amputees presented with PLP and approximately 22% suffer from RLP [79]. This study evaluated pain levels to assess if NMES intervention decreases chronic residual limb and or phantom pain in the residual limb.

1.2.2.3.1 Chronic Pain

RLP is common post-amputation. Nikolajsen reports in a prospective study of lower limb amputees, all participants reported RLP with a median intensity in the first week of amputation but the intensity and frequency diminished over time [21]. Furthermore, Nikolajsen revealed that severe or constant chronic pain is seen on average in 5-10% of amputees. This low percentage may be due to the fact there are many treatment modalities to control chronic pain. Furthermore, a study by Behr et al in 2009 reported that 81% of amputees surveyed reported RLP [80]. Ehde et.al., 2000, collected data from individuals six or more months since their amputation, revealing that RLP is as common as PLP, and was rated the worst pain problem by more participants than any other area on the body, such as the back, neck / shoulders, the other leg / foot, buttocks/ hip, arms/ hands, head and abdomen [76]. Further, 19% of these participants reported the pain as constant with variation to little variation in intensity [76]. Studies have shown that medication such as morphine and lidocaine have been found to ease RLP. Furthermore, isolated peripheral treatment with lidocaine can ease RLP but does not ease PLP in the same way as morphine [75].

1.2.2.3.2 Phantom Pain

During phantom limb pain (PLP) amputees report a stabbing, burning, aching, squeezing, shooting or throbbing to the limb that is no longer present. PLP occurs within days of the amputation and can continue for years [21]. In 1980, Sherman reported in a literature review

identifying 50 phantom limb pain treatment methods that were currently in use [78]. Today, there are various pharmacological treatments, common surgical procedures, psychological treatments and non-invasive treatments such as acupuncture, TENS, therapy modalities, such as applying heat or cold packs, mirror therapy, massage and ultrasound [81-83]. Furthermore, many amputees experience another phantom problem; phantom sensation. Phantom sensation is the feeling or movement of the missing limb, foot or toes and is not painful. Ehde, et al., 2000 reports over 70% of amputees describe the non-painful sensations as intermittent and brief, however 23% reported high disability and moderate to severe limitations from their phantom limb pain keeping them from their usual activities and interfering with daily, social, recreational and work activities. Phantom sensations are more frequent than phantom pain and have been experienced by almost all amputees but rarely present major clinical problems [21]. There are three known descriptors of phantom limb sensation. The first descriptor, kinetic perception; is the sense of movement of the absent limb. The second descriptor, kinesthetic perception; is the feeling that the phantom limb is a certain size or shape and that it may be in a position such as twisted or flexed. The third, exteroceptive perception, is the feeling of touch, pressure, temperature, itch or vibration of the missing body part [31]. Another phenomenon of phantom sensation is called telescoping. The amputee feels the phantom limb is retracting into the residual limb towards the body. This has been related with higher incidence of PLP**[31].**

1.2.2.4 Existing Solutions For Amputation Related-Pain

Various modalities are currently being used to control phantom pain but are costly, time consuming and can lead to additional side effects. Common methods used individually or more

often in combination to manage PLP are oral medications, noninvasive therapies such as applying hot and cold packs, massage, ultrasound, mirror therapy, psychological therapy, acupuncture, the prosthetic socket could be modified or liners / interfaces changed, nerves can be stimulated through a TENS device; or minimal invasive therapies such as nerve block injections or spinal cord stimulation can be tried [81-83]. Surgery, such as a neurectomy and stimulation implants, are final options if these other treatments have failed. Targeted nerve and muscle reinnervation may be another option that could relieve pain for the amputee. In targeted reinnervation, the limb nerves remaining after amputation are surgically redirected to new skin and muscle sites [84]. Targeted reinnervation was developed to provide intuitive sensory feedback and motor control mainly for upper-limb prosthetics[84]. A study by Kuiken et al reports that targeted muscle reinnervation provides a distal target for the transected axons to grow into, which represents a novel technique for the prevention and treatment of neuromas and their painful sequelae [85].

1.2.2.4.1 Pain medicines

Pain management for PLP from a pharmacological perspective most commonly involves the use of non-steroidal anti-inflammatory drugs (NSAIDS) and acetaminophen [77]. Other pain medications include antidepressants, anticonvulsants, narcotics, and N-methyl-d-aspartate receptor antagonists (NMDA) [75]. Nonsteroidal (NSAID's) therapy such as ibuprofen as with acetaminophen both decrease pain by inhibiting prostaglandin synthesis, an inflammatory mediator responsible for pain [77]. Another drug class, the tricyclic antidepressants, such as amitriptyline have been on the market for several years and work by inhibiting presynaptic

transporter proteins for serotonin and norepinephrine and in turn is thought to have an analgesic effect as well as improve a patient's affect and overall well-being [86]. Anticonvulsants such as gabapentin (Neurontin), pregabalin (Lyrica), and topiramate (Topamax) have been used to treat PLP, however at best mixed results with this class in pain reduction are evident [87]. Opioids such as morphine and Tramadol control pain at both the spinal level and in the cortex and thus have shown both short and long term efficacy in post amputation pain, however, concerns for side effects related to long term opioid use such as respiratory depression, constipation, and tiredness are apparent [75] as well as addiction. The NMDA or N-methyl-d-aspartate receptor antagonists such as ketamine work centrally on the nervous system to decrease pain sensation and have shown to reduce residual limb pain and PLP as well as increase pain threshold [33]. However, long term effective treatment beyond one year in controlling PLP is evident [87] Overall, the benefits to pharmacological management of PLP does support its use, however concerns for long term efficacy, tolerance and side effects related to the long term need for medication for controlling PLP has its concerns.

1.2.2.4.2 Physical and psychological therapy

Other less invasive and non-pharmacological therapies that exist to aide in reducing pain associated with amputation includes psychological therapy [81] and therapy modalities such as applying hot and cold packs, massage, ultrasound and therapeutic stretching [82, 83]. Therapy modalities often consist of strengthening and stretching exercises of the residual limb. It requires multiple visits and is costly [88]. Mirror box therapy is a well-known psychological therapy used to create an optical illusion; having the brain see both limbs intact [89]. It has been known to

reduce pain, reduce muscle spasms, decrease phantom pain and decrease phantom sensations by movement of the sound limb reflecting in a mirror to visualize two intact limbs moving while the amputated limb performs the same movement but is hidden from sight [33].

1.2.2.4.3 Acupuncture

Acupuncture is another well-known treatment for pain. It has been shown to reduce many forms of pain [90]. Very little is known about how a Traditional Chinese Medicine approach could be used to treat phantom limb pain. Currently, there is no standard acupuncture protocol in the literature to treat this syndrome [90]. However, Trevelyan et al., in 2016 recruited 15 lower limb amputees for a randomized feasibility study. They found a clinically meaningful decrease in group mean average pain and group mean worst pain in the acupuncture group [90]. Their future work includes a randomized control trial.

1.2.2.4.4 Socket modifications

When a person with an amputation cannot tolerate their socket due to pain, the clinician must modify the existing socket for comfort and to enhance function. Multi-durometer liners have been used with success to comfort a painful residual limb [91, 92]. Structural socket materials have changed since the introduction to thermoformable plastics in the 1980's [93]. Interfaces can be fabricated to provide relief to problem areas on the residual limb. Silicone distal end pads can be added to the socket to provide for comfort [30].

1.2.2.4.5 Transcutaneous Electrical Stimulation (TENS)

TENS is an alternate form of electrical stimulation that historically used high frequencies for pain relief [1] but is now also administered at very low frequencies (sensory level TENS, 2-10 Hz) [2]. TENS propagates along smaller afferent sensory fibers specifically to override pain impulses. When very low frequencies are used, TENS specifically targets sensory nerve fibers and does not activate motor fibers; therefore, no discernible muscle contraction is produced [3]. There has been a plethora of studies validating the positive effects of a TENS device [22, 94-97]. Studies have shown symptomatic relief and management of chronic, intractable pain adjunctive treatment for post-surgical and post-trauma acute pain [22, 94-98] and relief of pain associated with arthritis [99, 100]. Some studies have validated the use of TENS for pain. Some studies indicate that TENS is not effective alone to show a positive increase in function or strength compared to TENS plus exercise for individuals [101, 102].

There have been a few studies that used TENS intervention as a modality to treat amputee pain and limb healing [23, 94]. A study by Finsen, et al., in 1980 reported that TENS had a definite effect on limb healing to individuals within the first weeks of limb amputation. This study was a randomization of patients into three groups, a) sham TENS and chlorpromazine, b) sham TENS only c) active TENS device. Participants included individuals with symes, transtibial and through the knee amputations who were followed for at least one year or until re-amputation or death. The limb healing process was followed for nine weeks. The participants were given the TENS device to use for 30 minutes, twice daily for two weeks. Two electrodes were placed over the femoral nerve, anteriorly, and two electrodes were placed over the sciatic nerve, posteriorly. The frequency of the TENS unit was set to deliver “bursts of 7

pulses twice per second, pulse frequency of 100 Hz, and a pulse duration of 90μs [23]. The amplitude was increased per patient comfort level. Not all of the residual limbs in the TENS group healed in the nine-week timeframe, but the re-amputation rate was lower in the TENS group compared to the other two groups. In a more recent study, Mulvey, et al., 2012 reported that ten trans-tibial amputees wore a TENS device for 60 minutes with a continuous pulse pattern and a setting at pulse duration of 80μs and pulse frequency at 100Hz. The amplitude was increased per patient comfort level. The electrode pads were placed where the individual had the most pain. Participants scored their pain at baseline, 30 minutes and 60 minutes using a scale from 0-10 (0= no pain, 10= worst pain imaginable). Seven participants reported no pain after the 60 minutes of TENS treatment. A 48-hour telephone call was placed as a follow up with no adverse comments. The study demonstrated that TENS has potential for reducing phantom limb pain and residual limb pain at rest and during movement.

Although these treatments investigated had some success, these studies are preliminary and have not been mainstreamed or adopted to clinical practice to help amputees.

1.2.2.4.6 Nerve block injections

Another option to treat PLP is the use of nerve blocks either pre, during or post amputation of a limb in an effort to decrease or prevent amputation related pain [103]. Currently both epidural and peripheral nerve blocks have shown efficacy but are utilized for only a few days after amputation occurs, thus do not prevent the chronic pain associated with PLP from developing long term [103]. Data does show however that continuous peripheral nerve blockade that is initiated prior to surgery and continuous for up to one month post amputation has shown greater

long term pain management in studies up to 36 months post amputation [103]. Side effects and risks include chance of infection, bleeding, itching / rash, pain and bruising [104].

1.2.2.4.7 Spinal cord stimulation

This involves the process of inserting tiny electrodes along the spinal cord. A small electrical current is delivered to the spinal cord [105]. A case study done by Bunch et al., 2014 reports a successful outcome for a bilateral lower limb amputee who had suffered from intense residual limb pain, phantom pain and chronic back pain. Studies as far back as 1980 have shown benefits to amputees with chronic residual limb and phantom pain [106].

Implantable peripheral nerve stimulators (PNS) are another form of therapy that has been shown to control PLP related to amputation [103]. These devices are surgically implanted typically near the dorsal root ganglion outside of the spinal cord where small electrodes provide selective stimulation of sensory neurons that lead to decreased sensation of pain [104]. The clinical use of PNS is minimal due to a lack of clinical trials, cost, and skills needed to perform electrode placement [103].

1.2.2.4.8 Surgery

A more invasive option in managing pain associated with amputation includes surgical interventions. Historically, central nervous system procedures such as rhizotomy, dorsal root entry, spinal ganglionectomy, and cordotomy have shown significant risk for permanent damage to nerve tissue and are marked currently as procedures of last resort to manage PLP [77]. Peripherally, nerve surgery that has shown some benefits to PLP is neuroma removal at the stump site with implantation of the proximal nerve ending into adjacent muscle [107]. This has

led to many amputees that experience neuroma pain to report not only a decrease in pain at the site in general but also greater comfort in wearing of their prosthesis [108]. These surgical procedures involve anesthesia, and therefore can create side effects [109].

1.2.2.5 Abnormal Gait

Abnormal gait in amputees can be due to cascade of factors, such as muscle weakness, laxity or tightness, pain, poor gait habit, foot or componentry selection, poor prosthetic alignment and ill socket fit [30]. Since the ankle and foot complex is missing, prosthetic clinicians have a difficult time replicating the gait of the intact limb. If unlimited motion is granted in all four anatomical directions (multiaxial) stability of the amputee may be compromised [30]. However, researchers, engineers and manufacturers have been trying to solve this problem for decades. Currently, there are hundreds of prosthetic feet available but they do not fully mimic the physiologic function of unimpaired joints [110]. Microprocessor-controlled ankle systems have been developed to facilitate a more natural gait. Studies have shown that while these systems offer a more normal gait they are bulky, heavy and expensive [110].

Abnormal gait in amputees may also be due to weak or tight musculature of the knee extensors, knee flexors, hip extensors, hip flexors or gluteal muscles [30]. Therapy such as gait training, resistance training, and strength training are the most popular modalities in treating muscle impairments for persons with amputation [31, 39, 40]. Having an abnormal gait or improper prosthetic alignment can cause many problems [111]. Abnormal biomechanical prosthetic alignment forces the amputee to compensate and creates an abnormal gait. Gait deviations in individuals with transtibial amputation can include decreased walking velocity and

increased interlimb asymmetries in step length, swing time, stance time, and joint mechanics [30]. Due to a misaligned prosthesis and or abnormal gait, amputees may suffer from pain in a variety of locations throughout the body, including the lower back [39]. Gait biomechanics and activity matched prosthetic componentry must be considered to assure proper alignment of the transtibial prosthesis to avoid abnormal gait in prosthetic users [31].

2.0 PRELIMINARY STUDIES OF ELECTRICAL STIMULATION AND LIMB AMPUTATION

2.1 ELECTRICAL STIMULATION

Electrical stimulation (ES) has become popular in the rehabilitation field. Various forms of ES have served different purposes. Besides TENS, there are two other forms of ES that have reported beneficial; FES and NMES. NMES, used interchangeably with electrical stimulation (ES), is typically provided at higher frequencies than TENS to produce muscle tetany and contraction that can be used for "functional" purposes and can be found in literature as early as 1964 [4]. This combination is known as functional electrical stimulation (FES). FES is applied to innervate peripheral nerves that control and move specific muscles or muscle groups [112]. FES may activate musculature to assist with a function [3] or restore lost abilities such as ankle dorsiflexion, standing or grasping following neurological damage from Cerebrovascular Accident (CVA) or Spinal Cord Injury (SCI) [11, 112, 113].

FES primarily works through a closed loop system to assist people with a neurological impairment through the central or peripheral nervous system, such as cerebral palsy, multiple sclerosis, CVA, SCI [3]. The closed loop system involves a sensory input through nerve stimulation to illicit a muscle contraction. In order to effectively dorsiflex the foot at the proper

moment in gait, a sensor is plugged into the device or blue tooth technology is used to program the person's gait. The sensor detects the moment the foot needs to dorsiflex in order to avoid tripping, therefore a closed loop system is used. Once the device is programmed, the sensor is removed. FES consists of biphasic pulsed current, shorter frequency and lower amplitude than NMES.

NMES is primarily used to treat muscle atrophy and is limited to the treatment of patients with disuse atrophy where the nerve supply to the muscle is intact, which includes both the central and peripheral nervous systems and other non-neurological reasons for atrophy [9]. NMES is an open loop system that does not involve a sensory input mechanism.

There is a paucity of studies evaluating the use and efficacy of FES or NMES to treat edema and RLP or PLP in individuals with transtibial amputation. ES research has been widely conducted with persons with various medical conditions such as knee replacement, back pain, hemi paralysis and paraplegia [112, 114-116] .

Among the therapeutic modalities available, NMES is presented as a non-invasive technique used by physical therapists. Besides reducing pain, NMES has been used to enhance muscle strengthening, minimize muscle hypotrophy, reduce muscle spasm and or spasticity and increase range of motion in the joint. NMES has been suggested as an alternative therapy for quadriceps muscle strengthening in older adults with chronic pain and joint stiffness, which prevents them from engaging in voluntary exercises [117]. Studies that have investigated anterior cruciate ligament reconstruction have shown positive results when combining NMES with an exercise to strengthen the quadriceps muscles [17].

Studies have explored the usability and benefits of TENS but documentation is limited investigating the use of NMES. Many studies report the benefits of NMES primarily testing the quadriceps muscles. NMES has shown benefits to individuals with a range of diagnosis to improve limb function and muscle strength; SCI, CVA, cerebral palsy (CP), anterior cruciate ligament repair and arthritis [9-17]. NMES helps with improving quadriceps strength in various conditions such as chronic obstructive pulmonary disease and cardiovascular problems, improves blood flow to prevent deep vein thrombosis and to treat problems of neurologic damage such as poor circulation or slow wound healing in diabetic foot ulcers [24-27, 118-120]. Further studies have shown that intervention of NMES treatment with supervised volitional exercise increases quadriceps femoris muscle strength through knee extension isometric torque tests and improves quadriceps femoris muscle atrophy through the use of a CT imaging for persons with rheumatoid arthritis [13].

NMES has been proven to reduce pain in a variety of populations. Most studies show a reduction in pain for the knee due to osteoarthritis (OA), or a surgical procedure of the knee such as a total knee arthroplasty (TKA) or anterior cruciate ligament reconstruction (ACLR). Furthermore, studies show that NMES reduces pain for persons with low back pain [9, 28, 115, 121-123].

Previous studies have shown electrical stimulation helps with phantom pain [23, 94, 98]. The sensation of a limb when not present and or pain along with other paresthesia perceived in phantom limb pain are often thought to have developed from damaged peripheral nerve ending causing increased excitability in the central nervous system namely at the dorsal horn in the spinal cord [21, 75]. TENS has been proven to reduce hyperalgesia through both peripheral and

central mechanisms [124]. Studies have shown that high frequency TENS reduce dorsal horn neuron activity and reduces central neuron sensitization. High frequency TENS also reduces the release of the excitatory neurotransmitters glutamate and substance P, which is a small peptide that transmits pain signals from the sensory nerves to the central nervous system located in the spinal cord dorsal horn.

The mechanism of pain relief with TENS or NMES is explained by the gate control theory of pain [125] developed by Melzack and Wall. ES input transmits along large diameter afferent fibers, which, in turn, activates the inhibitory substantia gelatinosa (SG) (gray matter on the dorsal surface of the dorsal column of the spinal cord) interneurons, therefore closing the gate to the transmission of nociceptive (pain) information [2]. It is thought that TENS produces analgesia by activation of cutaneous afferent fibers at the site of application [126]. The ES interrupts the pain message to the brain similar to rubbing a bumped knee that may help relieve pain. Therefore, when NMES is applied to the area, it sends an electrical impulse which interrupts the message about pain sent from the nerves to the brain. The electricity blocks the activity of the pain receptors, which send the pain messages. If the brain doesn't get the messages from the nerves, it doesn't think that there is pain, so the person does not feel the pain.

There is strong evidence in the literature that strength training with NMES improves pain scores and functional outcomes in knee OA patients [9]. Vaz et al. reported an 8% improvement in isometric torque quadriceps strength and a reduction in pain of 38% in the NMES group [9]. Gaines, et al., 2004, studied a sample size of 43 participants 60 years of age or older with radiographic and clinical evidence of knee osteoarthritis. The study was a nurse managed home based program. Participants were randomized into the NMES group or education-only group.

NMES and was applied to the affected side quadriceps muscle and worn 15 minutes per day three days a week for 36 sessions. A decrease in pain was reported after 74% of the NMES sessions [127]. Although there was immediate decline in arthritis knee pain when NMES was used, results showed no significant group differences [127]. With a larger sample size, n=82, Imoto et al., in 2013 found significant differences between OA participants in the control group compared to the NMES group improving pain during an 8 week treatment program[122]. Laufer et al., in 2014 found that a significant group effect was demonstrated indicating a greater decrement in pain in the group receiving NMES in addition to an exercise program compared with the exercise-alone group [28].

Demircioglu et al., tested two groups of thirty individuals with OA who underwent total knee arthroplasty (TKA). Both groups were given home exercises, and the intervention group received NMES on the vastus medialis muscle five days a week for 30 minutes for 4-6 weeks. Results showed a statistically significant improvement in knee-related pain in the NMES groups at one month and three months post TKA [123].

Fitzgerald, et al., investigated quadriceps strength, activities of daily living scale (ADLS) and knee pain in a sample size of 43 subjects who had undergone anterior cruciate ligament reconstruction (ACLR). [15]. The group who received NMES showed a significant increase of strength and ADLS after 12 weeks whereas the knee pain rating did not show significant improvement [15]. NMES was deemed helpful but it was not reflected this in the pain outcome measure.

NMES has been shown to improve low pain back. Moore, et al., studied 24 persons with low back pain. Participants self-administered the treatment of a placebo, or a TENS device,

producing an asymmetrical biphasic square pulse, pulse width of 100 microseconds with a frequency of I00Hz, and adjustable amplitude within 0 to 60mA., or a NMES device, producing a symmetrical biphasic square pulse, pulse width of 200 microseconds with a frequency of 70Hz, and an adjustable amplitude within 0 to l00mA or subjects received a combination of TENS and NMES. Both the TENS and NMES visual analog scale of pain relief (VAS-R) scores were shown to be statistically significant. With NMES showing a 19% decrease in pain score [115].

It is difficult to exercise a muscle that has been amputated and is partially intact. ES conditions the muscle similar to exercise. It is difficult for TTA to exercise or strengthen the residual limb due to the short lever arm of the tibia. NMES is a good alternative exercise method for amputees to improve the health of the residual limb. NMES would allow for an intense workout of the muscle in lieu of a high intensity physical regimented workout. Our clinical experience has found improvements in knee function, socket fit, and reductions in pain with wearing NMES alone (see Preliminary Studies section). Should we demonstrate that NMES alone helps improve the outcome measures in this study; it could potentially be a highly practical, easy to use, cost effective treatment that can be done at home.

2.2 SIGNIFICANCE

According to a 2008 study, 1.6 million persons in the U.S. were living with limb loss in 2005 [128]. This number is expected to increase to 3.6 million by the year 2050 [128]. Of those with

limb amputation; approximately 80% develop residual limb pain or phantom pain within two years of their amputations [129].

Existing solutions for amputation-related chronic pain are pain medicines, nerve block injections, physical therapy, surgery and socket modifications [98]. All of these solutions have limitations in availability and cost, which prevents the widespread use of these treatments. In a recent systematic review, the use of TENS in amputation showed great promise to lessen phantom and residual limb pain but a number of limitations were raised with the studies found and no strong studies (e.g. randomized control trials) have yet been conducted [98].

Individuals with transtibial amputation have had their quality of life changed. Besides facing the overall pain from an amputation, transtibial amputees experience muscle weakness, muscle atrophy, prosthetic socket fit issues, learning to walk again and to live with a prosthesis that is not “part of their body”. If we can stabilize the residual limb volume, make the residual limb stronger, make gait more normal and decrease pain, this will solve many of the detriments the amputee faces.

This study is important because it is a starting point to develop a rehabilitation protocol that can help address the many functional deficits that occur after a transtibial amputation. With the information we learn from this study, we can apply it to future studies to help individuals with other levels of amputation and possibly improve immediate post-amputation care.

This study is a promising start to developing a cost-effective treatment for increasing residual limb strength, volume and decreasing pain which would improve the quality of life for many transtibial amputees.

2.3 PRELIMINARY STUDIES

Anecdotal evidence from a local prosthetist, Drew Buffat, CP, has shown positive results with the use of NMES in trans-tibial amputees. Several patients who were offered NMES at his clinic reported relief from chronic pain in their residual limb. Other changes were also noted in the residual limbs after continued use of NMES including a reduction in atrophy in the residual limb. A custom garment was measured (Figure 4) and fabricated to house the NMES surface electrodes. Electrodes were placed on the muscle belly of the quadriceps, hamstrings, anterior tibialis and the remaining muscles of the gastrocnemius muscles (Figure 5&Figure 6). The patient was instructed to wear the garment for up to 30 minutes continuously daily for three months. It is inconclusive how the stimulation was delivered and how improvements were seen by only one electrode being placed on the gastrocnemius and anterior tibialis muscles. There may have been a "transfer" effect from the electrical stimulation of the other muscle groups.

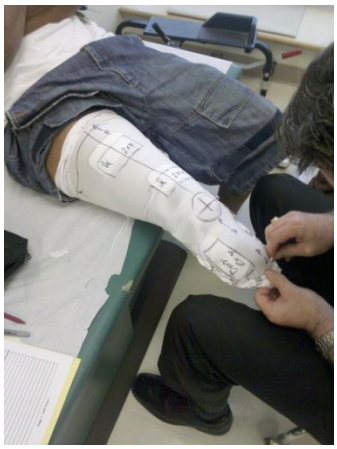


Figure 4 Pattern fabrication process

Figure 5 Final garment with electrodes in place, inside view

Figure 6 Final garment, outside view

In addition to pain reduction, for one patient, we noticed an increase in muscle density and size in the residual limb to the point where a new socket had to be fabricated. Due to this increase in muscle density the patient was able to wear the prosthesis for a longer period of time and eliminated the need to add socks to accommodate for volume reductions throughout the day. We believe that this happened due to an increase in muscle mass and better circulation in the limb. We also noticed the patient's skin became more resilient. We also suspect that the NMES treatment may have assisted with healing of reoccurring sores and in resolving his skin infections.

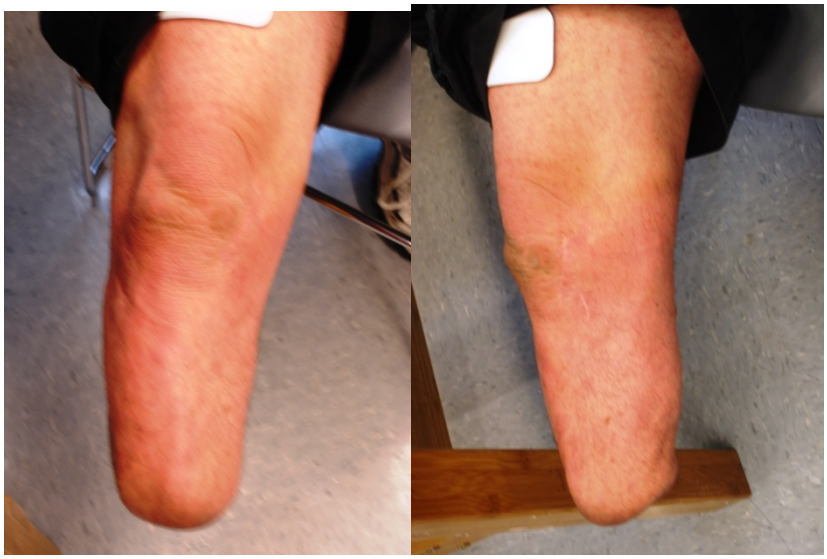


Figure 7 After an eight-week treatment with NMES, the participant reported toned limb, decrease of infection and hair growth.

Patients who used the NMES in general appeared to have a more solid and toned residual limb visually, an increase in a palatable muscle structure, changes in circumference measurements of the limb, increased circulation, a decrease in skin ulcerations and infections, increased wearing time of the prosthesis and experienced a better fitting prosthesis. Without using standardized outcome measures, our initial experiences and observations (anecdotal evidence) lead us to believe that NMES may be a positive treatment protocol for transtibial amputees.

3.0 RESEARCH DESIGN AND METHODS

3.1 PARTICIPANTS

3.1.1 Recruitment

Participants were recruited through our collaborations with local clinicians and with flyers and handouts at three specific sources. The first source was DeLaTorre Prosthetics & Orthotics; the second source was the Health South Harmarville Rehabilitation Hospital in Harmar, PA. The third source was direct referrals from local UPMC Physical Medicine and Rehabilitation Physicians. Posting for recruitment included various websites, newsletters and the CITI registry.

3.1.2 Inclusion/Exclusion Criteria

Participants were adult unilateral transtibial amputees with a minimum of a 4” residual limb length and enough limb length to accommodate two 2”x2” electrode pads. This length of amputation was chosen to provide for adequate surface area for the electrodes. Participants had no prior experience in using TENS or NMES in the past six months. The participants were a minimum of one year postoperative to insure proper healing of the limb has occurred. The

participants must have chronic limb pain (occurs longer than 3 months). We were unable to find any studies that specifically targeted persons with amputations with chronic pain to use as a reference for this study. Looking at other studies, we found that Santos, et al., considered persons with musculoskeletal pain for at least three months to have chronic pain [130].

Further, a study investigated by Dorn et al., included patients with chronic shoulder pain above 3, on a subjective number rating scale (NRS) from 0 – 10 for testing an exhausted non-invasive therapy [131]. Branchini et al., targeted participants who reported chronic low back pain with a mean score above 3 on the visual analog scale (VAS) rating scale from 0 to 10 [132]. The study defined chronic low back pain as pain and/or discomfort localized below the costal margin and above the gluteal folds with possible posterior thigh irradiations not extending below the knee; symptoms had to be present for over 3 months or longer [132].

In this study we classified participants who report chronic pain lasting for at least three months, rating the pain at least a 3 or above on the NRS.

All participants were given a monofilament test administered to check for loss of protected sensation. To our knowledge, there is no set protocol for testing a person with a transtibial amputation for loss of protected sensation. In a standard monofilament test, the participant must feel a minimum of 70% (7 out of 10) out of the areas where the pressure is applied. In past literature, Kosasih and Silver-Thorn report testing transtibial amputees by testing the following regions of the residual limb: patella tendon, popliteal, medial flare, lateral flare, fibular head, anterior tibial crest, distal end and incision site [133]. We tested these same areas and also added two areas, anterior distal thigh and anterior proximal thigh, to ensure that all areas receiving NMES are tested for sensation. The participant was seated for the testing with the

contralateral foot placed flat on the floor and the residual limb relaxed with 85° of knee flexion. The participant closed their eyes and informed the investigator when they sensed the monofilament pressure in the specific test location with a standard 5.07 / 10g monofilament. The participant must be able to feel 7 out of the 10 of the areas tested to be included in the study.

The exclusion criteria include having a cardiac condition, hypertension, congestive heart failure, etc. The exclusions were participants who have a pacemaker and an implanted cardiac defibrillator. The use of NMES may interfere with a pacemaker and is not suggested for people with a pacemaker. We also excluded persons with a BMI greater than 42 $kg/m^{2.}$ Literature shows that calculating the body mass index (BMI) for persons with amputations is different than calculating an abled bodied BMI. Dee and Lelovics, 2012, estimates amputee BMI with a simple formula with a correction for the amputation. Uncorrected BMI formula underestimates body fat in unilateral amputees [134].Himes, in 1995, reports that a transtibial amputation accounts for 5.9% of the body weight [135]. Therefore, a person with a transtibial amputation weighing 85kg would actually weigh: 85kg/ (1-.059) = 90.3kg if having a non-amputated leg. The person's height would then be calculated into the BMI formula:

Amputee adjusted BMI = weight (kg) / height (m^2) = 90.3 kg/172cm = 30.5 kg /m^2 versus standard BMI calculation for an abled body person of 85kg / 172cm = 28.7 kg/m^2.

Piva, et al., in 2013 reported that subjects with Rheumatoid Arthritis and a lower BMI are more likely to accept higher doses of NMES compared to obese subjects who have difficulty attaining high doses of NMES [136]. Stevens-Lapsley et al., 2010, tested subjects after a total knee arthroplasty, who had a BMI less than 40kg/m^2 and found that the BMI did not affect normalized quadriceps muscle strength or functional performance [137]. Furthermore, Stevens-

Lapsley reported that lower BMI in the NMES group may have expedited positive treatment outcomes because of the decrease amount of adipose tissue in their thighs [137]. Literature shows that if a person's BMI is over 40kg/m^2, more negative effects on muscle strength and functional performance may occur[137, 138].

Recent literature shows that NMES does not work effectively, due to excessive adipose tissue, with a BMI over 40 [138]. We need to take into consideration the amputation and adjust for our population. 40kg / m^2 + (0.059)(40kg/m^2). Therefore, in our study, we excluded participants of a BMI greater than 42 kg/m^2.

3.2 STUDY PROTOCOL

Participants interested in participation in the study were asked to call a phone number to obtain more information. At this time the investigator asked a series of questions from a prepared script to determine participant is eligibility. Through this phone call, the participants were screened on the level of amputation, level of cognition to comprehend and follow directions. The participant was then asked to come in for a face to face visit. A visual examination and a length measurement of the residual limb was performed. After the informed consent was obtained, the NMES electrode pads were placed on the participant in the designated areas to determine if the NMES could visually elicit a muscle contraction. If a muscle contraction occurred, the participant was randomized into one of the two groups after completion of the baseline visit. If the NMES failed to elicit a muscle contraction, the participant was withdrawn from the study.

We also noted if the person could voluntary contract their muscles. This study was implemented as a parallel, evaluator-blind RCT. Participants were randomized into either the intervention group or control group by selecting a sealed envelope with a piece of paper placed inside marked either intervention group or control group. Allocation was concealed until baseline measurements were completed. Only the participant and co-investigator knew which group the participant was randomized into. The student researcher was blinded throughout the study. Throughout the study, four MSPO students were trained to assist with collecting data and performing the procedures involving the Biodex, GAITRite® mat system and Omega scanner and hand measurements.

One group received the NMES intervention with training and the other group continued with their activities of daily living. The study consisted of a baseline visit and four follow up visits at 4 weeks, 8 weeks, 12 weeks and one final visit three months (six months after randomization) from the 12 week visit or from the discontinuation of the NMES. Outcome measures were taken for both groups during these visits. Each visit took no more than 2.5 hours.

The following procedures and outcome measures were obtained from a blinded assessor at the baseline visit.

1. The participant completed a pain questionnaire that addresses chronic residual limb pain, residual limb phantom pain and residual limb phantom sensation. The pain questionnaire was comprised of questions taken from the Graded Chronic Pain Scale and the short form McGill Pain Questionnaire [139-141].

The pain questionnaire was modified to reflect three separate outcomes; residual limb pain, phantom limb pain and phantom sensations based on a study on amputee pain conducted by

Ehde et al., 2000[76]. The first two outcomes were based on the Chronic Pain Grade scale [142]. Participants answered seven questions pertaining to residual limb pain and seven questions pertaining to phantom limb pain. The first three questions (1-3) addressed characteristic pain intensity. This was scored on a scale from 0-10, evaluating mean pain intensity rating at three sub-scales; pain right now, worst pain, average pain. The next three questions (4-6) addressed disability score. This was scored on a scale from 0-10, evaluating mean rating for difficulty of performing daily activities, social activities, work activities and the last question addressed disability days. The last score was the disability point score, which ranged from 0–3, and was derived from a combination of ranked categories of number of disability days and disability score.

From the pain questionnaire, three subscale scores; pain intensity, disability score and disability points, combined together resulted in a Chronic Pain Grade Score classification which was used to classify subjects into 1 of the 5 (grade 0-IV) pain severity grades. The three areas derive from the pain intensity items on the 11-point numerical rating pain scale, disability days; number of days the participant was kept from activities due to pain and inference items. The five categories are: grade 0, no pain problem, grade I, low disability, low pain intensity; grade II, low disability, high pain intensity; grade III, high disability that is moderately limiting; and grade IV, high disability that is severely limiting.

For the primary pain outcome, we based our hypothesis on comparing the responses on question number 2: “Please rate your residual limb pain / or phantom limb pain you experienced in the past month on a scale of 0-10”. This provided for an objective overall rating of the

participant's pain they have experienced over the past four weeks compared to the present time frame. The daily log journal will report any increased intensity of pain on a daily basis.

We scored residual limb pain separately from phantom limb pain. The grade classification was determined by the characteristic pain intensity score plus the disability points. Each participant was classified in a category between grades 0 to grade IV. As a secondary analysis which was exploratory, we analyzed the categories of the Chronic Pain Grade Scale at baseline, after 12 weeks and 3 months from the 12 week visit to see if there is a change in categories between grades 0 to grade IV.

The third area of our pain questionnaire addressed phantom limb sensation. The phantom limb sensation questionnaire consists of 24 descriptors taken from the Short-form McGill pain questionnaire [141] and the study done by Ehde et al., 2000 [76]. We asked the participant to rate the sensory (items 1-20) and affective (items 21-24) qualities of their phantom limb sensation. Each descriptor is rated by the respondent on an intensity scale ranging from none (0) to severe (3). This measure yields three scales: a sensory scale (range 0 to 60), an affective scale (range, 0 to 12), and a total scale (range, 0 to 72).

2. The residual limb volume was measured with the use of a hand held three-dimensional motion-tracking laser scanner system. Reflective markers were placed on a thin sock over the residual limb located at the tibial tubercle, fibular head, and anterior distal tibia. Circumferential measurements of the residual limb were taken with a tape measure (three times to find an average). Longitudinal measurements of the residual limb were taken with a straight ruler (three

times to find an average). The length measurement helped determine if the socket fit has changed. During surgery, the surgeon may suture the gastrocnemius muscle anterior around the distal end of the tibia. If the residual limb lacks distal total contact varicose hyperplasia may occur. If the limb becomes too long, boney contours of the socket and limb may not match causing pressure. These measurements are standard measurements that are used in clinical settings. Using a standard scale, participant's height and weight was recorded. We calculated the participant's body max index (BMI).

We took the volume / size measurements from two landmarks. During the scanner procedure, we started the measurements at the tibial tubercle as instructed on the Willow Wood software. When hand measurements we taken, we started at the medial tibia plateau which is anatomically proximal to the tibial tubercle.

3. Residual knee extension strength was tested using a standardized Biodex protocol and a custom attachment (System 4, Biodex Medical Systems, NY). Knee extension strength was tested with isometric and isokinetic exercises. The participant was first tested with the isometric test. The participant was asked to push as hard as they could for five seconds when prompted. They performed three repetitions of this exercise [9, 10, 143]. The Biodex machine was set up for the isokinetic exercise. The participant practiced first on the Biodex machine with their intact limb with an isokinetic test at velocity of 60° / second. We performed an isokinetic knee extension test on the residual limb at velocity of 60° / second for five repetitions for three sets similar to a study done by Pincivero et al.[144].

The participant performed both isometric and isokinetic tests. For the isometric evaluation, the individuals were positioned in the dynamometer, sitting with the trunk, pelvis and

thigh stabilized by belts. The back of the chair was inclined at an 85-degree angle and the rotational axis of the device was aligned with the rotational axis of the knee joint, at the level of the lateral epicondyle of the femur. The lever arm was positioned at 60 degrees of knee flexion, [13, 15, 137] with the support cushion modified for the amputee and positioned at the mid-calf.

Muscle performance was evaluated during static quadriceps extension contractions. To assure familiarization with the procedures the participant had one practice submaximal trial contraction. The test consisted of 3 contractions for 5 seconds each. There was a 60 second interval between each test. During evaluation, the participants were verbally instructed to move the lever of the dynamometer as powerfully as possible, trying to produce a maximum torque.

For the isokinetic evaluation with the Biodex, the individuals were positioned in the dynamometer, sitting with the trunk, pelvis and thigh stabilized by belts. The back of the chair will be inclined at an 85-degree angle and the rotational axis of the device was aligned with the rotational axis of the knee joint, at the level of the lateral epicondyle of the femur. The lever arm was positioned parallel to the leg, with the support cushion modified for the amputee and positioned at the mid-calf. The test was performed in a total range of motion of 90 degrees. The knee was positioned in 90 degrees of flexion to start and the participant was asked to extend their limb to full extension to 0 degrees [144, 145]. Custom adjustable plates were designed and fabricated by the HERL machine shop to provide for adjustability of the leg extension pieces for the amputee population (Figure 8). The plate connected the main arm of the knee extension piece to the calf pad to allow for length adjustability to achieve proper knee center. The plates, one for each limb, were fabricated by the machine shop team at the Human Engineering Research Lab (HERL) within the University of Pittsburgh. HERL is a department within the School of Health

and Rehabilitation Science whose mission is to continuously improve the mobility and function of people with disabilities through advanced engineering in clinical research and medical rehabilitation. The HERL lab has numerous resources and equipment to fabricate a variety of devices and products.

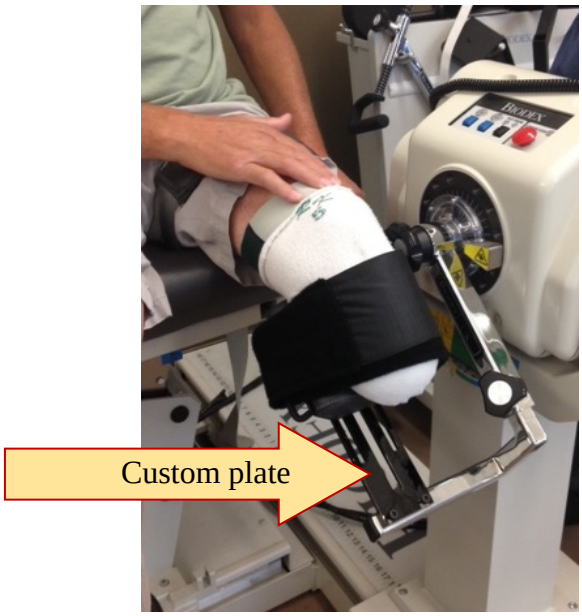


Figure 8 Transtibial participant positioned in Biodex with HERL fabricated adaptable plate.

Muscle performance was evaluated at angular velocity of 60°/second during concentric quadriceps contractions. To assure familiarization with the procedures the participant had two practice submaximal trial repetitions set at the velocity of 60°/second. The test consisted of five repetitions at 60°/ second for three sets. We selected the velocity of 60°/second because slow speeds have been considered the velocity to test strength. Whereas the higher speeds, above 180° / second are used to test endurance [144, 145], the lower velocity speed will produce a higher torque output [146].

Lund et al., referenced articles from Baltzopoulos & Brodie, 1989; Thompson et al., 1989; Gross et al., 1991, reporting that the highest reproducibility for measured isokinetic muscle strength is achieved at velocities below 100° / second. It is, furthermore, possible to extrapolate muscle strength to the strength of a selected velocity for the individual subject from results achieved with different velocities [147]. This implies that measurement at only one angle velocity may be necessary [147].

There was a 60 second interval rest between the sets tested. During evaluation, the participants were verbally instructed to move the lever of the dynamometer as fast and as powerfully as possible, trying to produce a maximum torque.

4. The participant was asked to walk on the GAITRite® mat system (Figure 9). Data was collected on step length on the amputated side, velocity and percentage in stance time on the amputated side. We evaluated the spatial temporal parameters of the transtibial amputee with the use of the GAITRite® Walkway system. We evaluated the difference in step length, percentage in stance time and velocity between baseline and post NMES training. Outcome measures that have been analyzed in previous studies include step length on the amputated side, velocity and percentage in stance time on the amputated side [148-150].

Participants performed a 10-meter walk test. Participants will be asked to walk 5 meters in each direction on the GAITRite® mat at a self-selected walking speed. The participant performed the task three times and a rest was provided as needed. Velocity, step length and percentage of stance time was collected.

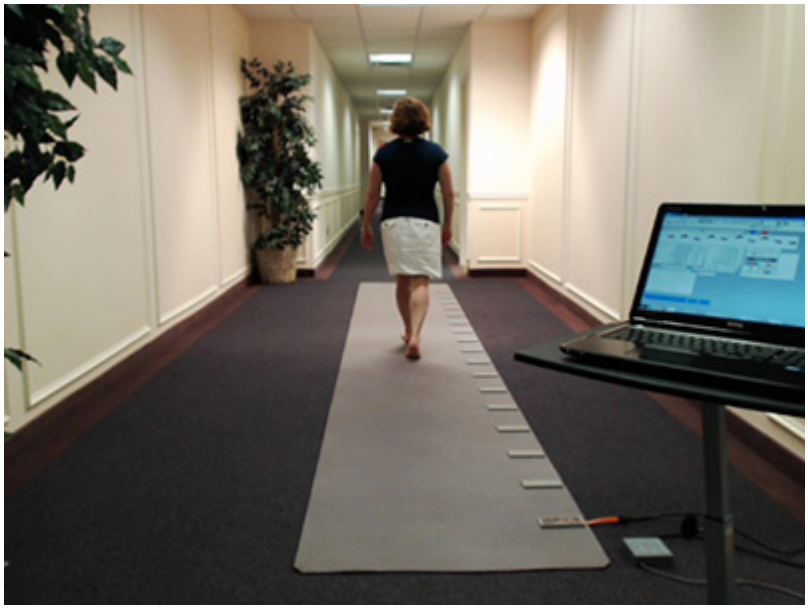


Figure 9 Participant walking on portable GAITRite® Walkway System

Study participants who were randomized into the intervention group were fit with a portable commercially available surface NMES device. A trained investigator, Sara Peterson, CPO, fit the electrode pads and explained the function of the NMES device to each intervention group participant.

The investigator prepared the skin by checking for abrasions or openings. Once deemed intact, the use of an alcohol pad was used. Any excess hair was removed to assure for good electrode contact. The electrode pads were placed with the lead wires away from anatomical knee joint and electrode placement will be smooth and provide total contact. Electrode polarity and configuration will be set according to protocol. We used the recommended standard protocol settings in the NMES product manual to strengthen and re-educate the atrophied muscles. The participant was given one NMES device.

To optimize NMES delivery, the electrode pads were strategically placed over the motor points of the muscles to increase motor branch excitation, therefore maximizing muscle tension and muscle oxygen consumption [151] and minimizing higher than needed current levels to reach the appropriate dosage [152]. Botter et al. recruited 53 healthy subjects both men and women, ages 18-50, to identify motor points in ten muscles of the lower limb of the dominant side. The motor points for the medial and lateral quadriceps are shown in (Figure 10) [153]. The motor points for the anterior tibialis muscle and gastrocnemius muscle are shown in (Figure 11) [153].

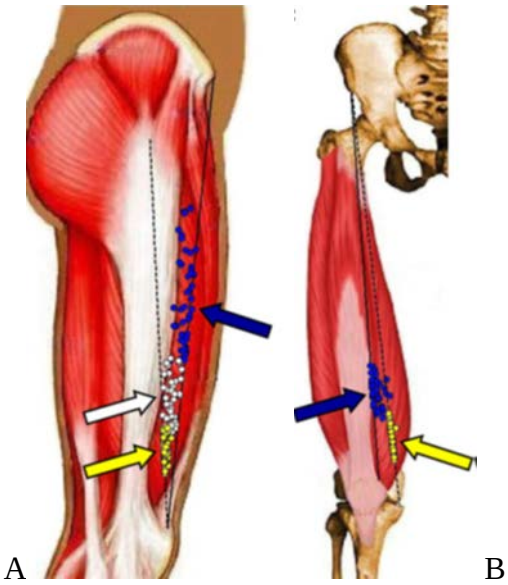


Figure 10 Motor points in upper leg

The arrows indicate the average positions of the motor points along the respective reference lines. A) Motor points identified in the vastus lateralis (blue circles, proximal motor point; white circles, central motor point; yellow circles, distal motor point). Continuous black line is the

reference line for the proximal motor point, while dashed black line is the reference line for the central and distal motor point. B) Motor points identified in the vastus medialis (blue circles, proximal motor point; yellow circles, distal motor point). Continuous black line is the reference line for the proximal motor point, while dashed black line is the reference line for the distal motor point.

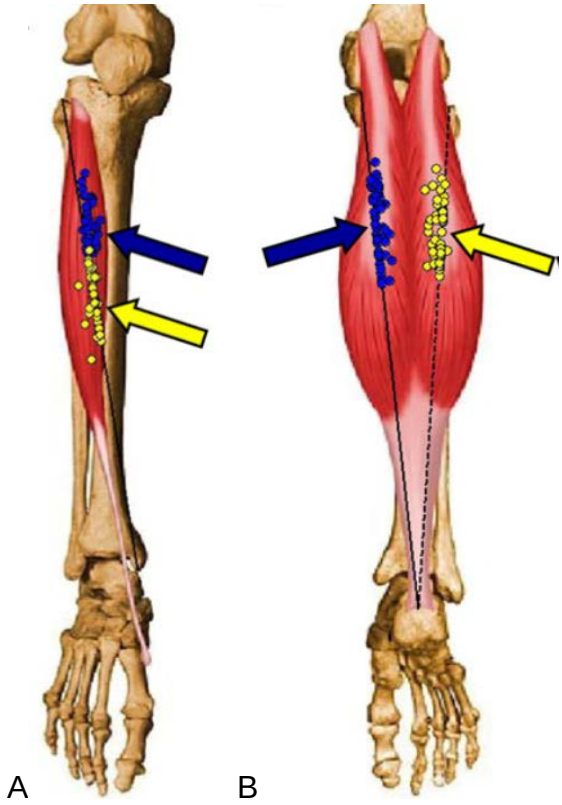


Figure 11 Motor points in lower leg

A) Tibialis anterior (blue circles, proximal motor point; yellow circles, distal motor point) B) medial (blue circles) and lateral (yellow circles) gastrocnemii. The arrows indicate the average positions of the motor points along the respective reference lines.

The participant was trained to use the EMPI Continuum, EMPI, Inc., Clear Lake, SD, device on both the large and small muscle settings. Three muscle groups were trained with NMES per session; one muscle group was trained at a time. The participant first wore one EMPI oval 2.75” x 5” electrode pad on the muscle belly of the vastus medialis muscle and one EMPI oval 2.75”x 5” electrode pad on the muscle belly of the vastus lateralis muscle simultaneously for 15 muscle contractions similar to a study done by Snyder-Mackler et al. while testing strength of the quadriceps after anterior cruciate ligament reconstruction [14] (Figure 12). We used larger electrodes on the quadriceps because they are more likely to stimulate a greater muscle cross-sectional area and produce more force at a given level of comfort [151]. The electrode placement was repositioned and adjusted over motor points at each location per individual based on limb length, limb shape etc. to achieve an optimal strong muscle contraction. Once decided upon at the initial visit, participants were educated precisely at which location they need to position their own pads for each session. We asked the participant to wear the NMES the same time of day each day the NMES device is worn.

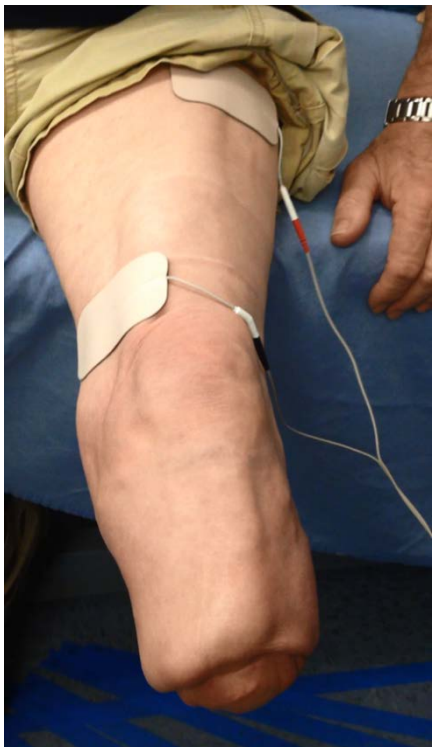


Figure 12 Transtibial participant with NMES on quadriceps muscles

To ensure the proper dose of electrical stimulation was given each time, the assessor looked for a visible upward glide of the patella, a visual muscle contraction of the quadriceps, without excessive muscle spasms, and full knee extension [154]. We increased the stimulation to as much stimulation as tolerated to optimize the dosage. We encouraged the participant to increase the dose intensity throughout each NMES application treatment.

We started with the setting for the quadriceps muscles set at 300μs pulse width and 50Hz for the pulse rate as per guidelines from EMPI Continuum manual. Settings for the large muscle quadriceps was as follows: lag delay of 3 seconds, ramp up time of 3 seconds, on time of 12 seconds and ramp down of 2 seconds. There was a rest time of 42 seconds. This completed a one-minute cycle. The participant was instructed to wear the NMES device for 15 minutes per

day at the quadriceps location [14]. We trained and encouraged participants to increase the amplitude intensity to the highest setting as tolerated during the NMES treatment.

When complete, the participants removed the oval electrode pads and applied two EMPI square 2”x2” electrode pads over the anterior tibialis muscle. We used smaller electrodes to stimulate only specific muscles in isolation. We instructed the patient to place one electrode as proximal as possible on the muscle and the second electrode at least one inch distal to the first electrode on the muscle belly of the anterior tibialis muscle (Figure 13).

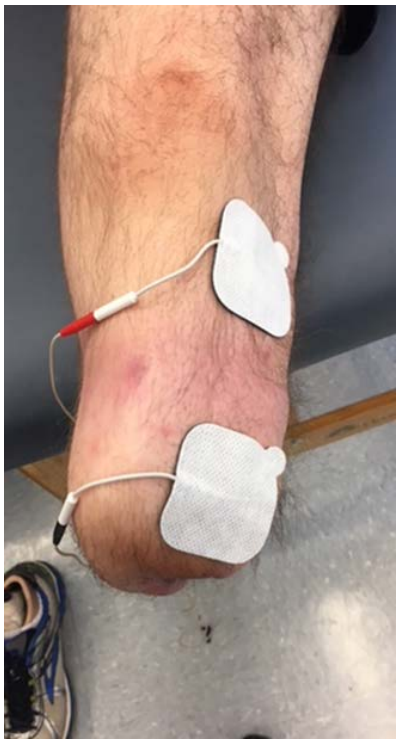


Figure 13 Two 2”x2” electrodes over the anterior tibialis muscle approximately 1”-2” apart.

For the anterior tibialis muscle, we started with the setting of the 300μs pulse width and 35 Hz for the pulse rate. The participant was instructed to wear these electrodes for 15 minutes per day. [14]. The assessor visibly looked for a strong muscle contraction at maximum tolerated dose. Settings for the small muscles, anterior tibialis was as follows: ramp up time of 2 seconds, on time of 10 seconds, ramp down time of 2 seconds with a 46 second rest time.

Upon completion, participants removed the EMPI square 2”x2” electrode pads and placed two EMPI square 2” x2” electrode pads over the medial gastrocnemius muscle (one over the head and one over the belly) and two EMPI square 2”x2” electrode pads over the lateral gastrocnemius muscle (one over the head and one over the belly) (Figure 14). The participant was instructed to wear these electrodes for 15 muscle contractions or 15 minutes [14].

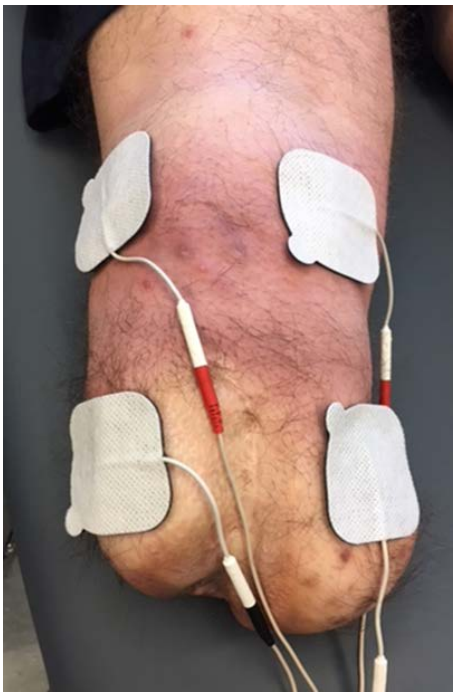


Figure 14 Electrode pads placed on the medial and lateral heads of gastrocnemii and gastrocnemii muscle belly.

For the gastrocnemius muscle we started with the setting of the 300μs pulse width and 35 Hz for the pulse rate. The participant was instructed to wear these electrodes for 15 minutes per day. [14]. The assessor visibly looked for a strong muscle contraction at maximum tolerated dose. Settings for the small muscles, gastrocnemius were as follows: ramp up time of 2 seconds, on time of 10 seconds, ramp down time of 2 seconds with a 46 second rest time. The NMES settings were individualized for eliciting a strong muscle contraction of the residual limb while maintaining a tolerable level of stimulation. Participants were taught how to position the electrodes and how to operate / program the unit for each muscle group. They were instructed to apply and wear the NMES during a time of rest (non-active times of the day) and were given extra sets of electrodes and batteries to use throughout the study. They were provided a photo of their limb with electrode pads in place and a pamphlet with simple instructions including photos on where and how to place, remove and store the electrodes. The instructions included the wearing schedule and who to contact if they have questions.

We recorded the settings at each visit and instructed the patient to not change the pulse width and Hertz settings Both study groups were given daily logs to record daily prosthetic wearing times, changes in health, medications, activity, pain levels and NMES use (the intervention group only).

Participants were instructed to wear the intervention on 3 sets of muscles, the device counted each single wear time as 1 sessions, a complete treatment would be 3 sessions. We asked the participants to wear the intervention a minimum of 5 days out of 7 days per week, totaling 60 sessions per 4-week (1 month) period. With these instructions we were hoping that they would wear the NMES device 2 to 3 times a week. Adherence with the NMES intervention was

monitored with the patient daily log book and an onboard data logger in the NMES device. The participant had to complete the entire 15-minute treatment and wait for the device to complete the full session before being turned off. Adherence was defined as the percentage of prescribed sessions (5 times per week) that were completed according to the daily log book and NMES data logger. The subject was asked to bring the NMES to each visit to check the data logger, batteries and electrode pads. We compared the data from each device to the daily log book and have reported compliance with the overall results.

Intervention group participants were directed to wear the NMES at each three muscle groups separately for 15 strong muscle contractions five times per week similar to a study done by Snyder-Mackler et al. [14]. We set up a face-to-face visit with the NMES intervention participants at the end of the first week and called the NMES participants every two weeks thereafter to check on compliance and address any issues with using the device. The participants were instructed to wear the NMES for 12 weeks. When the participant reported an ill-fitting prosthesis during the study, we referred the participant to their local prosthetist for proper adjustments. If the prosthetic socket caused pain on the participant's residual limb, the participant was withdrawn from the NMES intervention but was still enrolled in the study and performed the above mentioned outcome measures at the 3-month final follow up visit.

As a feasibility study, emphasis was on feasibility and not on statistical significance of results. Compliance with the protocol was examined through number counts on drop outs or numbers of missed visits, completion rates of outcome measures, and deviation from the protocol. Data were collected on adverse events and participant's positive and negative feedback.

Details of participants who were excluded from the study was recorded and exclusion was distinguished from attrition.

We aimed to test each participant at the same time of day to ensure consistency with limb volume. We offered the NMES to the control group after completion of the study per participant request.

Feasibility indicators

Feasibility indictors included *process, resource, management, and treatment parameters* collected throughout and at the end of the study.

The *process parameters* were recruitment, consent, retention and intervention group participants' perceived benefit from the NMES. Perceived benefit was explored using an exit questionnaire rated on a 0-5 scale with the following questions about whether: 1) they found the NMES intervention useful in overall improvement of the involved limb, in regard to strength, 2) pain, 3) limb size and 4) walking ability, 5) if they felt that the health of the residual limb changed in a positive way, 6) the device was easy to use, 7) if they wanted to continue using the NMES device at home on a continuous basis and 8) if they would recommend the NMES intervention to other amputees. The response options were from 0 (strongly disagree) to 5 (strongly agree). Participant's written and verbal comments were also collected.

The *resource parameters* were compliance rates measured by the number of sessions completed over the total number of sessions times 100.

The *management parameters* were participant processing, informed consent was signed the same day as the baseline visit, the number of sessions to which the evaluators remained

blinded, the time of day the participant wore the intervention and the time of day the participant was tested.

The *treatment parameters* were adverse events, mean (SD) intervention self-reported pain at all time points, and estimates of the mean change treatment parameters: effect size and variance.

Follow up

All participants returned for follow-up visits to assess residual limb strength, residual limb volume, pain and gait. The participants in the control group had four (4) follow up visits. The participants in the intervention group had five (5) follow up visits. The extra visit occurred one week after the intervention group participants received the NMES device and used it. This visit was to check the NMES device settings and to make sure that the participant understood where to place the electrodes.

At each follow up visit the log book entries was collected and reviewed. The participant completed the pain and questionnaire at all four follow-up visits. A second investigator inspected the residual limb and check the fit of the prosthetic socket, record residual limb knee extension strength on the Biodex machine, took anthropometric measurements (circumferences and lengths) of the residual limb with a tape measure and ruler, and scanned the limb with the hand held three-dimensional motion-tracking laser scanner system. The participant walked on the GAITRite® mat system to record gait parameters.

Each visit included:

- Checked documentation in daily log and collect the log book entries from the previous visit
- Completed a pain questionnaire

- (IG only) Checked proper use of NMES device by patient and checked compliance through device data logger
- Inspected the residual limb and check the fit of the prosthesis[155]
- Measured circumference and length of limb
- Scanned residual limb with hand held three-dimensional motion tacking laser scanner system
- Biodex machine exercises, isometric and isokinetic
- Gait analysis with GAITRite® System [148, 156]

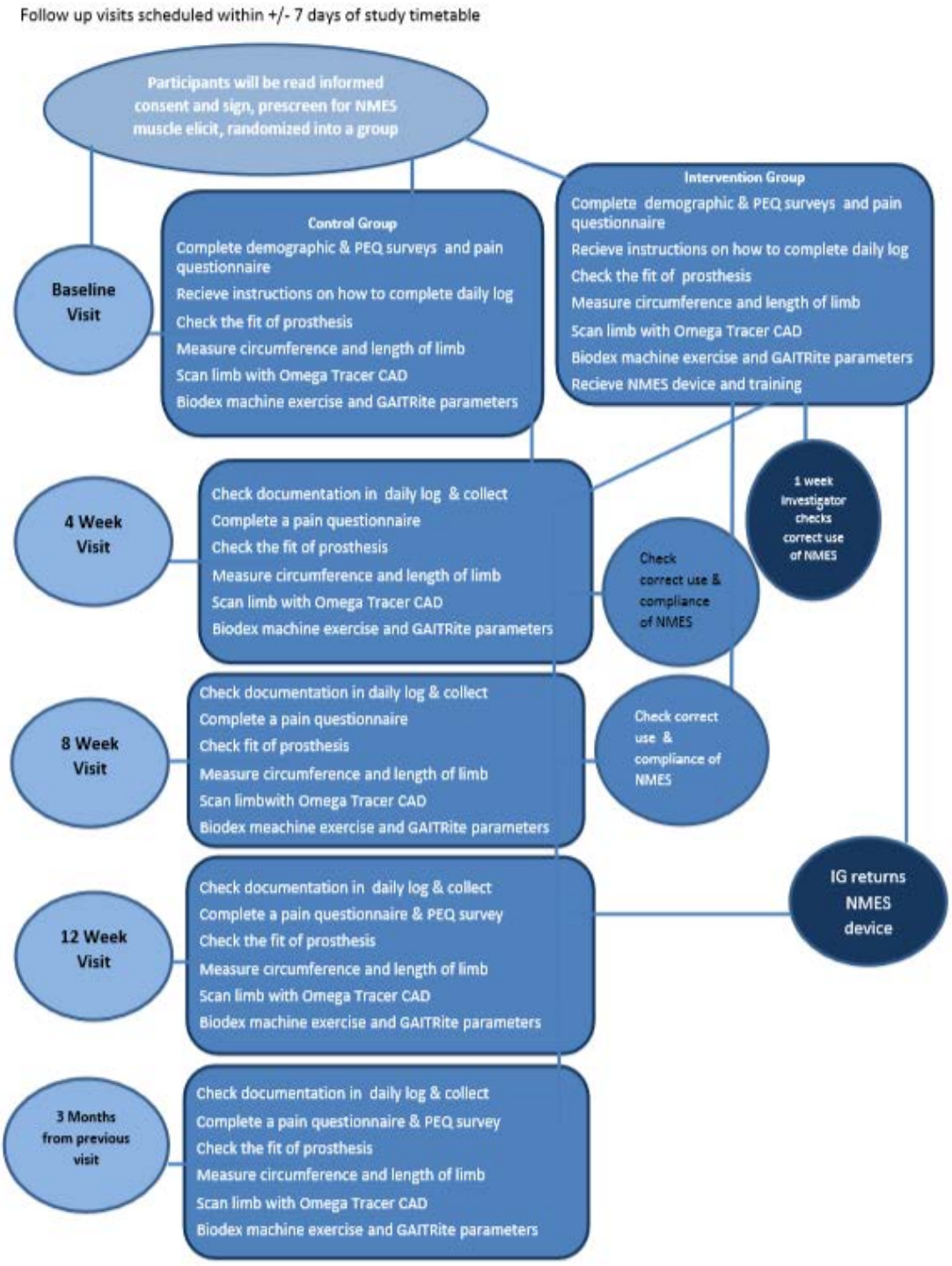


Figure 15 Flow Chart

3.3 EQUIPMENT

3.3.1 EMPI Continuum™

Continuum is a multi-functional dual channel, electrotherapy device offering proven adjunctive rehabilitation therapies, including muscle re-education (NMES), pain control, and stimulation of local blood circulation. The device produces a mild electrical current transmitted via lead wires to electrodes placed on the skin over the motor point of the targeted muscle. Stimulation of motor end plates causes nerve depolarization and activation of muscle fibers, resulting in a muscle contraction. The Continuum has two independent digital intensity controls. The device has thirteen pre-programmed and three custom regimens for NMES, TENS and Pulsed DC current therapies. The Continuum offers treatment duration time up to 60 minutes cycled or continuous, offers symmetrical and asymmetrical waveform types, adjustable pulse rates up to 150 Hz and pulse width durations up to 400 μs, off times, channel ramp times and on time. Device has a data and parameter logger. This allows monitoring of patient usage of the device. It also has an automatic lock so the control buttons do not get accidently pushed changing the

intensity. The device is battery operated with two AA rechargeable NiMH batteries. A battery charger and extra batteries are included with the device.

3.3.2 Biodex System 4 Pro™

Biodex System was used to measure isometric and isokinetic peak torque of knee extension. The Biodex provides objective data in graph format for easy interpretation. Biodex equipment marks the highest correlation coefficients for reliability, accuracy, validity and repeatability. McCleary & Andersen 1992, performed Biodex test-retest reliability on uninjured male athletes, [157] to our knowledge, no reliability test-retest study has been performed on amputees. Therefore, we added the isometric testing to our protocol to eliminate variable effects of limb motion and motor control on the reliability of the strength measures.

3.3.3 Omega Tracer Cad/Cam from WillowWood

Residual limb volume was measured with the use of a hand held three-dimensional motion-tracking laser scanner system. Measurements can be read from the 3D image at designated anatomical landmarks on the residual limb. The scanner has been tested for reliability with a repeated measures test with amputees by Dr.de Boer-Wilzing VG et al., 2011 [158].

3.3.4 Standard Tape Measure and Straight Ruler

Circumference and length measurements were taken in three times in centimeters, averaged and recorded. These are standardized measurements we take in the classroom and clinical setting.

3.3.5 GAITRite® Walkway System

The GAITRite® is a portable gait analysis tool for automated measurement of temporal (timing) and spatial (two-dimension geometric position) parameters of its pressure activated sensors. Encapsulated within the electronic walkway are sensor pads.

Spatiotemporal parameters of gait were measured using a pressure-sensitive mat (GAITRite®, CIR Systems, Clifton, NJ). The GAITRite® mat is approximately 580 cm (5.8m) in length and 90 cm in width. Each sensor pad has an active area of 24 inches square (61cm square) and contains 2,304 sensors. The system records footfalls by the location of activated sensors and also the time of activation/deactivation. Data was sampled at 30 Hz and stored in a personal computer that will calculate spatial and temporal parameters using SPSS application software.

As the subject ambulates across the walkway, the pressure exerted by the feet onto the walkway activates the sensors. The walkway does not only sense the geometry of the activating objects but also the relative arrangement between them in a two-dimensional space. In addition, the walkway senses the vertical component of the relative pressure exerted by the objects. This walkway for gait analysis uniqueness is the special algorithms built into the system. The algorithms isolate the objects and identify them as footprints.

3.4 PRIMARY ANALYSIS

We planned to do an ANOVA but due to the amount of missing data (see results section) we chose to explore descriptive and non-parametric statistics. Descriptive statistics were computed for the sociodemographic variables which were assessed for significant differences at baseline between groups using chi square and unpaired t-tests. We computed changes in individual mean scores from baseline to 12 weeks, confidence intervals, effect size and power. We also showed comparisons through box plots and scatter plots. We also ran a Mann Whitney U non-parametric test to examine mean differences between groups at 8 and 12 weeks compared to baseline. A Shapiro-Wilk test was used to check the data for normality. We found three variables, with not normally distributed data at baseline and one variable with not normally distributed data at 12 weeks. For the intervention group, the data was not normally distributed for the change score in phantom limb pain (PLP) (at baseline) and the phantom limb chronic pain grade score (at baseline and 12 weeks) and for the control group it was not normally distributed for the change

in total phantom limb sensation score at baseline. Because we used the Mann-Whitney U test to look at differences we did not need the data to be normal. Because this was a proof of concept study, we didn't correct for type I errors to preserve power and our ability to detect statistical differences.

Box plots were created to show group results at baseline, twelve weeks and twenty-four weeks. Scatter plots were created to compare compliance with the main outcome measures at the three timepoints of 4 weeks, 8 weeks and 12 weeks. Scatter plots were also created at week 8 and at 12 weeks to compare intervention group comparisons of change in strength versus change in limb size, change in pain versus change in velocity, change in strength versus change in velocity, change in limb size versus change in velocity, and change in pain versus change in strength. We used SPSS software for all data analysis.

The study goal was to recruit up to twenty-five participants and retain at least eight per group. This study provided insight into the actual attrition rates expected for a larger study. Because testing outcomes were obtained at interim times during the intervention period (at 4 and 8 weeks) we explored how much NMES use was needed to begin to see clinically relevant changes in the measures in less than the 12-week allotted intervention period. We examined and assessed the characteristics of the subjects who complied and stayed in the study to those who dropped out. We also compared characteristics of the subjects in the intervention group who experienced larger positive changes to those who did not to gain insight into best candidates for future studies and for prescription of this type of therapy. Characteristics of the participants under consideration include gender, age, dosage, amount of time NMES was worn, self-reported activity level through the questionnaire, BMI and date of amputation.

3.4.1 Aim 1:

3.4.2 Hypothesis 1

The following outcome measures tested were peak knee isometric torque and isokinetic extension torque at 60°/ second averaged over the three trials and normalized by body weight in kilograms. Literature shows that knee extension strength can be increased through NMES intervention. Studies have shown that unilateral transtibial residual limb extension strength is less than the sound side extension strength.

Isakov et al., 1996, tested both male and female unilateral transtibial amputees' isometric quadriceps strength and reported a mean and standard deviation value of 46.0+26.4Nm [7]. The participant's sound side was also tested and the results were comparable to a healthy person, 93.0+34.0Nm [7]. Harbo et al., 2012, performed isometric testing on 178 participants on the Biodex 3 and found mean and standard deviation scores for both males and females respectively, 246.6+56.3Nm, 166.6+38.2Nm. The torque values from these studies reveal that amputees have strength deficits of the quadriceps muscles compared to healthy people [143].

Isakov et al., 1996, tested both male and female unilateral transtibial amputees' isometric quadriceps strength at 60°/ second and reported a mean and standard deviation value of 40.4+20.5Nm [7]. Lund, et al., 2005, performed a study comparing dynameters and tested healthy people on the Biodex 3 and reported mean and standard deviation values during quadriceps isokinetic strength test at 60°/ second as 154+36.34Nm [147]. In a similar study, Gross et al., 1991, tested for reliability on a lightly younger group on the Biodex and reported values of 182+60.34Nm [147]. Neder et al., performed quadriceps isokinetic strength test at 60°/

second on both female and male participants ages 20 to 80 years old on the Biodex 3 system and found mean and standard deviation values to be 109.5+32.4Nm (females) and 172.05+41.35Nm (males) with their right leg tested as slightly stronger [24].

Talbot, et al., in 2003, found a 9% statistically significance increase in knee extension isometric strength torque in post-test NMES intervention group in older adults with osteoarthritis of the knee [159]. A similar strength result was found by Caggiano et al., in a study among older healthy men [160].Fitzgerald et al., found a 9% statistically significant increase in people who used NMES for 12 weeks as part of their rehabilitation following an anterior cruciate ligament reconstruction compared to those who did not receive NMES [15]. Vaz et al., in 2012, found an 8% statistically significant increase in isometric strength in patients with OA, furthermore, researchers found that post-NMES training strength values of the intervention group were close to the values of the heathy group [9]. Therefore, NMES had a statistical and clinical significance.

For our study, we accepted that an 8% increase clinically in both isometric and isokinetic strength torque will be significant.

3.4.3 Hypothesis 2

The following outcome measures tested were circumference measurements at five levels of the residual limb, anterior-posterior / medial-lateral width measurements, a length measurement of the tibia, an overall residual limb length, and total volume and mass of the residual limb.

Prosthetists often need to fabricate a new socket for an amputee due to volume change. The main changes come from either decreased/ increased edema or muscle atrophy. Lilga and

Oberg found that significant volume reductions warranted a new socket when needing to add a one or two 5 ply sock. The researchers calculated this to be 5 to 10 percent change respectively [161]. This correlates with a study by Fernie and Holiday who found that two 5 ply socks was the maximum acceptable volume change before a new socket was warranted [162]. The same study suggests that difficulty donning a prosthetic socket occurs when the residual limb volume increases by 3 to 5 percent [162]. We aimed to find that the intervention of NMES would increase hypertrophy. For this study we accepted that a 3 to 5 percent increase in volume measurements will be clinically significant and may warrant socket adjustments or a new socket for the intervention group. A reduction of 5 percent decrease in volume, or a 5-ply sock fit, will also warrant socket adjustments or a new socket. We were looking for a greater than 5% increase or decreases in size in more than one area on the residual limb. Once we saw this, we discontinued the NMES intervention.

3.4.4 Hypothesis 3

The following outcome measures tested included chronic residual limb pain, phantom limb pain and phantom sensation over the past month.

To test our hypothesis 3, we compared the responses on question number 2: “Please rate your residual limb pain / or phantom limb pain you experienced in the past month on a scale of 0-10 (0 being no pain and 10 being the worst pain possible)” at baseline, after 4 weeks, 8 weeks, 12 weeks and 3 months from the 12 week visit within and between the groups.

We also analyzed the total score (0-72) for phantom sensation comparing the baseline score and the score post intervention at 12 weeks and 3 months.

To my knowledge, there are no set standards for rating amputee phantom pain. There are set standards for rating chronic pain. Therefore, we will treat all data the same. Stratford and Spadoni, 2001, investigated the reliability, consistency, and clinical application of a numeric pain rating scale and reported clinically important differences. Researchers assessed pain on two occasions within 7 days and reported a raw change of 3 points or 27% (percent of raw in total = 3 points/11points) was required for a minimal detectable change [163]. Another study by Childs, J. D., Piva, S. R., et al., (2005) used a 15-point Global Rating of Change scale and reported a minimal detectable change of 2 points based on a 95% confidence interval in persons with low back pain [164]. Furthermore, Childs, J. D., Piva, S. R., et al., (2005) reported a minimally clinically importance difference from 1 week of physical therapy treatment (2.2 points) to 4 weeks of physical therapy treatment (1.5 points)=0.7 points [164].

Farrar et al., (2001); examined data for 2724 patients enrolled in 10 double-blind, placebo-controlled, parallel, multi-center chronic pain studies that utilized the same study design and procedures. The studies included subjects with varying diagnoses including fibromyalgia, diabetic neuropathy, post-herpetic neuralgia, chronic low back pain and osteoarthritis and found 1.7 points or a reduction of 27.9% (raw change/baseline x 100) resulting in minimally clinically importance [165].

A systematic review by Cherian, et al., 2016 looked at the effects of various physical non-operative modalities on the pain in osteoarthritis of the knee. The review found that of the

seven NMES studies, the standardized mean difference (SMD) in pain after treatment with NMES was 1.924, which represented a significant reduction in pain [166].

The numeric pain rating scale that was used is an 11-point scale from 0-10, “0” = no pain to “10” = the worst pain possible. Patients will select a value that is most in line with the intensity of pain that they are presently experiencing or experienced in the past month. None= 0, mild = 1-3, moderate = 4-7, severe = 8-10 for rating worst pain possible. This scale has a good sensitivity while producing data that can be statistically analyzed [167].

For this study, we accepted a raw change of 3 points (27%) as a relevant clinical change.

3.4.5 Hypothesis 4

The following outcome measures tested were step length of the amputated side, velocity and percentage in stance time on the amputated side. All spatiotemporal values were averaged over the 3 walking trials; gait symmetry measures (step length, stance time) were calculated as ratios of the spatiotemporal values from the left and right limbs.

Velocity: Velocity was obtained after dividing the distance traveled by the ambulation time. It was converted to meters per second (m/s).

We investigated gait parameters with a 10-meter walk test (10MWT) while using the GAITRite® system. The 10MWT is selected due to its good intra-rater and interrater reliability [168]. A study by Bohannon in 1997 reported comfortable walking rate using the 10 meter walk test in 230 healthy persons, mean velocity for participants ages 20-70 was 1.37 m/s [169]. Self-selected walking velocity of amputees is lower than mean normal values. [170-172] Bateni

reports confirm that self-selected speed of walking among amputees is lower than mean normal, at 1.11 m/s. Kegel et al., indicated that the self-selected walking velocity of amputees improve (13%) by muscle strengthening through isometric exercises [5]. Kegel et al., also concluded that amputees have lower than normal walking velocity even after an isometric exercise program [5].

Minimally clinically important differences (MCID) were found in literature ranging from 0.05 m/s – 0.16 m/s. Musselman, et al., 2009, found a MCID > or = 0.05 m/s in persons with incomplete SCI [173]. Tilson, et al., 2010, found a MCID = 0.16 m/s improvement in post-stroke participants [174]. Lam et al., 2008, tests SCI participants and found a change of 0.13 m/s to detect significant clinical change for the 10MWT [175]. For this study we hoped to find an increase in velocity of 0.15 m/s or approximately 13%.

Step Length: Step length was measured along the length of the walkway, from the heel center of the current footprint to the heel center of the previous footprint on the opposite foot. The step length can be a negative value if the subject fails to bring the landing foot heel point forward of the stationary foot heel point. The unit of measure is centimeters.

Prosthetic step length and stride length of transtibial amputees has been frequently found to be slightly shorter than the sound limb [149, 176]. Step length from heel strike of the uninvolved lower extremity to heel strike of the involved (prosthetic) extremity is typically greater and accomplished in less time than the opposite step. Houdijk, et al, reported prosthetics step length of eleven transtibial amputees during comfortable walking speed as 0.77m $\pm$0.07m [178]. Houdijk et al., compared the transtibial amputee participants to matched able bodied participants by age, height and body mass and reported a step length of 0.83m +0.10m [178]. Hafner et al., found a similar result when testing seven transtibial amputees while using an

energy storing foot with a prosthetic step length of 0.78m ±.085m [179]. We will accept a 10% increase as a clinically significant difference in step length.

Stance phase: The stance phase is the weight bearing portion of each gait cycle. It is initiated by heel contact and ends with toe off of the same foot. It is the time elapsed between the first contact and the last contact of two consecutive footfalls on the same foot.

During gait, normal walking consists of 60% of time spent in single leg stance phase and 40% of time spent in swing phase. Due to an amputation, these percentages can vary. It has been reported that amputees spend more time in stance phase on their uninvolved limb and more time in swing phase on their amputated side. Breakey reported that single limb support time was 37% of the gait cycle for the affected limb and 43% for the unaffected limb [177]. A cause of this may be a lack of trust of the affected side for weight bearing. Amputees often try to transfer weight to the unaffected side, therefore making the stance phase of the affected side shorter. Bateni and Olney compared transtibial unilateral amputees to healthy people and reported asymmetry in stance phase gait, but was not statistically important [150]. We aimed to achieve data close to normal values post-intervention but accepted a 5% increase in stance time on the affected side as a clinically relevant finding.

The below chart summarizes clinically relevant changes we planned to see, non-amputee normal values and amputee values found in literature. Clinical thresholds were used to help identify if we had any clinically relevant changes in outcomes. Clinical thresholds used in the study were taken from previous existing literature from similar outcome measures.

Table 1 Clinically relevant changes per outcome measure, literature and source of amputee values (SD), non – amputee normal values, group baseline (SD) values of our study.

Outcome Measure	*Clinical statistical change*	*Literature review with transtibial amputees*	*Source*	*Non-amputee normal value*	*Baseline Amputee value Mean (SD) n=20*
Isometric knee extension strength (Nm/Kg) 60° / sec	8%	46.0 (±26.4) n=18*	Isokov {7}	2.7-38.4 range** 2.7-31.7 female** 28.7-38.4 male**	12.36 (±5.39) n=20 7.44 (±2.29) female 13.99 (±5.14) male
Isokinetic knee extension strength (Nm/Kg) 60° / sec	8%	40.4(±20.5) n=18*	Isokov {7}	2.7-38.4 range** 2.7-31.7 female** 28.7-38.4 male**	11.73 (±4.62) n=20 7.21 (±2.35) female 13.23 (±4.21) male
Residual Limb Pain (0-10 scale)	27%	5.4 (±2.7) n=188	Ehde {28}	NA	4.80 (±2.69)
Phantom Limb Pain (0-10 scale)	27%	5.1 (±2.6) n=183	Ehde {28}	NA	4.2 (±3.05)
Phantom Sensation (0-72 scale)	27%	14.5 (±8.9) n=183	Ehde {28}	NA	15.20 (±12.96)
Chronic Pain Grade Score (RLP)	16.67% / 1 grade	NA	NA	NA	1.35 (±1.09)
Chronic Pain Grade Score (PLP)	16.67% / 1 grade	1.88 (±1.08) n=183	Ehde {28}	NA	1.30 (±0.98)
Velocity (m/s)	13%	1.11 m/s	Bateni {147}	1.37 m/s	0.86 (±.25) n=18
Stance phase on amputated side (%)	5%	37%	Breaky {174}	60%	62.42 (±6.55)
Step length (m) on amputated side	10%	0.77 (±0.07)	Houdijk {178}	0.83 (±0.10)	0.53 (±.12) n=18
Residual limb mean circumference (cm)	3-5%	NA	NA	NA	33.15 (±4.93)
Volume (cm^3)	3-5%	NA	NA	NA	949.88 (±388.37)

* not normalized by body weight

*** Biodex standards*

3.5 POWER ANALYSIS

With 10 participants in each group and using a mixed model ANOVA, 80% power, alpha less than 0.05 and knee extension strength as the primary outcome we were powered to detect an effect size of .25 which is a medium effect size. Other studies on NMES in other populations have found effect sizes of between 0.32 and 5.5 [29, 159, 178, 179] for knee extension strength. Because of the pilot nature of the study the data were used to calculate effect sizes, mean

differences and confidence intervals to aid in determining sample size needed for a full-scale RCT.

3.6 TIMELINE

The study took two years to complete. The study was funded by the Orthotic & Prosthetic Educational Research Foundation (OPERF) and the SHRS Research Development Fund. The study received approval from the University of Pittsburgh's Internal Review Board's (IRB). Subjects were enrolled from August 2016 through August 2017 (Q1-Q5). Running the study and data collection took place over eighteen months (Q2-Q7). Data analyses and publishing and reporting the results took place in the last three-month quarter (Q8).

Item	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Recruitment	■	■	■	■	■			
Instrumentation	■							
Preparation of documentation	■							
Running the study		■	■	■	■	■	■	
Data collection		■	■	■	■	■	■	
Data Analyses								■
Publishing and reporting results								■

Figure 16 Study Timeline

4.0 RESULTS

Thirty-two subjects were interviewed for the study. Twelve subjects were excluded from the study for various reasons. The participants either did not meet the criteria by having a pace maker, had a bilateral transtibial amputation, were a severe diabetic or did not meet the BMI requirement. One subject declined to participate as he said the study was too time consuming. There were subjects that were interested in the project but did not have reliable transportation to arrive to each visit timely. One subject signed the informed consent but failed to arrive for the baseline visit. The below flow chart depicts enrollment, allocation, follow up and data analysis.

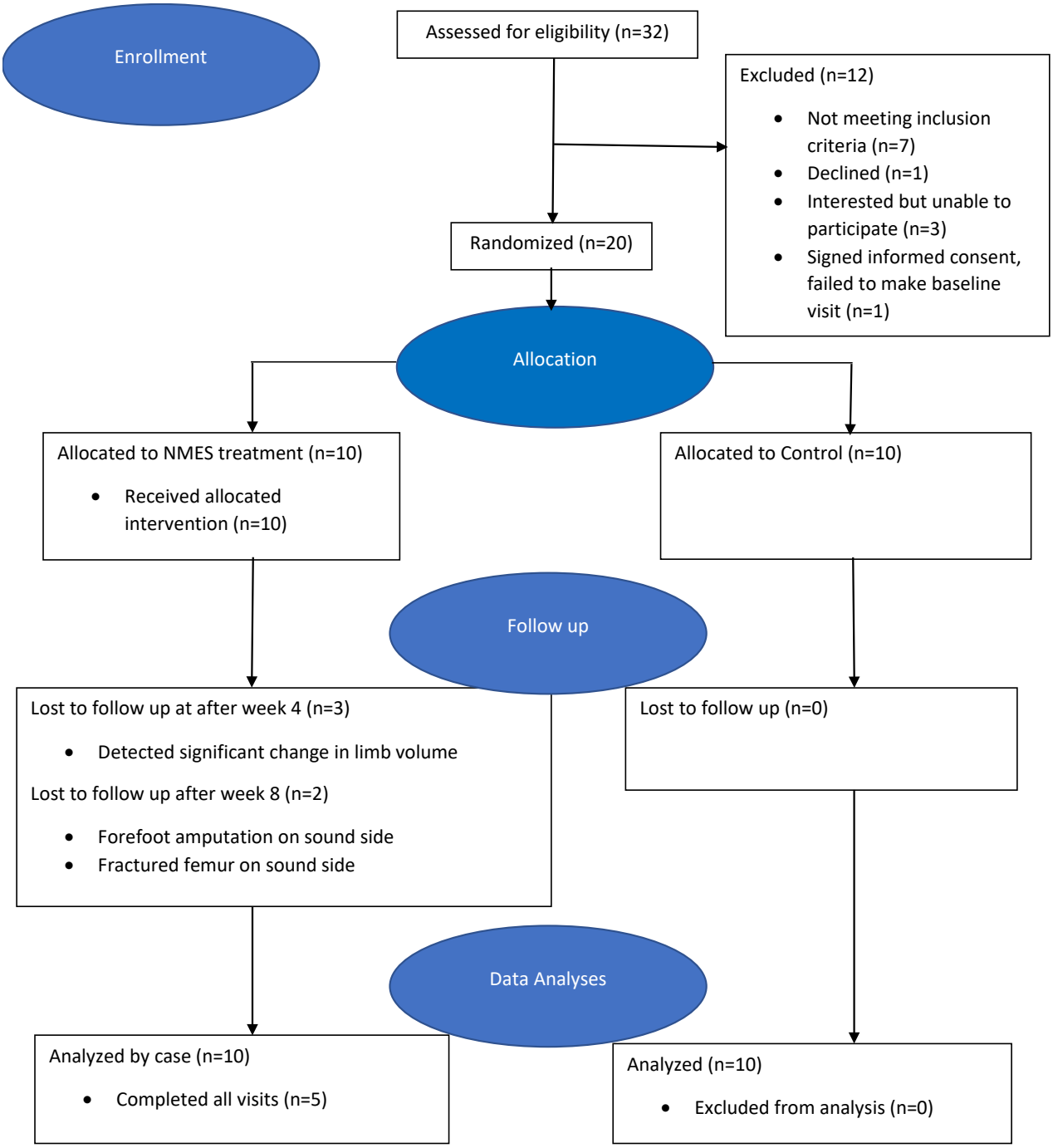


Figure 17 Study Enrollment

We had a 10% overall attrition rate in the study. In the control group we had zero subjects drop out and all subjects completed all visits over all time points. In the intervention group, we had two subjects (20%) drop out after week 8 and three subjects stopped the NMES treatment at the week 4 visit, as the intervention showed positive results, we saw a clinically significant change in residual limb size. These three subjects returned for their final visit 3 months (12 weeks) post intervention. This left five people in the intervention group in which we had a complete set of data. Due to only having five complete data sets, it was too small of a number to do an inferential statistic. We were unable to apply an ANOVA to the treatment group as the results would not be as meaningful or accurate with the small sample size. For these reasons we decided not to run the ANOVA test and to perform descriptive statistics, examine trends in the data graphically and describe the outcomes on a case to case basis.

4.1 MEASUREMENTS:

4.1.1 Demographic and baseline outcome measurement data.

Demographic data were collected at baseline. The demographic data includes gender, ethnicity, age, years since amputation, weight, height, body max index (BMI), residual limb length from

medial tibial plateau to distal end and self-reported cause of amputation. Phantom limb pain and phantom limb sensation outcome measures at baseline showed a statistical significance at baseline between groups.

Table 2 Control group and intervention group means at baseline demographics, outcome measures and NMES intensity level.

Demographic and baseline outcome measurement data				
	Control (n=10)	Std	Intervention (n=10)	Std
Gender:	8 M 2F		7M 3F	
Ethnicity:				
Caucasion	8		9	
African American	2		1	
Age (years):	49.9	16.78	55.7	10.38
Years since amputation:	7.1	6.23	8.66	8.06
Weight (kg):	89.27	22.02	90.57	19.34
Height (cm)	178.18	10	174.1	14
BMI	30.6	6.5	30.7	5.4
Length of amputation at baseline (cm):	14	2.9	14.6	1.4
Cause of amputation:				
Trauma	4		5	
Vascular	2		1	
Diabetes related	1		3	
Malignancy / Cancer	1		0	
Infection	2		1	
Knee Extension Strength (Nm/Kg):				
Isometric Strength	10.77	4.56	13.94	5.9
Isokinetic Strength	9.68	3.37	13.78	4.94
Pain:				
Residial Limb Pain (0-10 scale)	4.4	2.99	5.2	2.44
Phantom Limb Pain (0-10 scale)	2.4	2.63	6*	2.36
Phantom Sensation (0-72 scale)	7	4.62	23.4*	13.55
Chronic Pain Grade Scale (RLP) (0-4)	1.1	0.99	1.6	1.2
Chronic Pain Grade Scale (PLP) (0-4)	0.9	0.74	1.7	1.06
Gait:				
Velocity (m/s)	0.80	0.28	0.91	0.22
Stance phase on amputated side (%)	64.18	5.43	61.02	7.29
Step length (m) on amputated side	0.50	0.11	0.56	0.13
Residual limb size:				
Mean Limb Circumference (cm)	32.62	4.61	33.68	5.42
Volume (cm^3)	900.96	397.98	998.79	393.4
Intensity of NMES (0-100) (Intervention Group):				
Quadriceps Muscle	NA	NA	55.7	4.47
Anterior Tibial Muscle	NA	NA	69.3	4.76
Gastrocnemius Muscle	NA	NA	65.8	24.86

*p<0.05

n=8 control group gait variables

4.2 CHANGES IN OUTCOMES OVER TIME

Outcome measures for each of the four hypotheses are listed below showing group individual baseline mean and standard deviation (pre-intervention) and 12 week mean with standard deviation (post intervention). Also included are the mean changes (difference), standard deviations and confidence intervals. The effect sizes associated with each change score were determined based on an independent t-test. Mann Whitney U test compared week 8 to baseline showed a trend towards significance (p=0.082) in phantom limb pain. At 12 weeks compared to baseline we found a significant decrease in residual limb pain (p=0.03) and a trend towards a significant decrease (p=0.069) in the residual limb pain chronic pain grade scale.

Table 3 Changes in outcome over Time in Control and Intervention Groups with Effect size

	Control				Intervention					
			Change					Change		Effect
	Baseline Mean (SD) n=10	12 Week Mean (SD) n=10	Mean (SD)	(95% CI)	Baseline Mean (SD) n=10	Baseline Mean (SD) n=5	12 Week Mean (SD) n=5	Mean (SD)	(95% CI)	size Power
Knee Extension Strength (Nm/Kg):										
Isometric strengh (Nm/Kg)	10.77(4.56)	12.99(5.17)	2.22 (4.98)	-1.35 to 5.78	13.94 (5.9)	10.23 (4.01)	10.10 (3.22)	-0.13 (4.7)	-5.97 to 5.70	0.49 0.13
Isokinetic strength (Nm/Kg)	9.68 (3.37)	10.76 (4.10)	1.08 (2.61)	-0.78 to 2.96	13.78 (4.94)	11.19 (4.46)	12.06 (2.94)	0.87 (2.98)	-2.84 to 4.57	0.08 0.05
Pain:										
Residial Limb Pain (0-10 scale)	4.4 (2.99)	5.9 (3.18)	1.5 (2.92)	-0.59 to 3.59	5.20 (2.44)	5.40 (3.29)	4.20 (3.35)	-1.20 (1.30)	-2.82 to 0.42	1.09 0.45
Phantom Limb Pain (0-10 scale)	2.4 (2.63)	3.60 (3.47)	1.2 (2.94)	-0.90 to 3.3	6.00 (2.36)	6.00 (2.24)	3.00 (2.35)	-3.0 (4.3)	-8.34 to 2.34	1.14 0.49
Phantom Sensation (0-72 scale)	7.00 (4.62)	9.30 (8.96)	2.30 (6.40)	-2.27 to 6.87	23.40 (13.56)	20.00(4.3)	14.60 (12.88)	-5.40 (12.03)	-20.34 to 9.54	0.8 0.27
Chronic Pain Grade Scale (RLP) (0-4)	1.1 (0.99)	1.3 (0.823)	0.20 (0.79)	-0.36 to 0.76	1.6 (1.2)	1.40 (1.14)	0.60 (0.55)	-0.80 (0.84)	-1.84 to 0.24	1.23 0.55
Chronic Pain Grade Scale (PLP) (0-4)	0.90 (0.74)	0.70 (0.68)	-0.20 (0.92)	-0.86 to 0.46	1.7 (1.06)	1.40 (0.55)	0.60 (0.55)	-0.80 (0.44)	-1.36 to -0.25	0.83 0.29
Gait:										
Velocity (m/s)*	0.80 (0.28)	0.85 (0.22)	0.05 (0.21)	-0.12 to 0.22	0.91 (0.22)	0.86 (0.22)	0.98 (0.14)	0.11 (0.17)	-0.10 to 0.32	0.31 0.08
Stance phase on amputated side*	64.18 (5.43)	59.84 (7.49)	-4.34 (7.22)	-10.38 to 1.70	61.02 (7.29)	62.17 (6.5)	56.26 (5.81)	-5.91 (10.61)	-19.09 to 7.26	0.17 0.06
Step length (m) on amputated side*	0.50 (0.11)	0.51 (0.09)	0.01 (0.06)	-0.04 to 0.06	0.56 (0.13)	0.55 (0.16)	0.61 (0.06)	0.06 (0.11)	-0.08 to 0.20	0.56 0.15
Residial Limb Size:										
Mean Limb Circumference (cm)	32.62 (4.61)	31.99 (4.85)	-0.62 (1.08)	-1.39 to 0.15	33.68 (5.42)	33.40 (7.00)	33.17 (6.67)	-0.47 (0.97)	-1.68 to 0.73	0.15 0.06
Volume (cm^3)	900.96 (397.98)	924.89 (478.66)	23.93(89.03)	-39.76 to 87.61	998.79 (393.4)	957.66 (508.71)	956.02 (515.74)	-1.64 (9.9)	-13.93 to 10.66	0.4 0.1

* n=8 control group, gait variables

4.3 GROUP COMPARISON OF MEDIAN SCORES OVER TIME

Box plots were created to review the main twelve outcome measures score between groups and over time points baseline, 12 weeks and 24 weeks. Box plots show distribution of scores for each outcome. The upper and lower margins of the box indicate the interquartile range (Q3-Q1) which are the 25th and 75th percentiles. The center line sits at the median score (50th percentile). The outer bars (whiskers) indicate the range of scores at each end of the distribution with circles indicating outliers beyond three standard deviations from the mean.

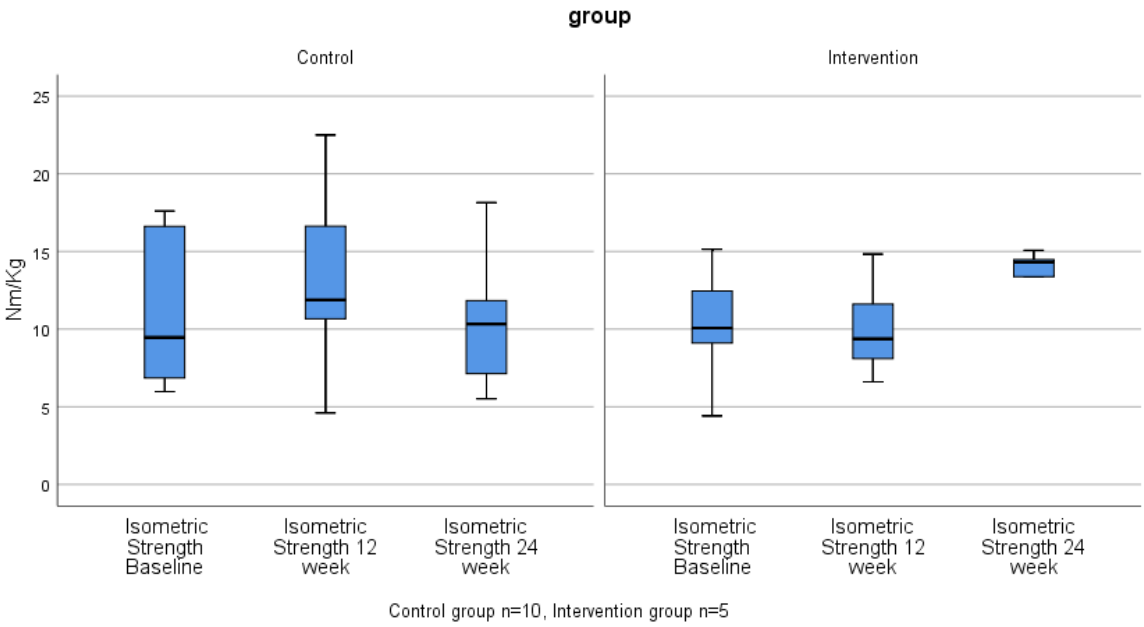


Figure 18 Isometric strength box plot

Isometric strength data showed very little change in the control group. The intervention group had little change between baseline and 12 weeks but at 24 weeks there was notable increase in isometric strength.

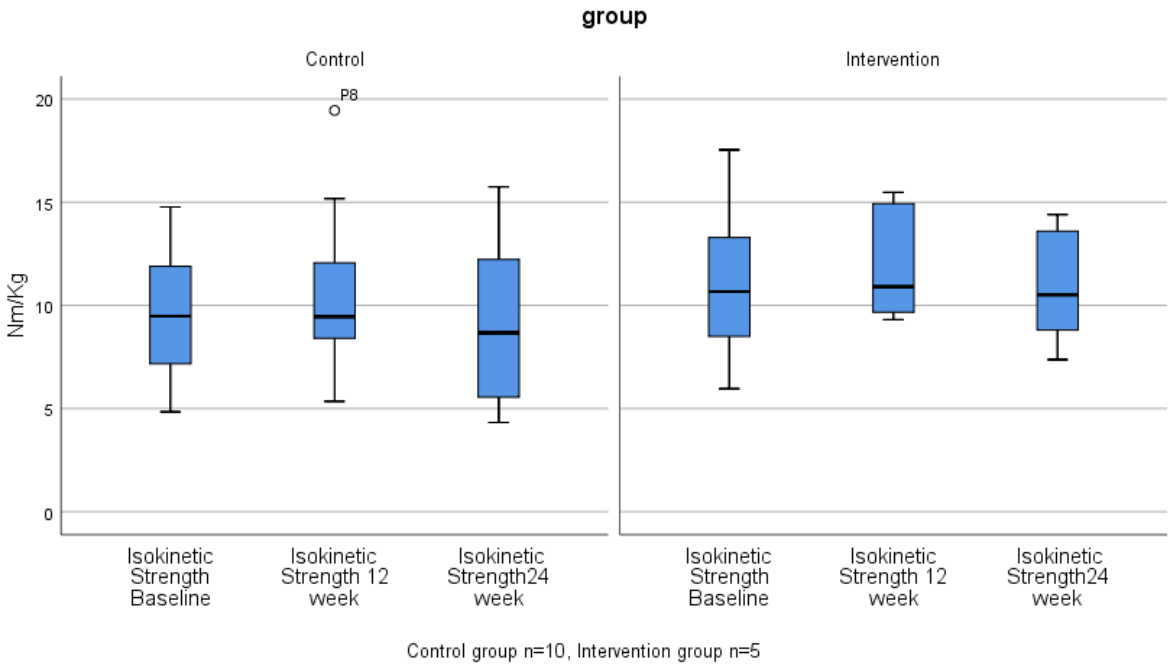


Figure 19 Isokinetic strength box plot

For both groups, the respective median values were stable throughout all time points. Both the minimum and maximum values fluctuated more in the Intervention group than they did in the Control group. In the Intervention group, the maximum value decreased twice, while in the control group it increased twice. Also, the decrease in maximum value in intervention was larger than the increase in the Control group. The respective minimum values did not follow an upward or downward trend but again the change in Intervention group scores was more dynamic. In the

end the box plot for Intervention group at 24 weeks is quite symmetrical, with interquartile ranges that remained fairly stable.

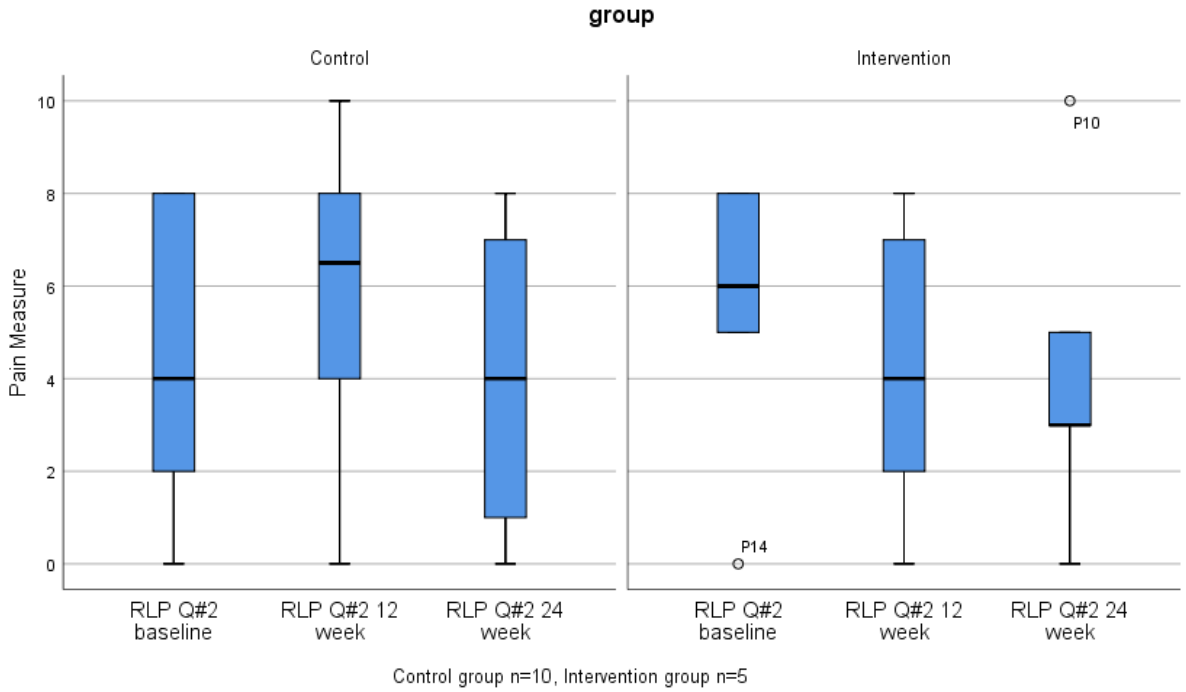


Figure 20 Residual limb pain question 2 from pain questionnaire box plot

Residual limb pain data shows the Intervention group reported a higher level of pain at baseline and reported a decrease in pain through week 24 lower than the control group. Meanwhile, the high scores of the Control group increased (as evidenced by the strong increase in the median) but decreased close to baseline level at 24 weeks. The median for Intervention group had a significant decrease over time. Also, at 12 and 24 weeks more than one participant in Intervention group reported low or no pain, with multiple participants of the Control group reporting low to no pain over all time points. We saw a 3 point score decrease or a 50% decrease

in pain score in the Intervention group which showed a statistical significance with the Mann Whitney Test.

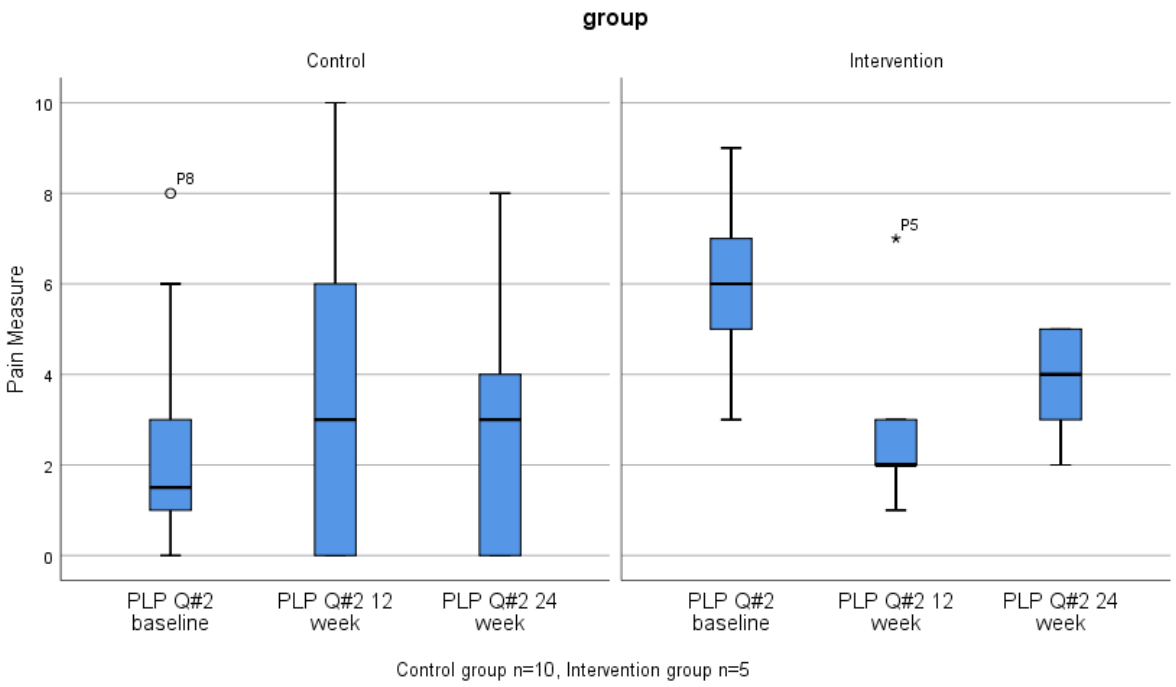


Figure 21 Phantom limb pain question 2 from pain questionnaire box plot

Phantom limb pain data shows the Intervention group reported a significantly higher level of pain at baseline than the Control group. The intervention group did show a decrease in phantom limb pain lower than the control group at week 12. The median PLP score of the Control group increased at 12 weeks then remained stable at 24 weeks. Scores were low to start with (P8 reported strong pain at baseline, and is marked as an outlier), increased, then decreased below the median for a majority of participants. The values for the Intervention group had strong

and different fluctuations. The median score drastically decreased at 12 weeks and increased at 24 weeks. Also, through each change, the variance was lower, with only one outlier (P5) at 12 weeks. Although the phantom limb pain score increased at week 24 for the Intervention group, the score remained clinically significantly lower than the baseline score.

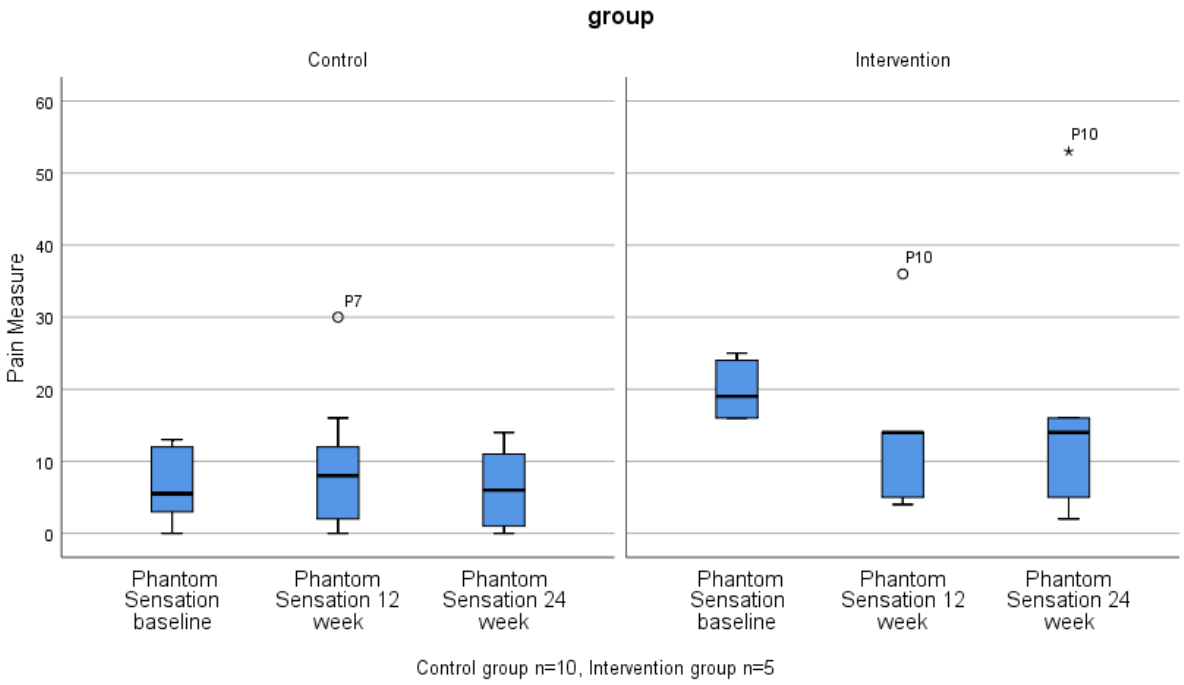


Figure 22 Phantom sensation box plot

The phantom limb sensation scores stayed relatively the same for the Control group at each time point, with a high outlier singled out at 12 weeks. The data set of the Intervention group which started markedly higher than the Control group at baseline, decreased at 12 weeks, and remained similar at 24 weeks. P10 was considered an outlier due to having much higher phantom limb sensation than the others at both the 12-week and 24-week time points. There was not a clinically significant decrease found.

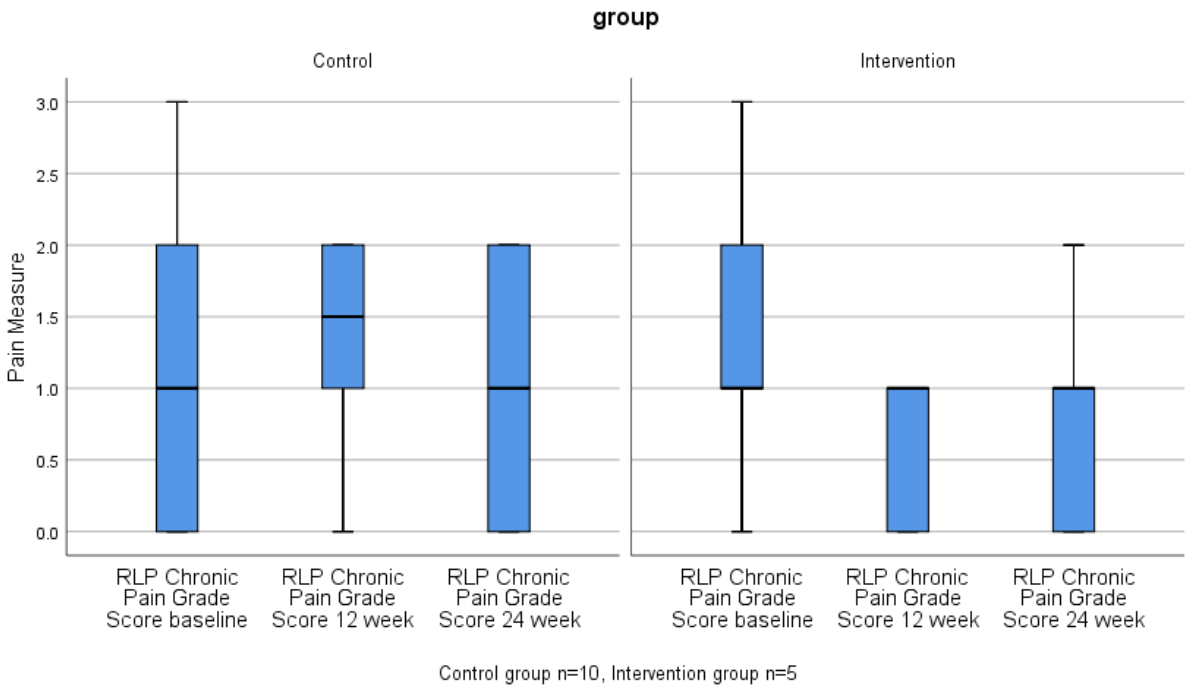


Figure 23 Residual limb pain (RLP), chronic pain grade scale (CPGS) box plot

At baseline, the Intervention group showed slightly higher scores compared to the Control group. The Control group scores at baseline showed higher variance than the baseline Intervention scores. At 12 weeks the Control group scores showed an increased median from baseline whereas the Intervention group showed a similar median however many participant scores fell below the median indicating improvement in this pain measure. At 24 weeks, the Intervention group scores mostly remained in that bottom half quartile with just the maximum number at 2.0 equal to the 75% quartile from baseline. The Intervention group ended with a narrower /lower range of scores than Control group.

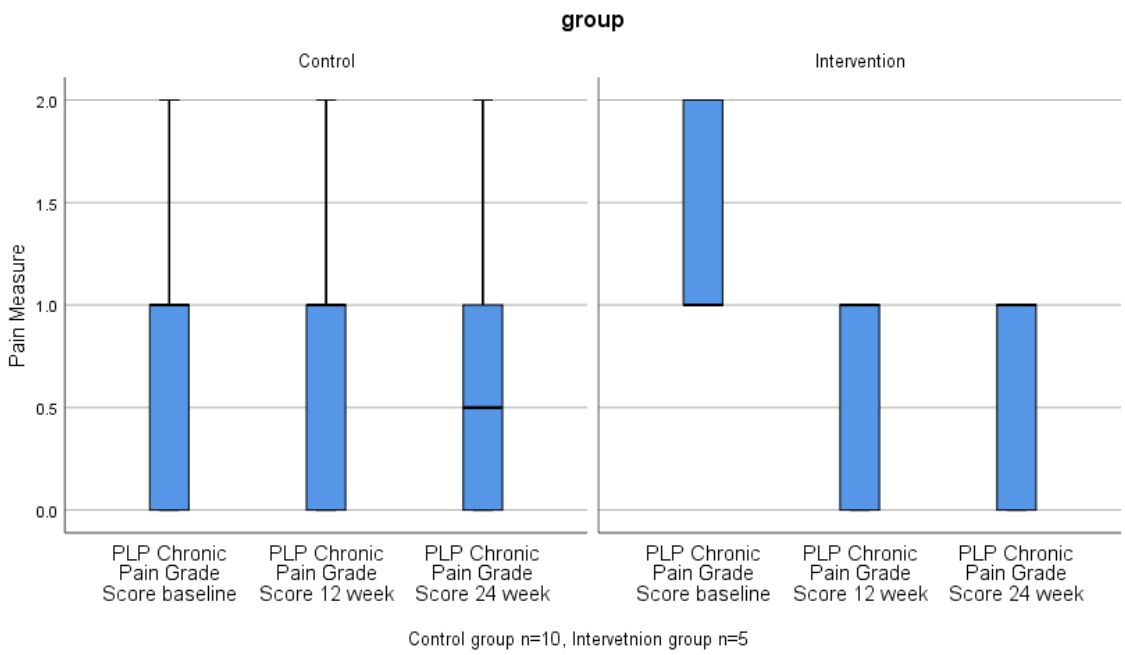


Figure 24 Phantom limb pain (PLP), chronic pain grade scale (CPGS) box plot

Phantom limb pain (PLP) chronic pain grade scale data shows that the intervention group reported higher scores at baseline. The levels of the control group remained stable over time. The Intervention group median score remained at 1.0 across time points but the data shows skewness. At baseline all of the data fell close together in the upper 25% quartile (Q3). At 12 weeks and 24 weeks more than half of the data fell below the median. Data shows that there were no clinically significant changes over time.

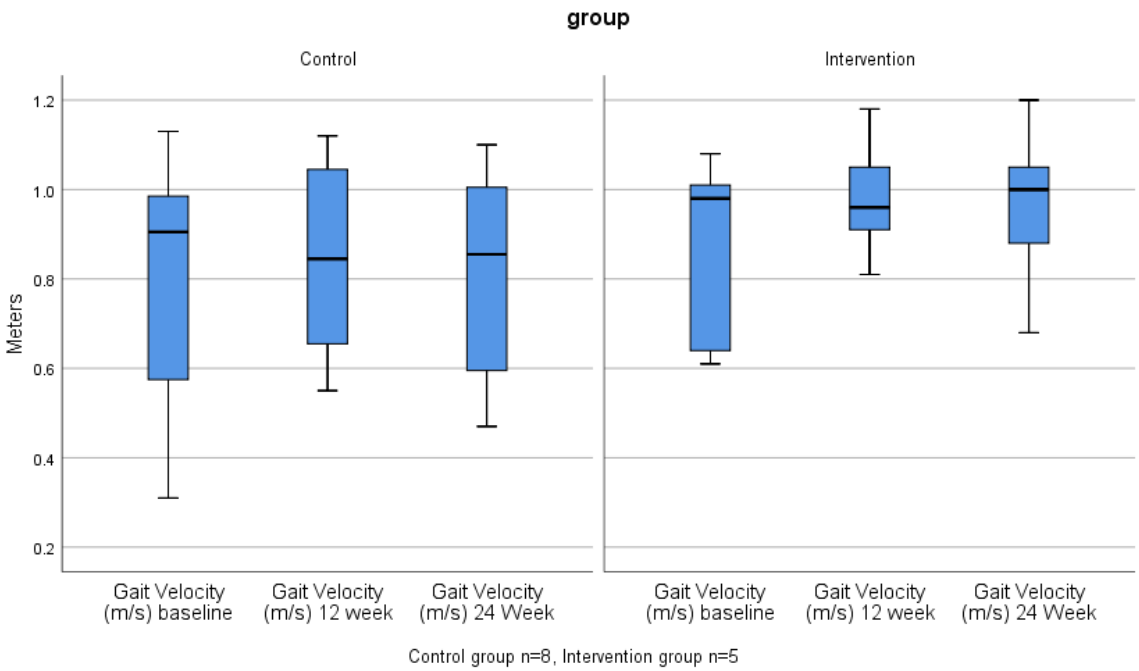


Figure 25 Velocity box plot

The intervention group walked slightly faster than the control group at baseline. For the intervention group, although the maximum and minimum values fluctuated throughout the study, the median remained within the same close range, and at 12 and 24 the variance was reduced relative to baseline. In the end, the values of the intervention group are more closely ranged and slightly higher than those of the control group. No statistical significance or clinical relevance was found.

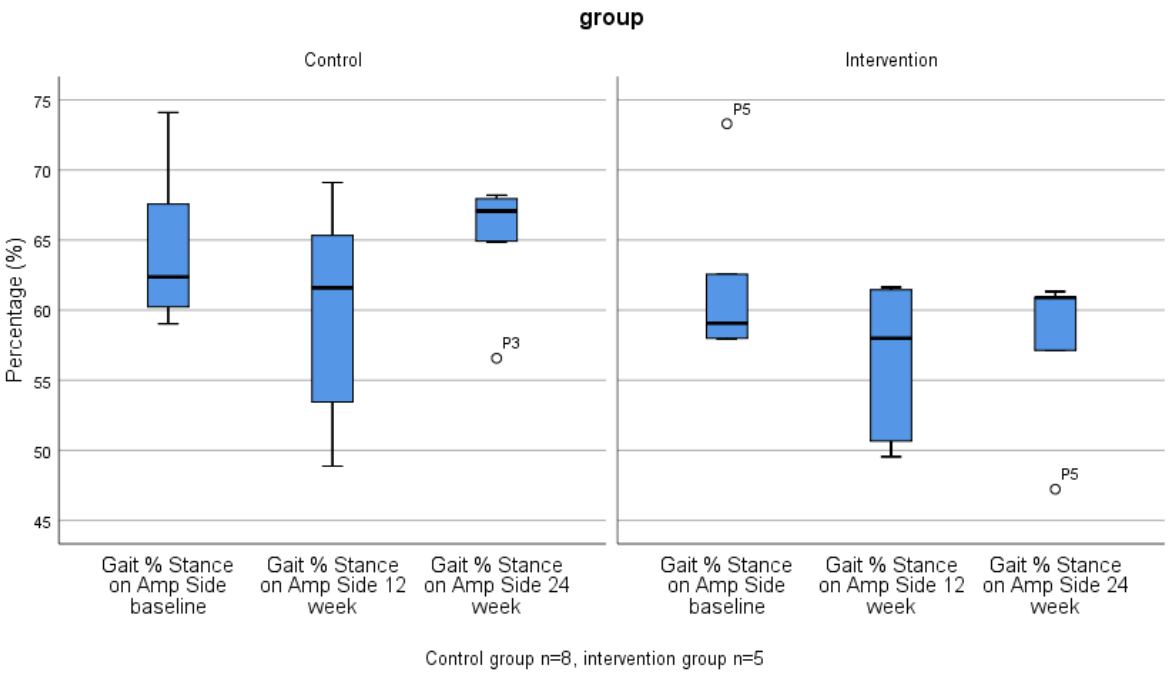


Figure 26 Time in stance box plot

The median of the Intervention group followed a similar pattern as the median of the control group. Each median of the intervention group was lower than that of the control group. The control group showed notable increased percent time in stance at 24 weeks compared to the 12 weeks. P5 from the Intervention group was marked as an outlier twice because P5's gait percentage in stance was much higher than the other participants at baseline yet much lower than the other participants at 24 weeks.

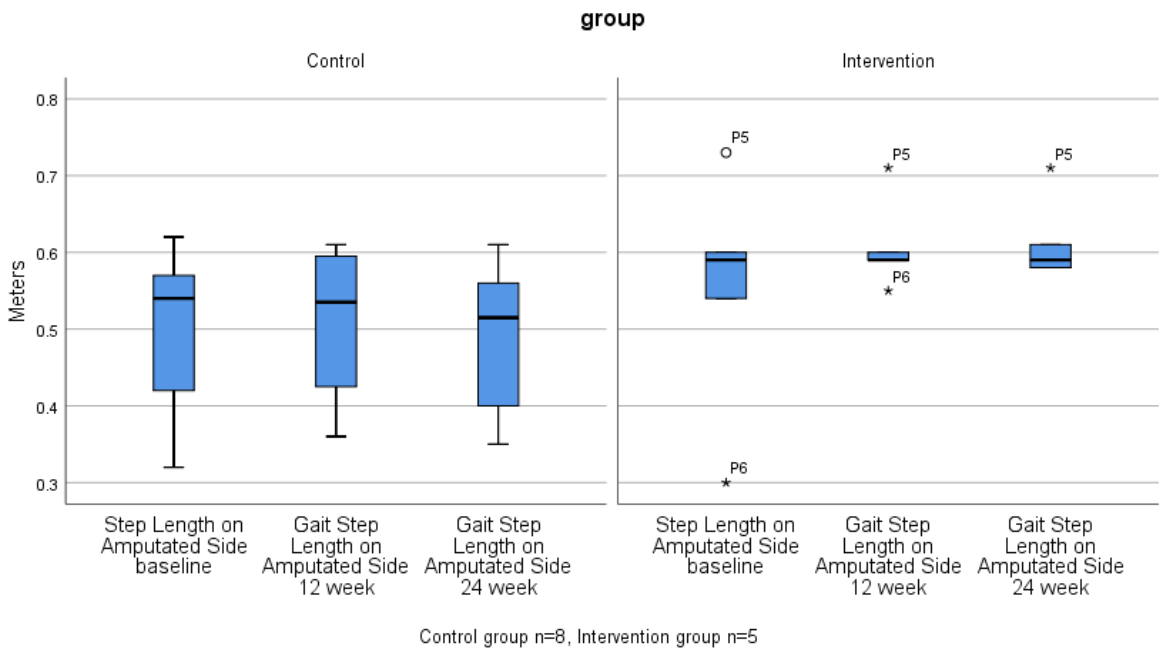


Figure 27 Step length box plot

Step length on the amputated side data showed that participants of the intervention group had longer step lengths at all three time points relative to the control group. But both groups remained stable across all time marks, with the Intervention numbers being much closer in range, with the exception of P5, who was consistently a high outlier. There was no clinical significance found.

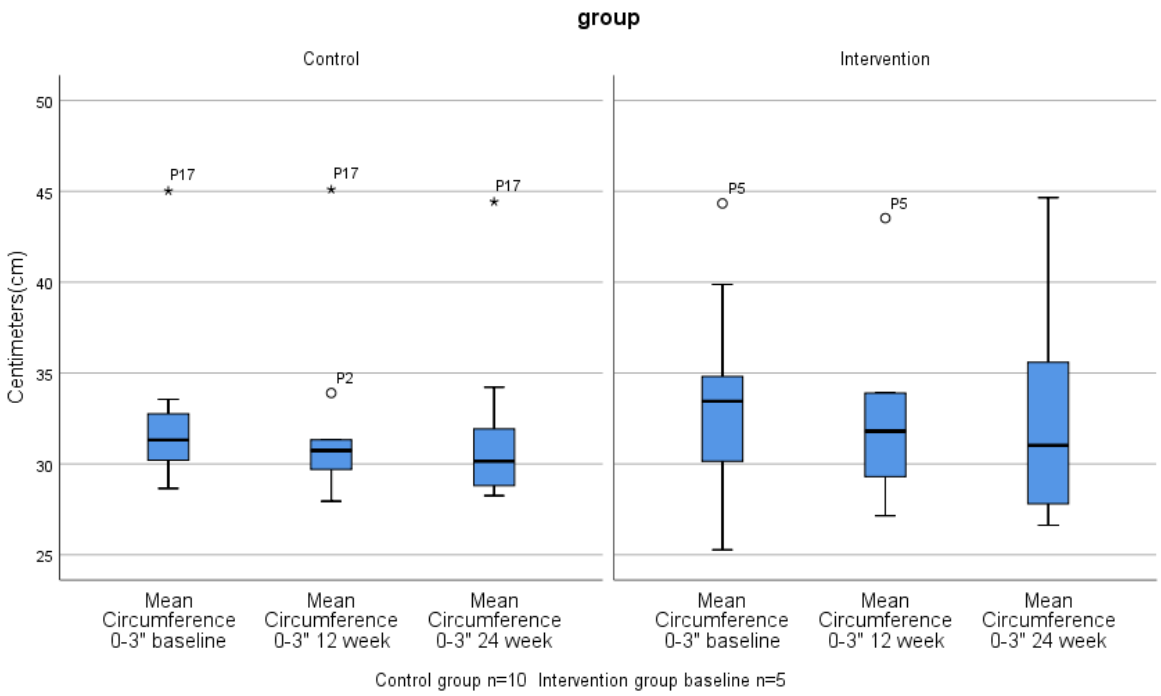


Figure 28 Residual limb mean circumference box plot

Mean circumference measurements were calculated by averaging the residual limb circumferences at tibial tubercle, one, two and three inches distal to tibial tubercle. At baseline, the intervention group showed a slightly larger residual limb size with a wider range mean score compared to the control group. Both group's residual limb size mean score remained fairly stable over time and was not found to be clinically significant. Both groups had one outlier who had large circumferences.

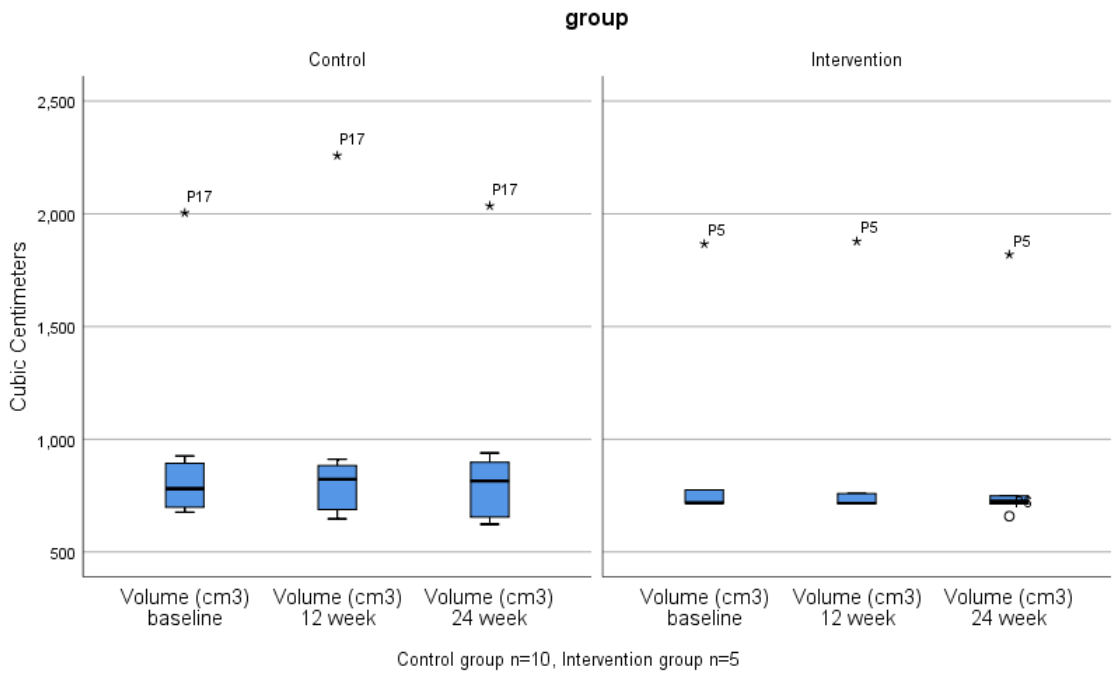


Figure 29 Residual limb volume box plot

Volume in cubic centimeters was taken at the patella tendon bar to the distal end of the residual limb. Both groups remained stable and within the same range, but the interquartile ranges for the Intervention group reduced each time to the point that at 24 weeks the volumes were almost identical for all subjects. Again, P17 from Control group and P5 from Intervention group were consistently high outliers. No clinically significant change was found for either group.

4.4 NMES COMPLIANCE AND INTENSITY

We found that seven of the ten participants were compliant with the set protocol. We asked the participants to wear the NMES for 5 times a week in hopes that they would wear it 2-3 times per week. The group mean days wearing the intervention over the 12-week period was 4.10 to 4.37 times per week.

Table 4 Intervention group compliance number of days worn over each time point

	Number of days per week		
Participant number	0-4 weeks	0-8 weeks	0-12 weeks
4	7	4.5	N/A
5	1.5	0.83	1
6	5.25	6.5	6.25
10	6	6	5.25
12	1.25	2.25	N/A
13	4.83	N/A	N/A
14	7	4.91	7
15	1.91	N/A	N/A
16	5	N/A	N/A
18	4	5.42	1
Mean Average	4.37	4.34	4.1

The intervention group had varied NMES intensity settings at baseline. Setting 100 was the maximum setting the NMES device could be programmed. Figure 30, Figure 31, and Figure 32 shows average NMES intensity level for all participants over each time point. In Figure 30, the five participants who wore the intervention through all time points were able to gradually increase their intensity throughout the study and seemed to tolerate the intervention. Reviewing anterior tibialis intensity levels (Figure 31) the five participants who completed the study had little fluctuation in the NMES intensity. Participant 13 (P13) could not tolerate the dosage

compared to the baseline visit. We saw a change in limb size and P13 complained about socket discomfort, so he was discontinued from the NMES. For the gastrocnemius muscle (Figure 32) there were some small inconsistencies, but the majority of the intensities remained stable across the time points.

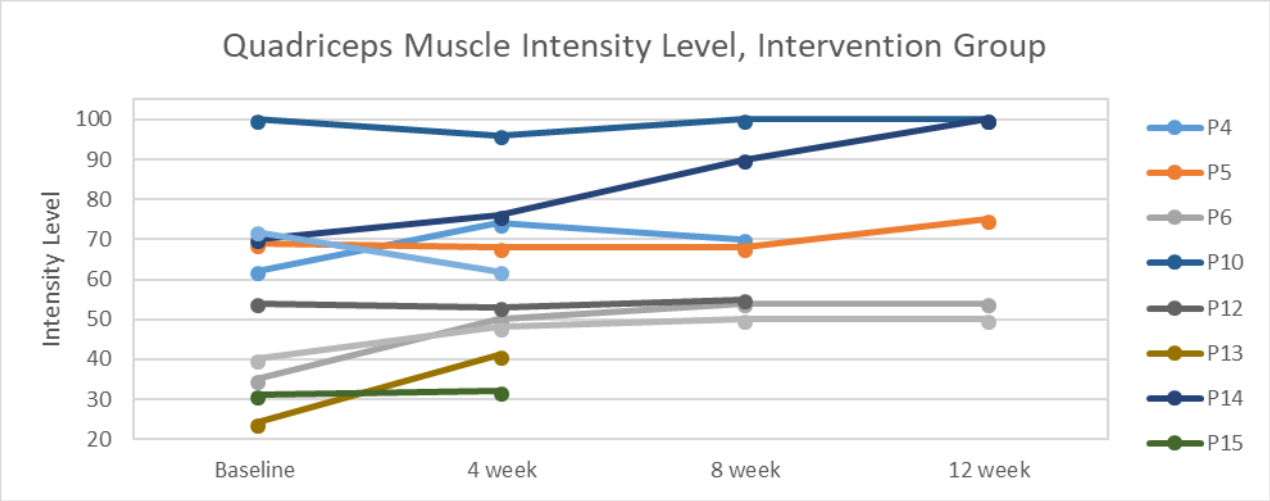


Figure 30 Quadriceps muscle intensity level over each time point, intervention group

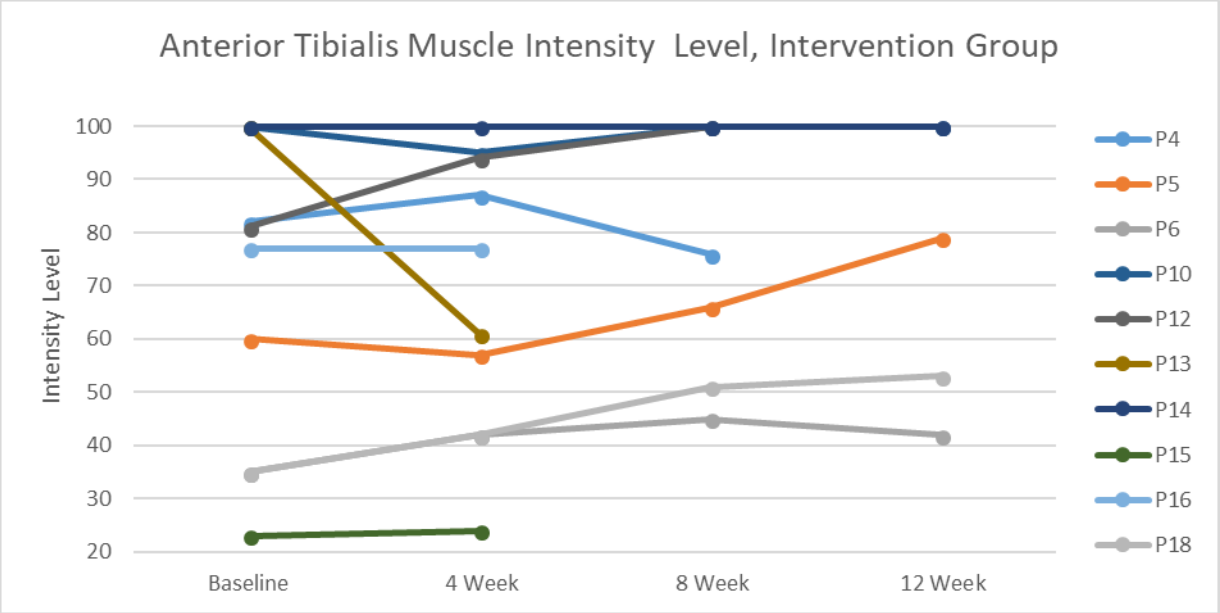


Figure 31 Anterior Tibialis muscle intensity level over each time point, intervention group

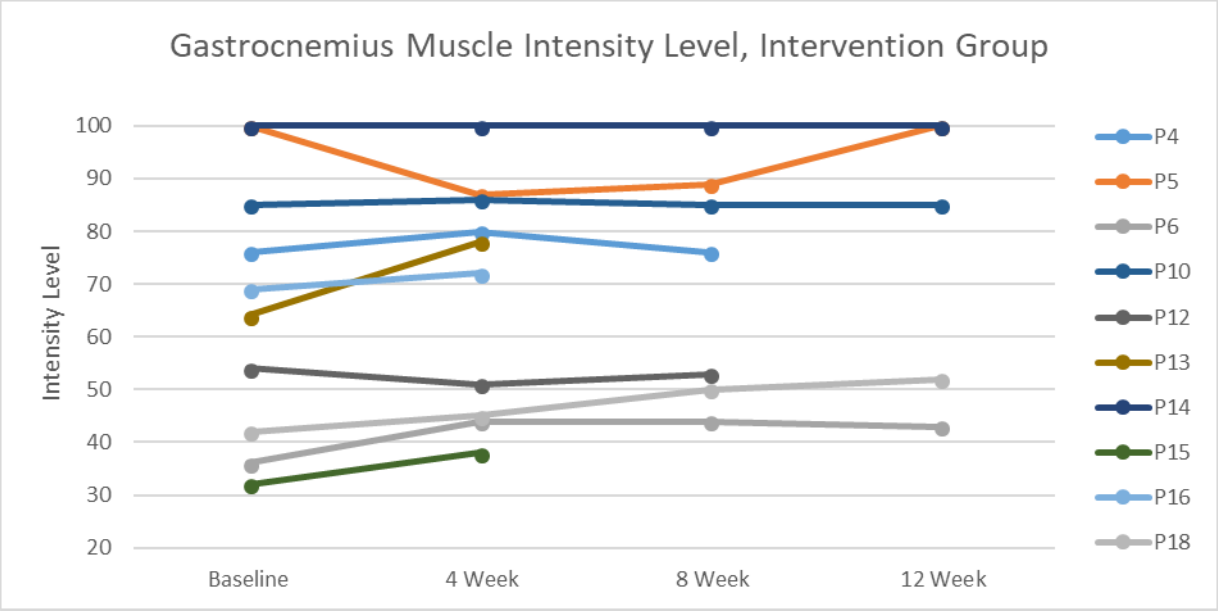


Figure 32 Gastrocnemius muscle intensity level over each time point, intervention group

4.4.1 Scatter plots of compliance, Intervention group

The relationship between compliance on changes in selected outcome measures was examined with scatter plots. A line was drawn on each plot to show the clinically significant threshold for each outcome measure (e.g. strength 8%, residual limb pain (RPL) phantom limb pain (PLP) and phantom sensation -27%; velocity 13%; percentage in stance on the amputated side 5%; step length on the amputated side 10%; mean circumference and volume 5%). The x-axis compliance was labeled from 1 to 7 days and the data shows how many days a week the intervention was worn on average by each person during each time frame in the study (0-4 weeks, 4-8 weeks or 8-12 weeks).

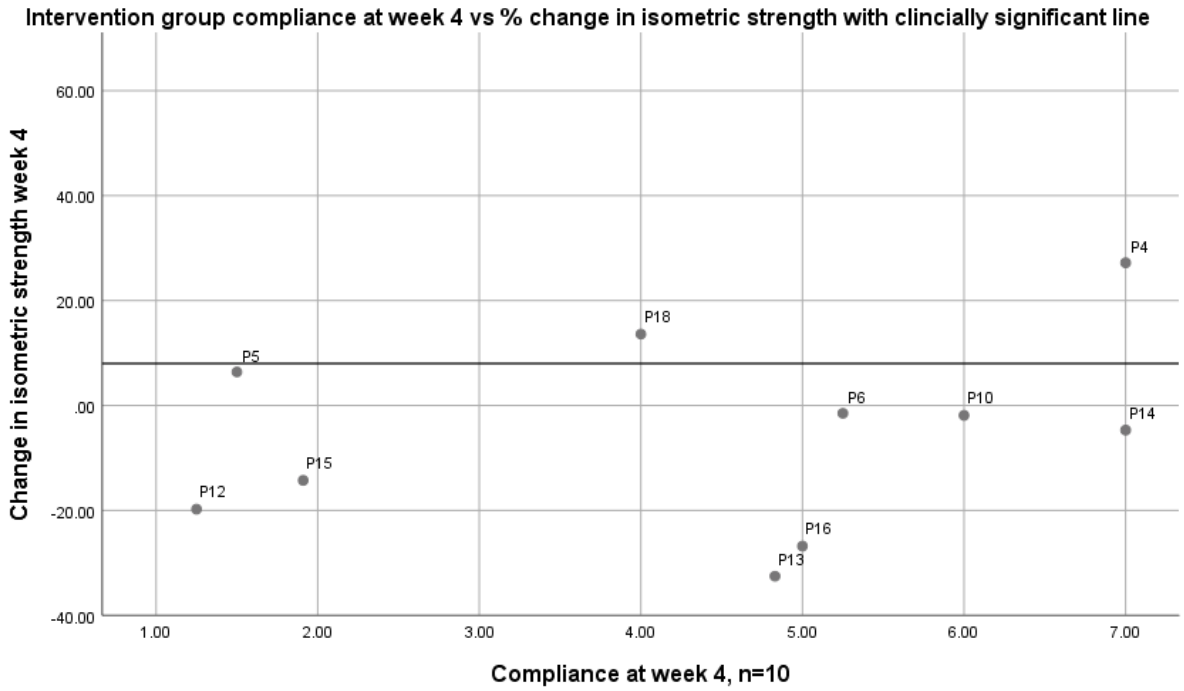


Figure 33 Intervention group compliance at week 4 verses change in isometric strength

After four weeks of treatment, two participant's isometric strength showed a clinical significance. One participant wore the intervention for four days a week and the second participant wore the intervention seven days a week. We saw two participants experience a significant decrease in strength at week four. P16 was about to have surgery due to cervical myelopathy, which decreased his strength and P13 reported experiencing residual limb pain due to a tight fitting prosthetic socket due to residual limb volume increases.

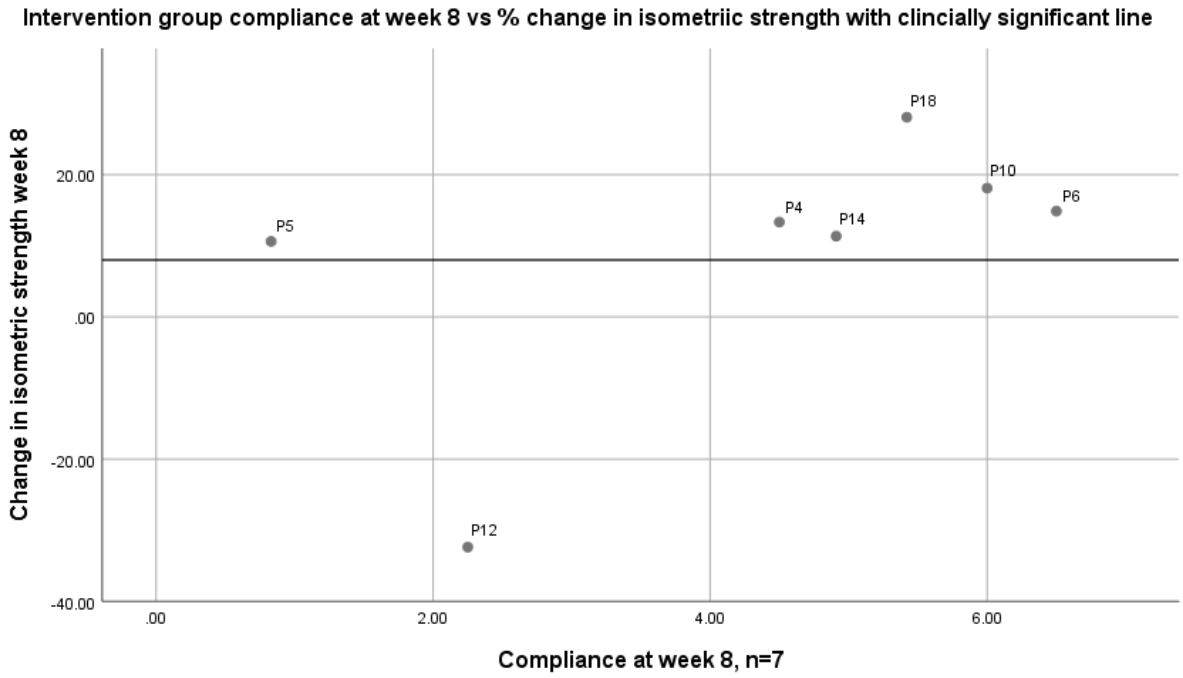


Figure 34 Intervention group compliance at week 8 verses change in isometric strength

Six participants showed a clinically significant increase in isometric strength, with five of the six wearing the intervention between 4 and 6.5 times a week.

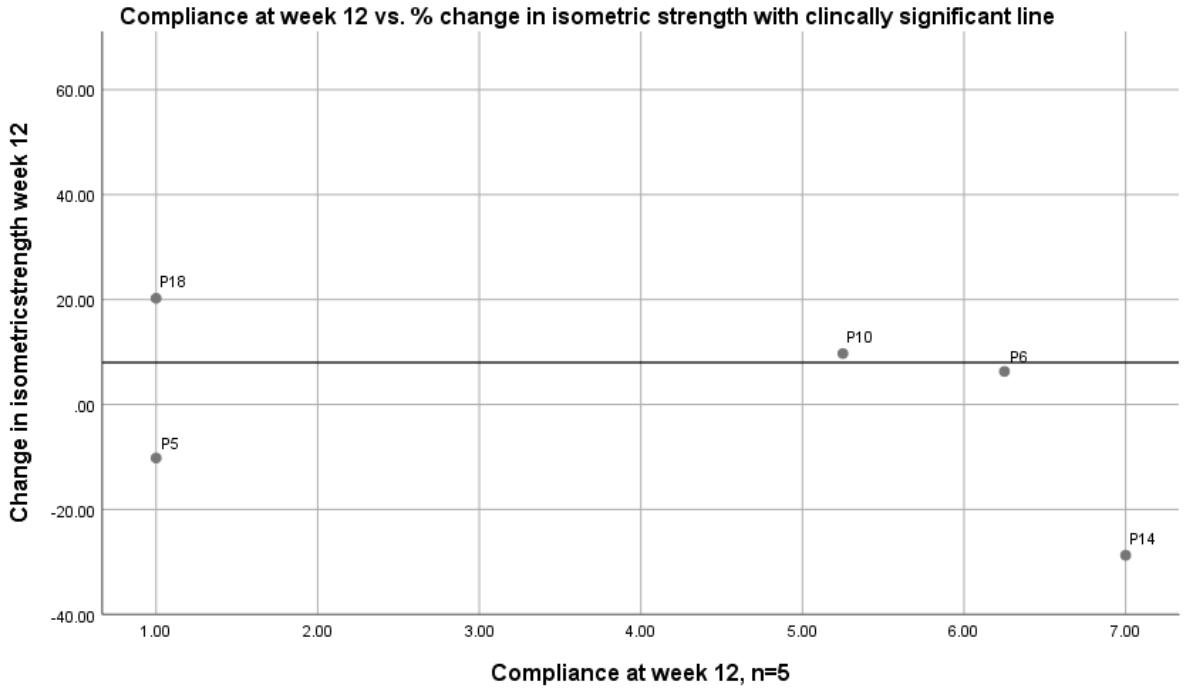


Figure 35 Intervention group compliance at week 12 verses change in isometric strength

Three participants continued to see an increase in isometric strength at week 12. Two participants wore the intervention between 5 to 6.5 times a week. Participant 18 had previuosly wore the intervention 4 to 5 times a week. She still showed an increase in isometric strength at week 12 with only wearing the intervention 1 time a week.

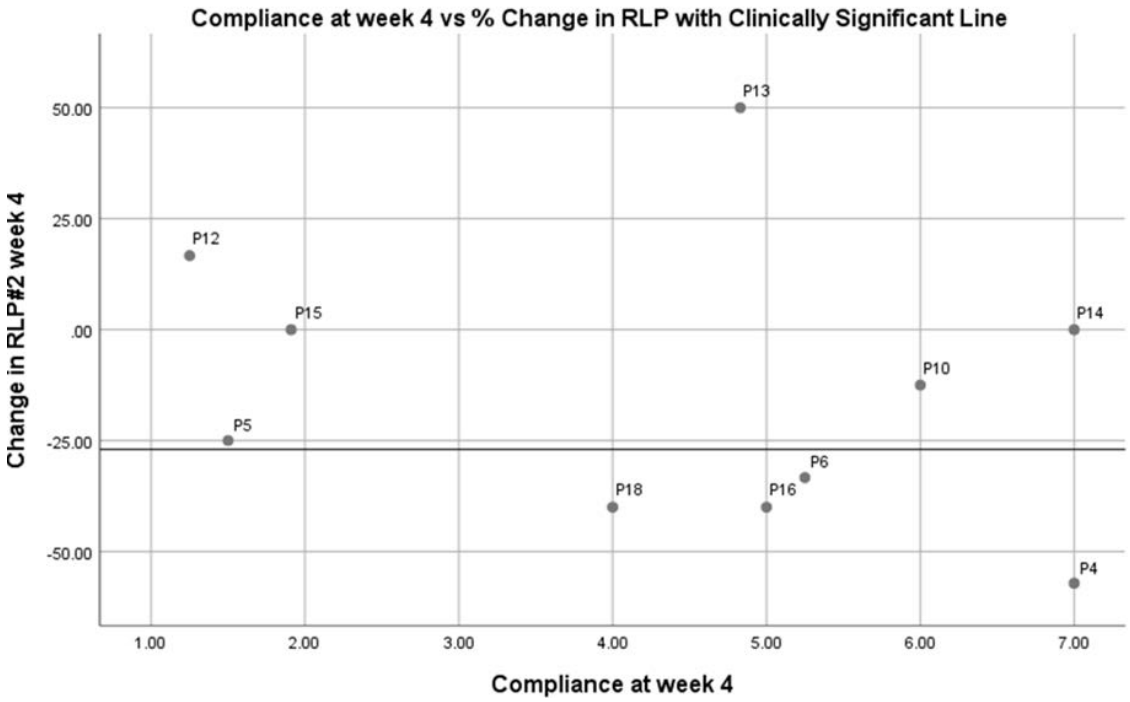


Figure 36 Intervention group compliance at week 4 verses change in residual limb pain question 2, n=10

At week four, four participants showed a clinically significant decrease of at least 27% in residual limb pain with wearing the intervention 4-7 times a week.

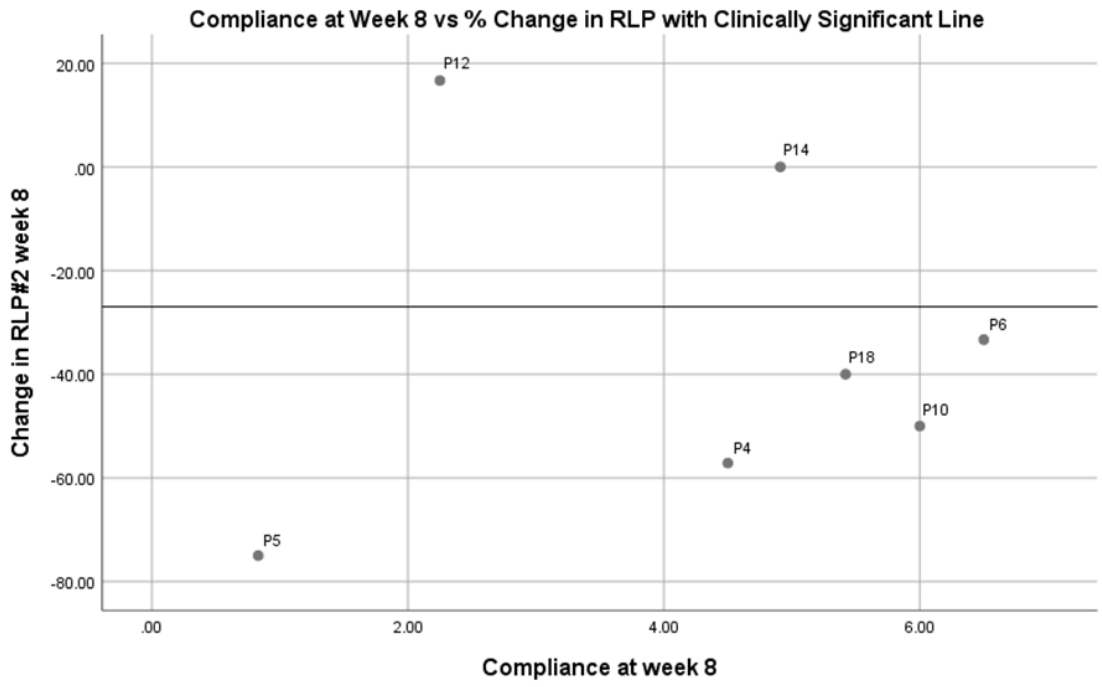


Figure 37 Intervention group compliance at week 8 verses change in residual limb pain question 2, n=7

At week eight, five participants showed a clinically significant decrease in residual limb pain. Participant 5 who only wore the intervention 1 time a week reported the largest decrease in RLP. The four others wore the intervention between 4 and 7 times per week.

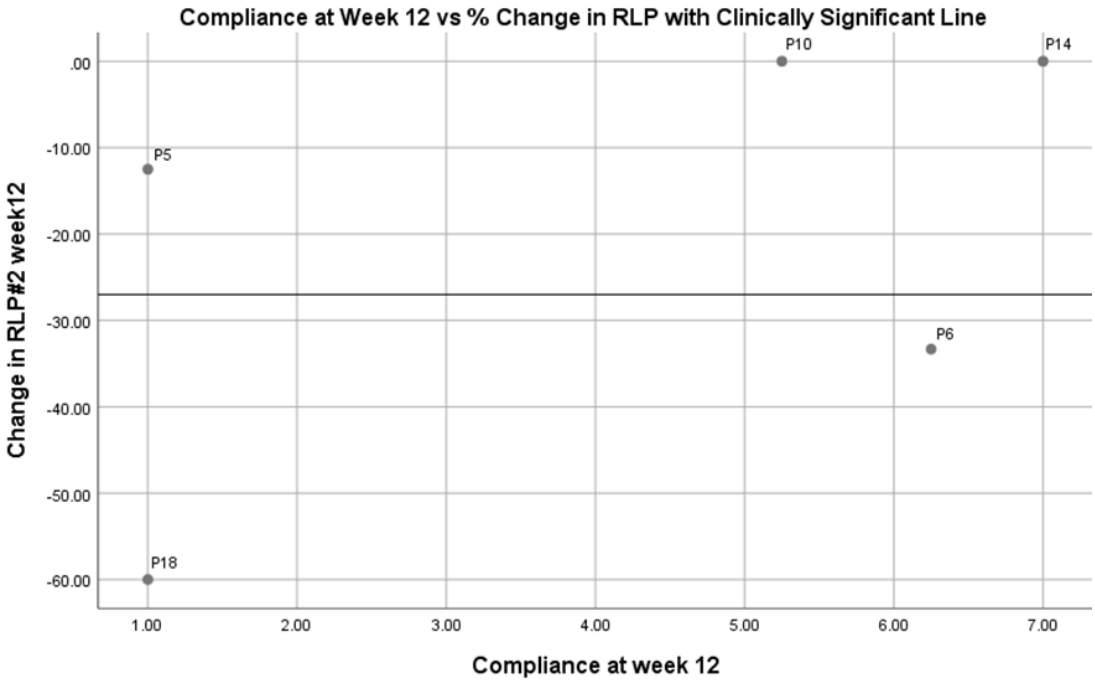


Figure 38 Intervention group compliance at week 12 verses change in residual limb pain question 2, n=5

Compliance at week twelve showed that two participants RLP did not change from baseline and two participants RLP showed a clinical significant decrease with participant 18 with the largest decrease wearing the intervention one time a week. Participant 18 was very compliant in wearing the intervention in the past, 4 to 5 times a week.

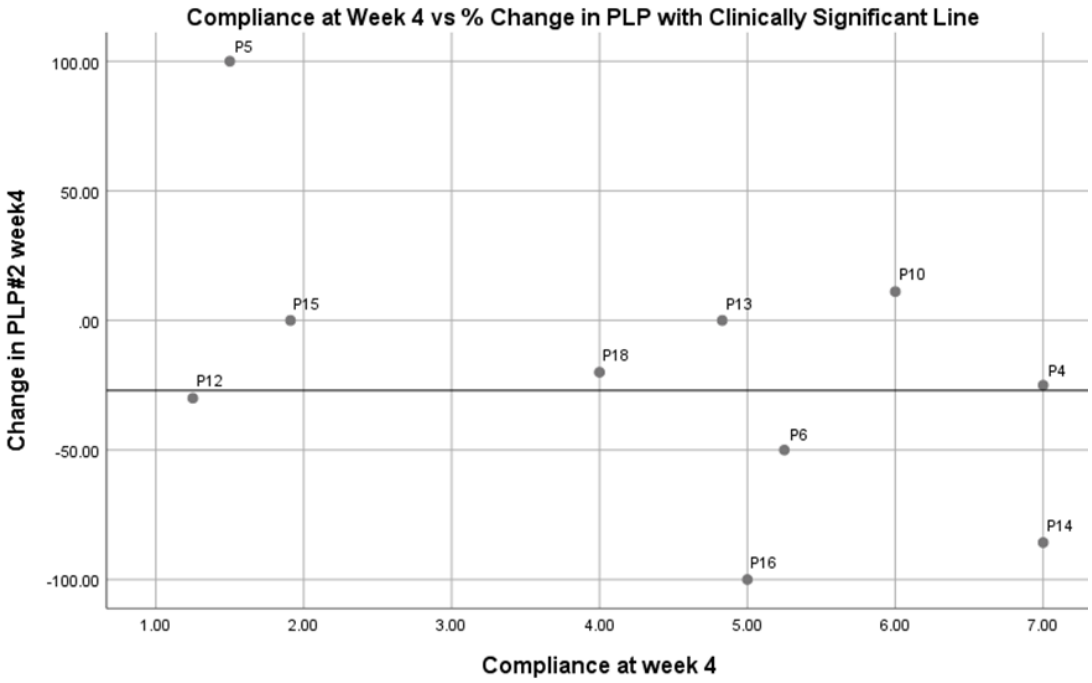


Figure 39 Intervention group compliance at week 4 verses change in phantom limb pain question 2, n=10

At week four, four participants reported a clinically significant decrease in phantom limb pain (PLP), three of these participants wore the intervention 5 to 7 times a week. Participant 4 reported a 25% decrease in pain which was close to clinical significance and wore the intervention 7 days a week.

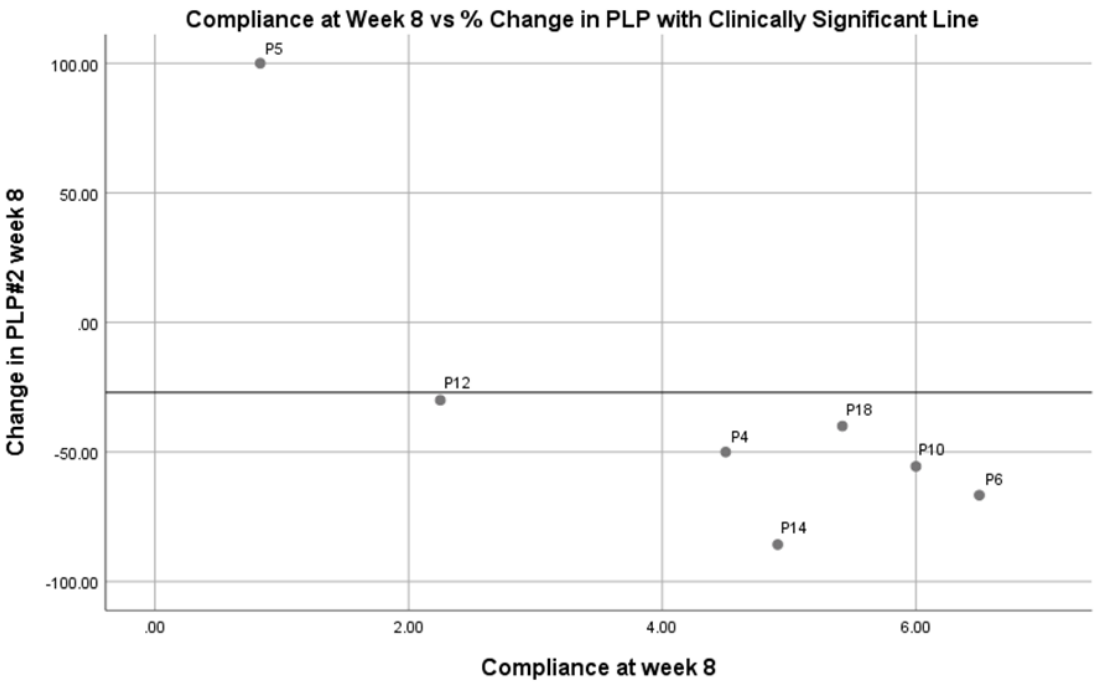


Figure 40 Intervention group compliance at week 8 verses change in phantom limb pain question 2, n=7

At week eight, six participants reported a decrease in PLP, with five of the participants wearing the intervention 4 to 6.5 days a week.

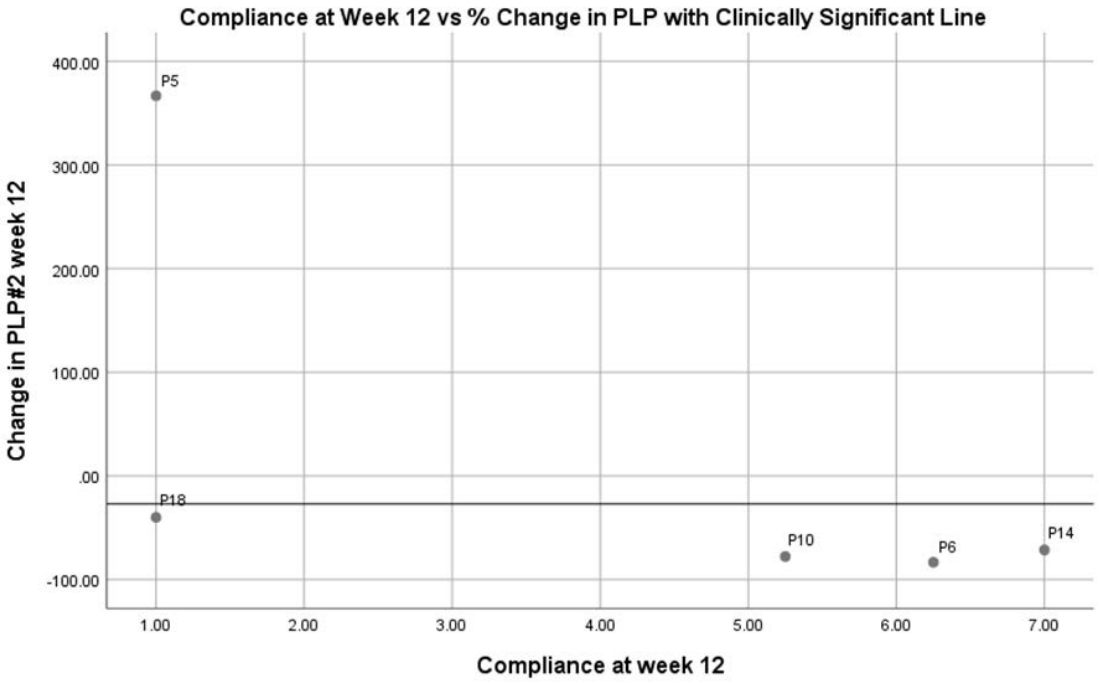


Figure 41 Intervention group compliance at week 12 verses change in phantom limb pain question 2, n=5

At week twelve four participants reported a clinically significant decrease in PLP, with three participants wearing the intervention 5 to 7 times a week.

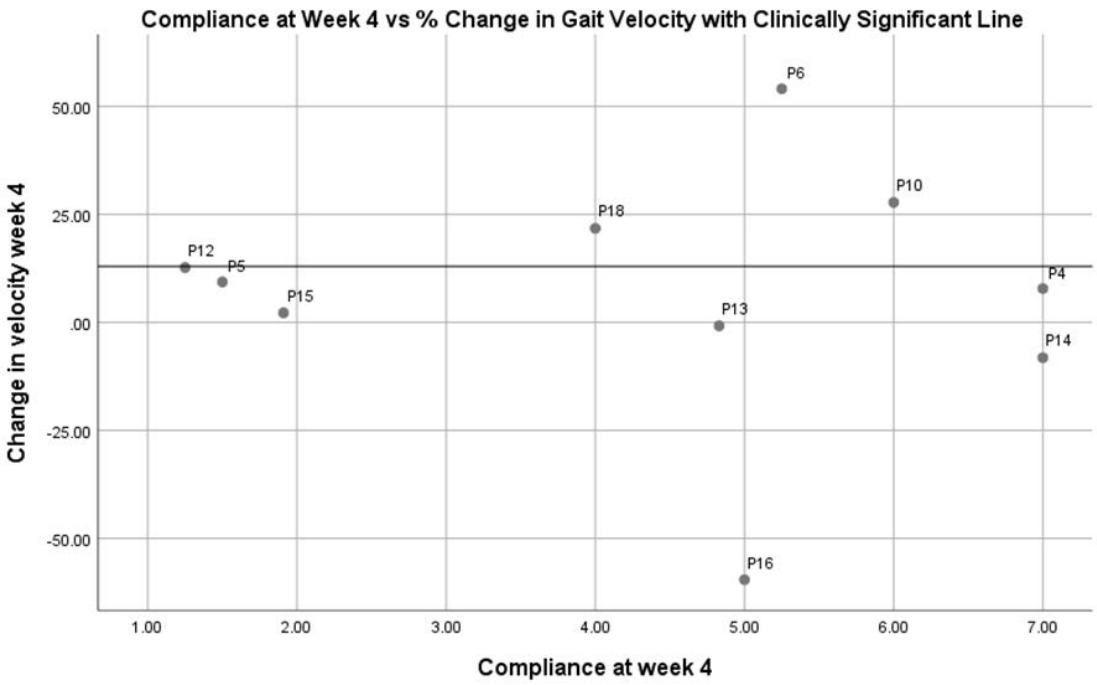


Figure 42 Intervention group compliance at week 4 verses change in velocity, n=10

At week four, four out of the ten participant's velocity increased, with three of the participants wearing the intervention 4 to 6 times a week.

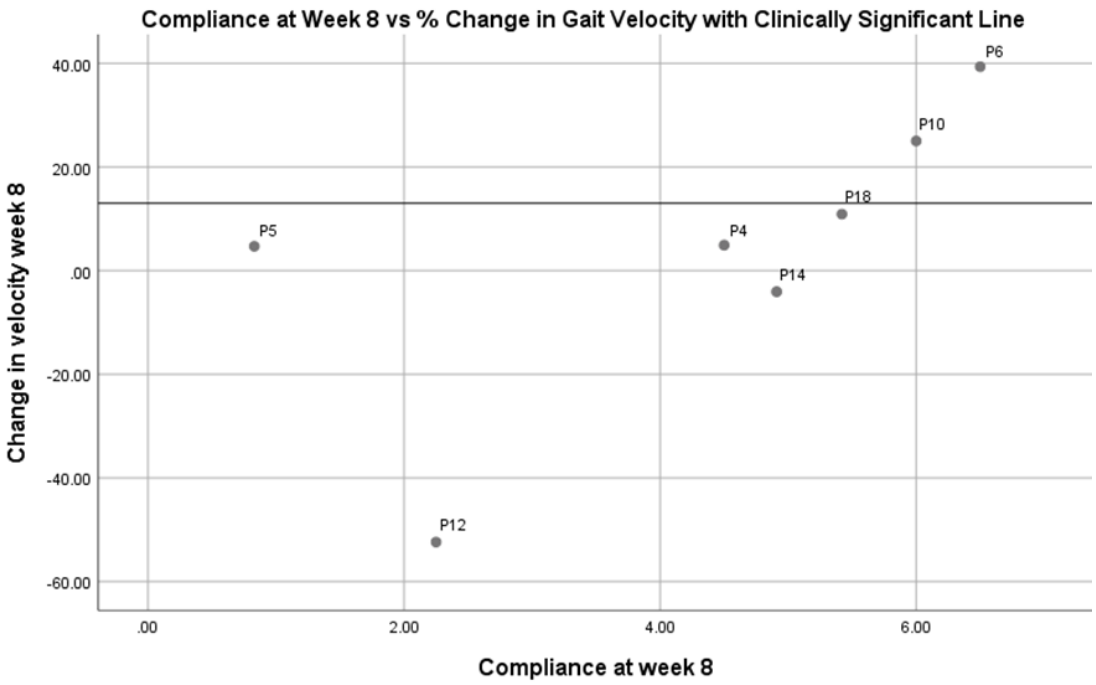


Figure 43 Intervention group compliance at week 8 verses change in velocity, n=7

At week 8, two out of seven participants velocity increased while wearing the intervention 6 to 7 time a week. With the exception of P5 this plot shows there was generally a trend for increasing velocity with increased compliance.

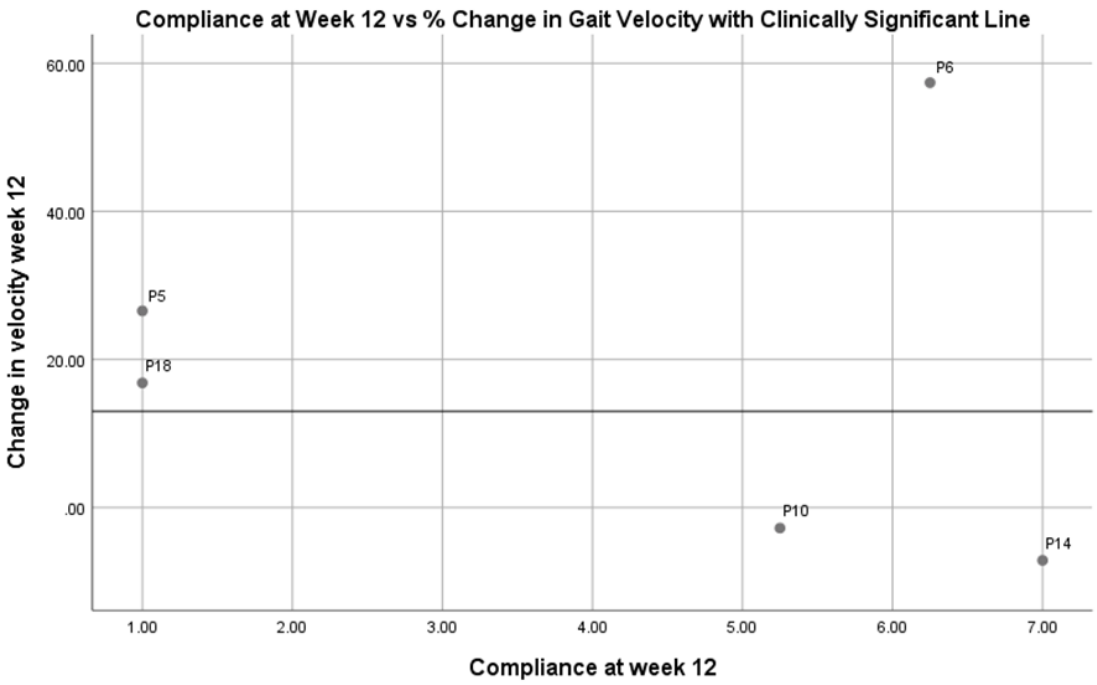


Figure 44 Intervention group compliance at week 12 verses change in velocity, n=5

Three of the participant's velocity increased, two of them wore the intervention one time a week and the third who showed the largest change wore the NMES 6 days per week.

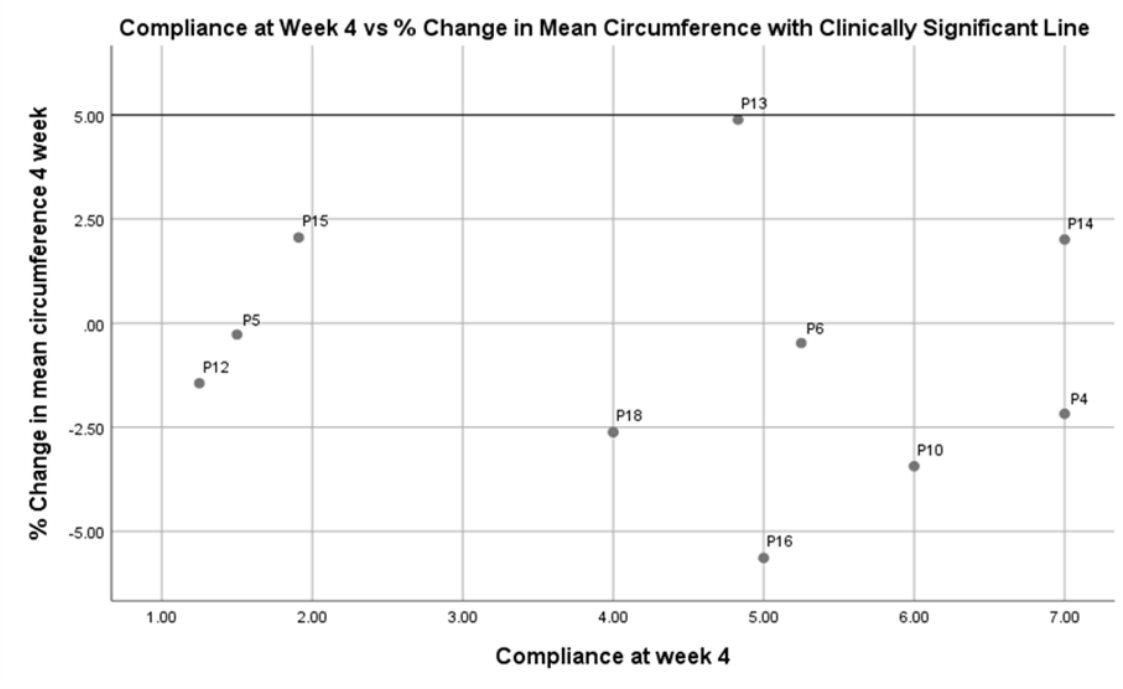


Figure 45 Intervention group compliance at week 4 verses change in mean circumference, n=10

At week 4, no clinical significance was found in mean circumference size change compared to compliance.

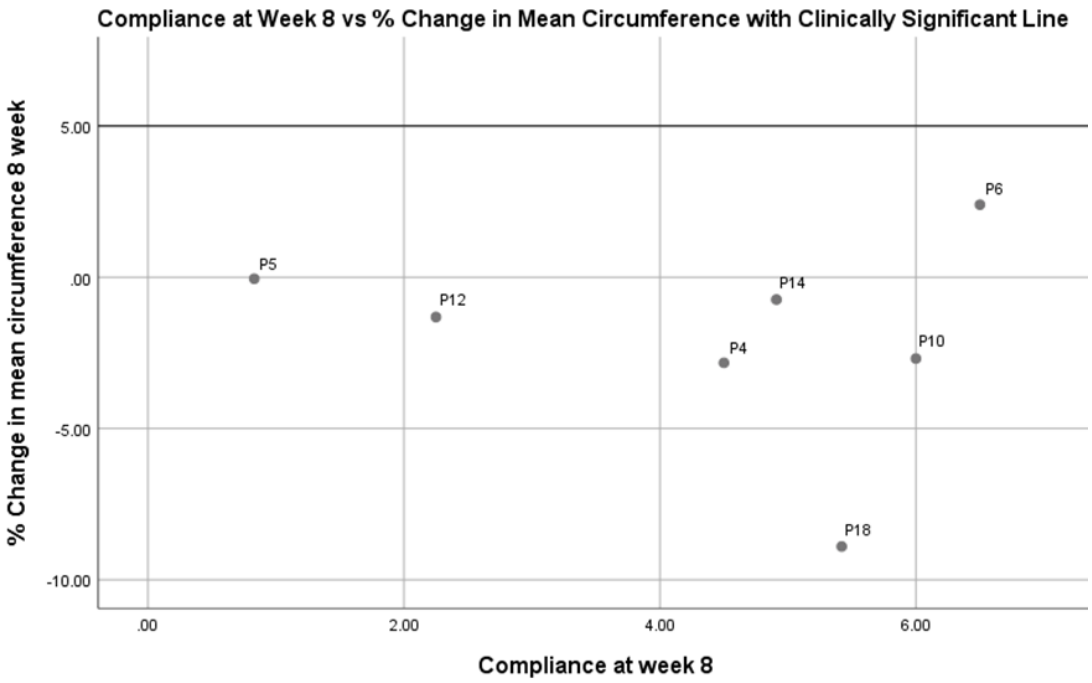


Figure 46 Intervention group compliance at week 8 verses change in mean circumference, n=7

At week 8, no clinical significance was found in mean circumference size change compared to compliance.

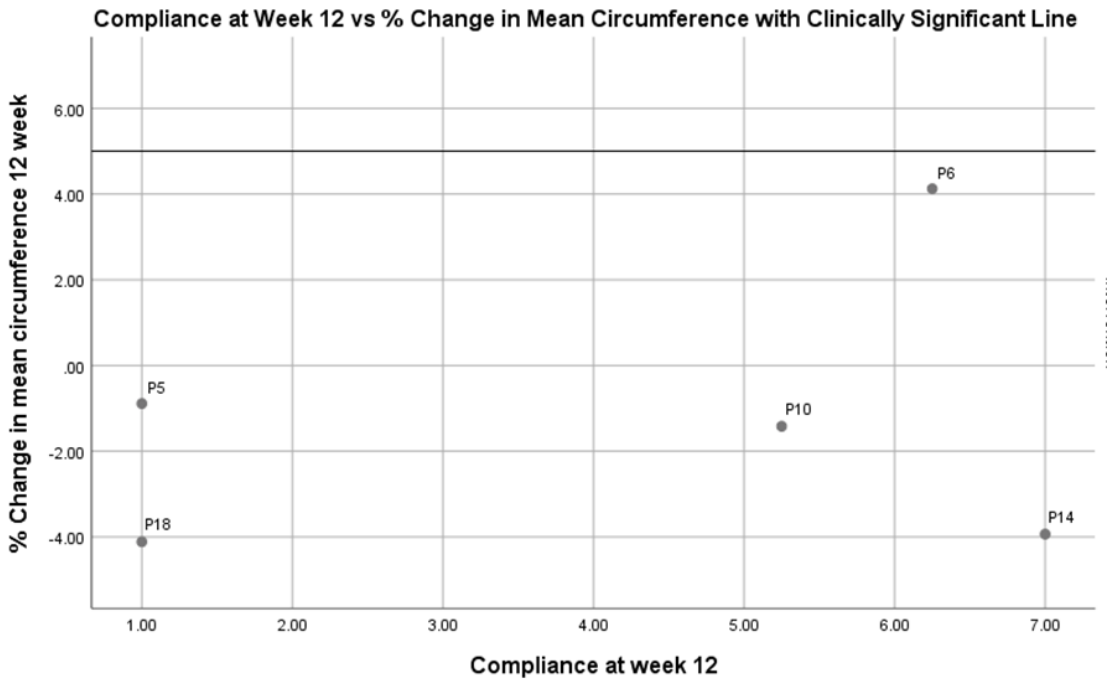


Figure 47 Intervention group compliance at week 12 verses change in mean circumference, n=5

At week 12, no clinical significance was found in mean circumference size change compared to compliance.

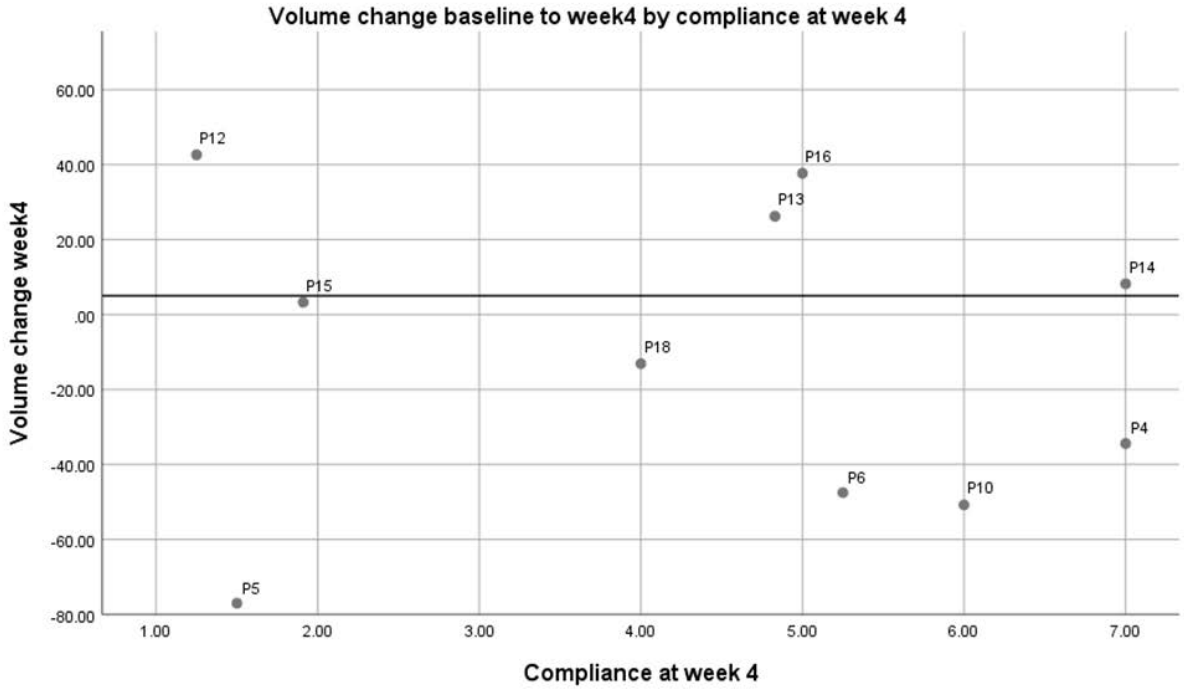


Figure 48 Intervention group compliance at week 4 verses change in volume, n=10

At week 4, four participants showed a clinical significant increase change in volume. Three of those participants wore the intervention approximately five to seven times a week.

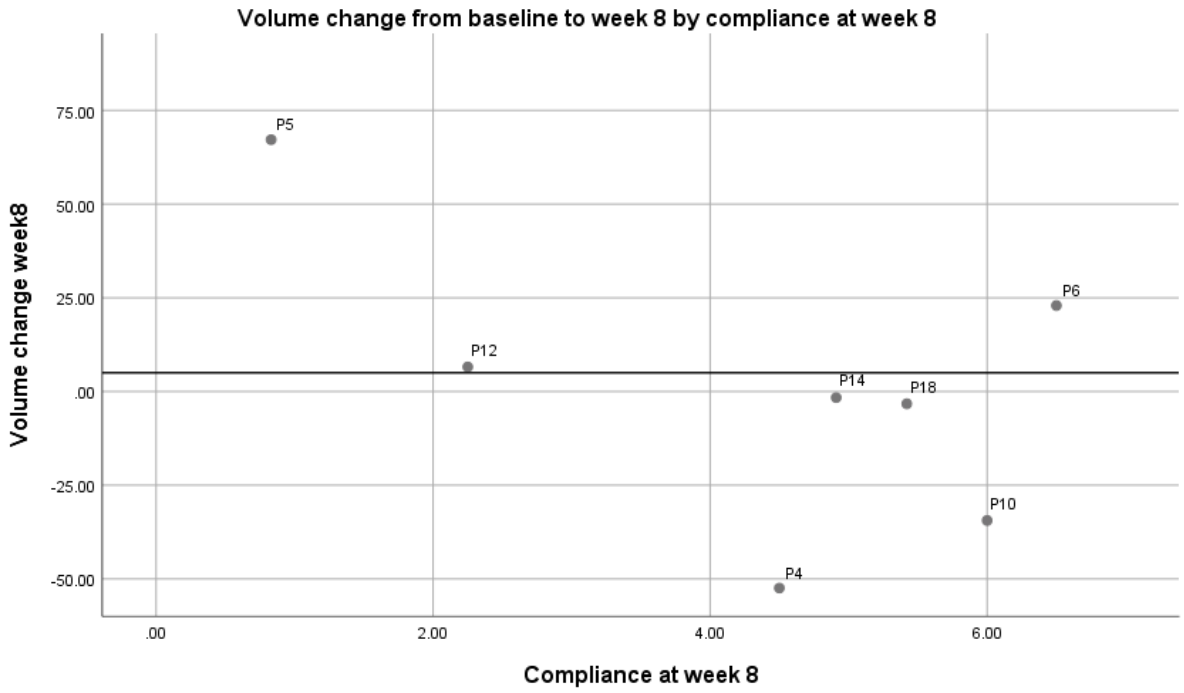


Figure 49 Intervention group compliance at week 8 verses change in volume, n=7

At week 8, three participants showed a clinical significant increase in volume.

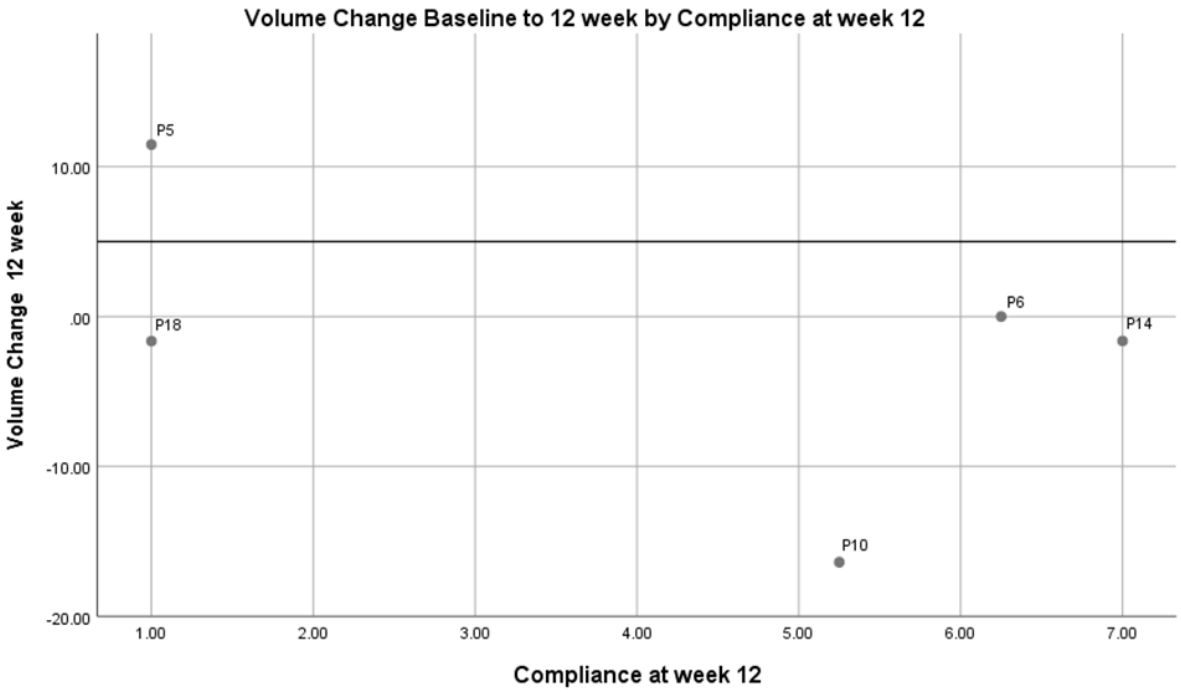


Figure 50 Intervention group compliance at week 12 verses change in volume, n=5

At week 12, one person showed a clinical significant increase in volume.

4.5 SCATTER PLOTS COMPARING OUTCOME MEASURES

In further investigating the effects of changes on the outcomes we conducted additional exploratory analyses (scatterplots) with the week 8 intervention data (n=7) to examine:

1. Association between increased volume changes and isometric strength gains after 8 weeks.

2. Association between increased residual limb mean circumference change and strength gains after 8 weeks.

3. Association between decreases in residual limb pain and velocity increases after 8 weeks.

4. Association between strength gains and increased velocity after 8 weeks

5. Association between increased volume changes and velocity increases after 8 weeks.

6. Association between decreased residual limb pain changes and isometric strength gains after 8 weeks.

7. Association between decreased phantom limb pain changes and isometric strength gains after 8 weeks.

8. Association between decreased phantom limb pain changes and velocity increases after 8 weeks.

9. Association between an increase in residual limb mean circumference and velocity increases after 8 weeks.

A moderate association ($r^2 > 0.3$) was observed in three of these relationships. Greater increases in velocity was associated with greater reductions in residual limb pain ($r^2 = 0.4$) and increases in isometric strength ($r^2 = 0.3$). Greater reductions in residual limb pain was associated with greater increases in isometric strength ($r^2 = 0.3$).

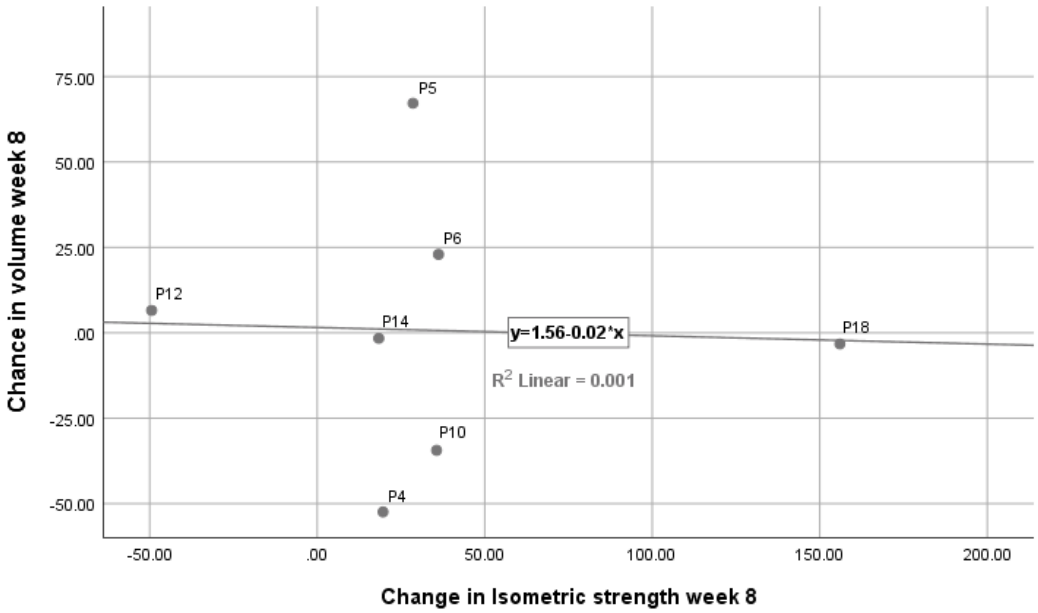


Figure 51 Change in mean volume baseline to week 8 by change in mean isometric strength baseline to week 8, intervention group

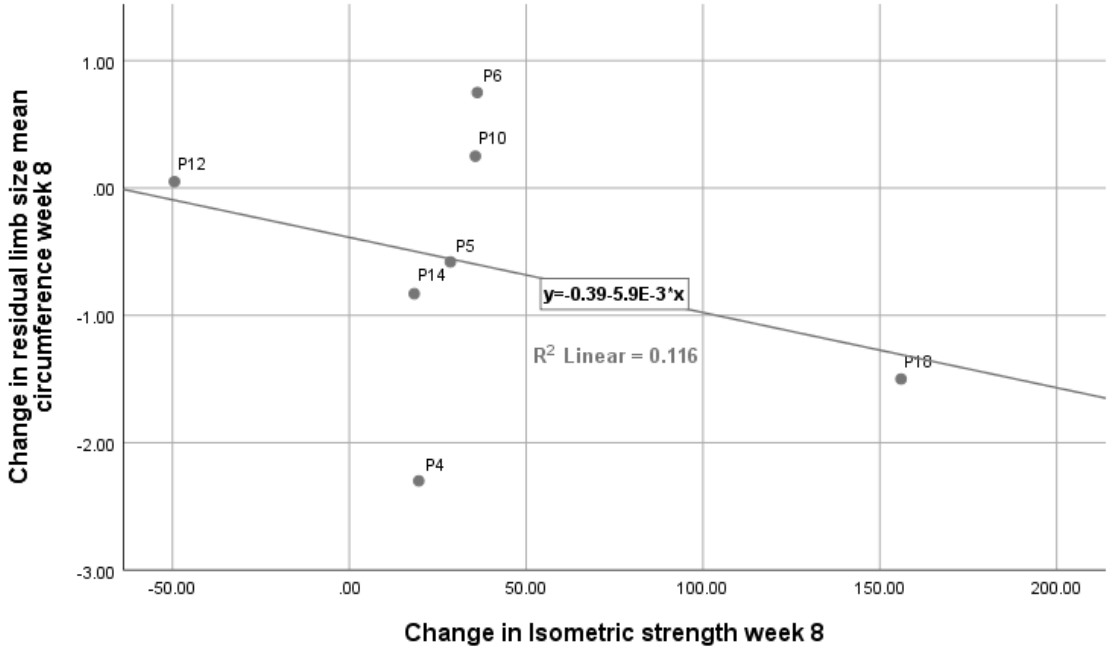


Figure 52 Change in residual limb size mean circumference by change in men isometric strength, intervention group

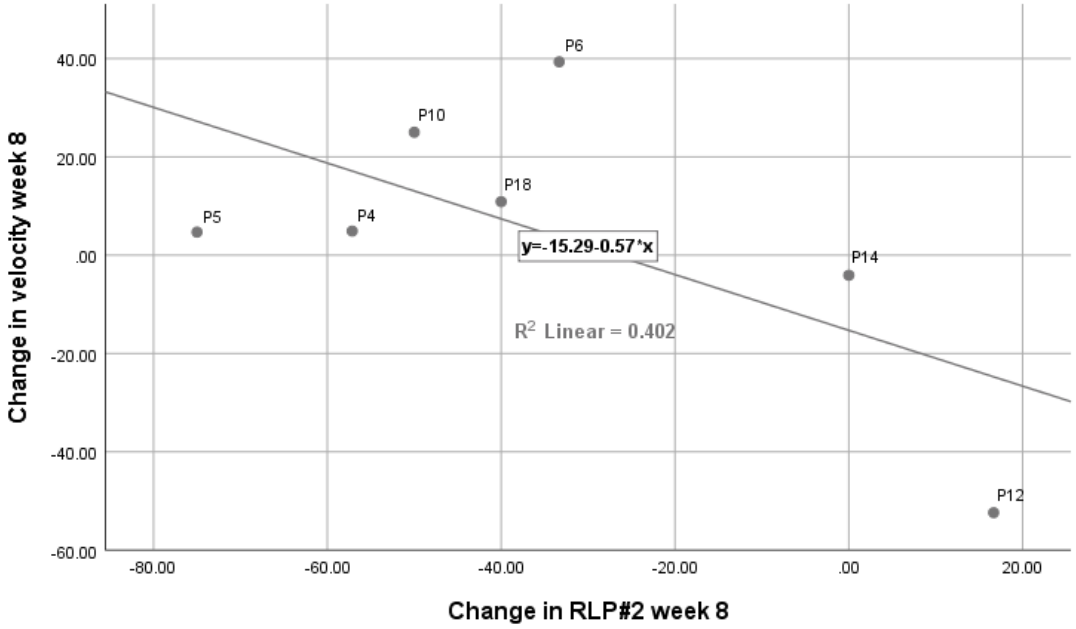


Figure 53 Change in mean velocity week 8 by change in mean residual limb pain question 2 week 8, intervention group

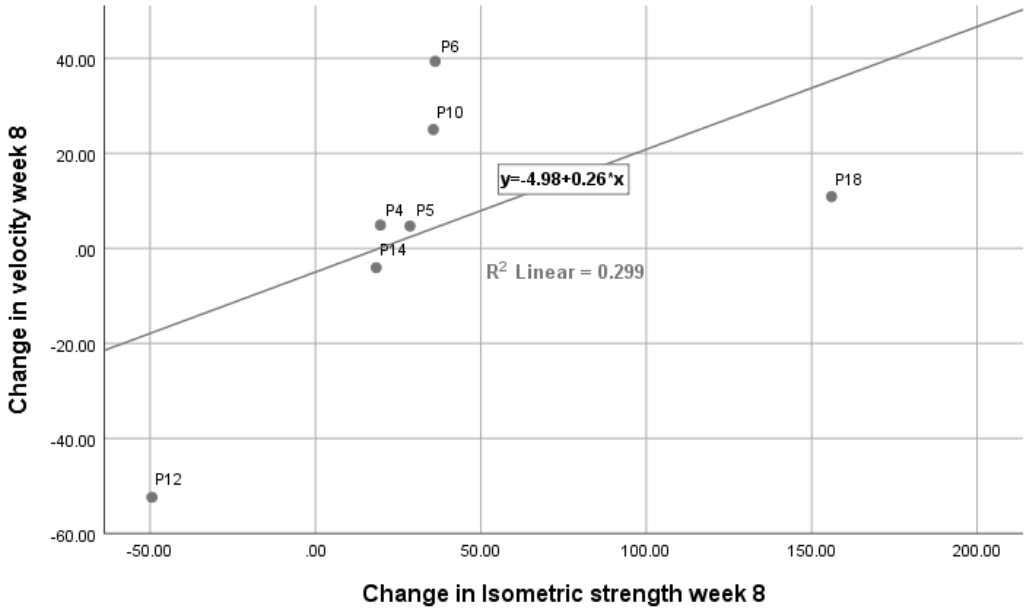


Figure 54 Change in mean velocity week 8 by change in mean isometric strength week 8, intervention group

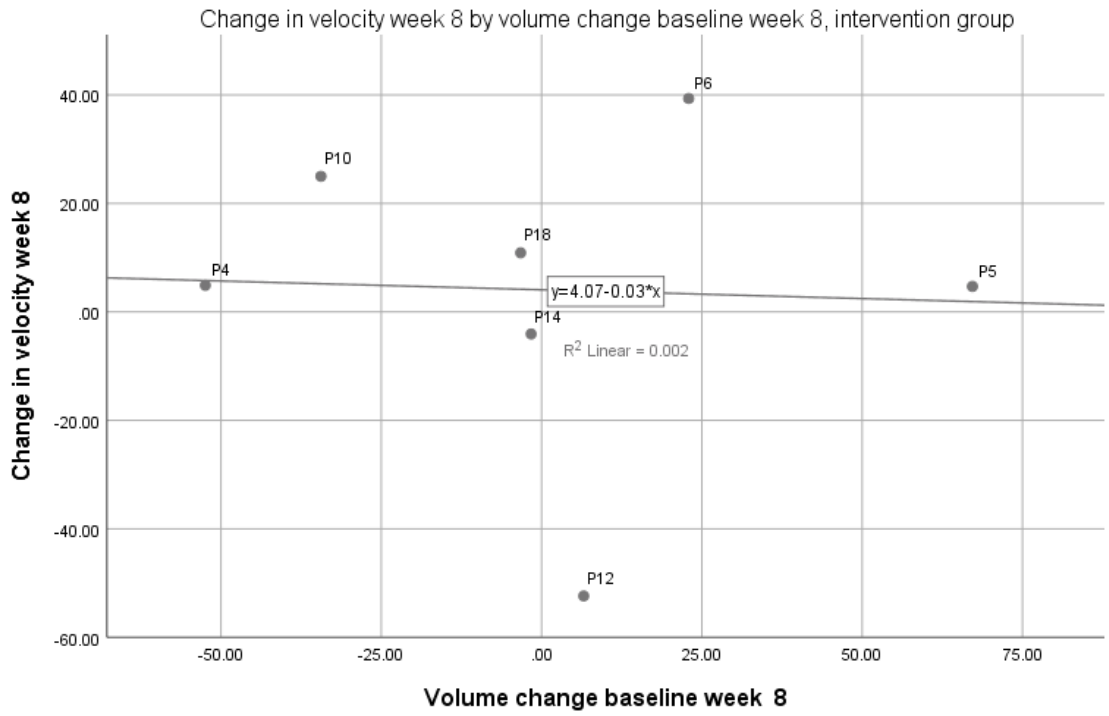


Figure 55 Change in Velocity week 8 by volume change baseline week 8, intervention

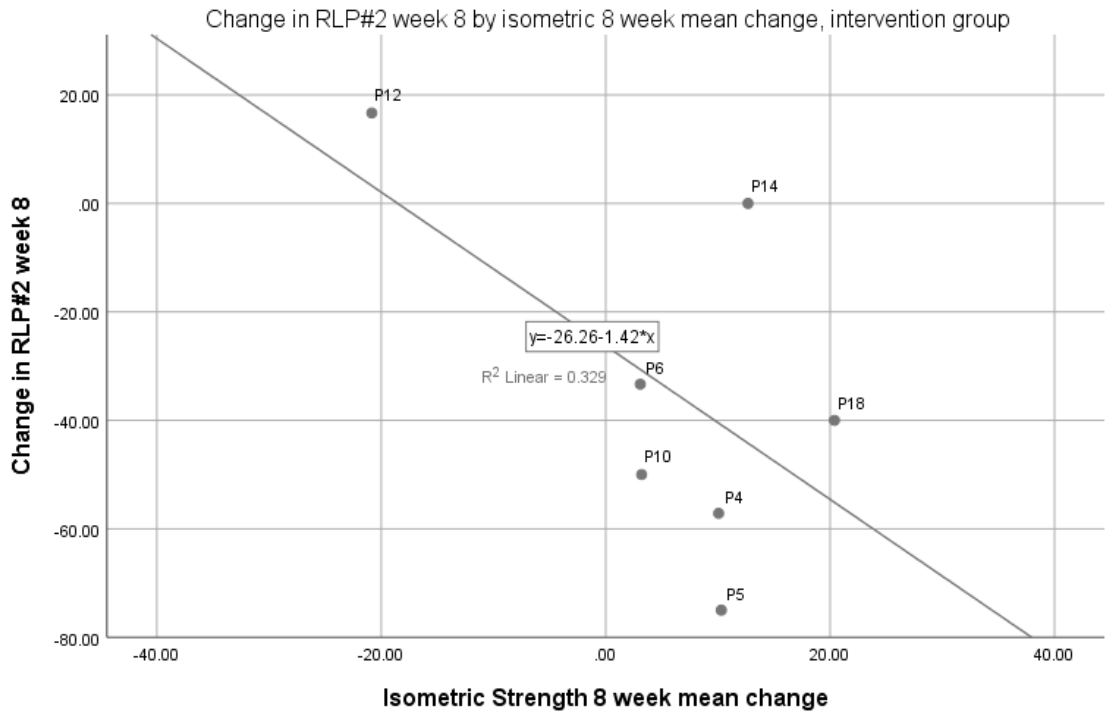


Figure 56 Change in RLP#2 week 8 by isometric 8 week mean change, intervention group

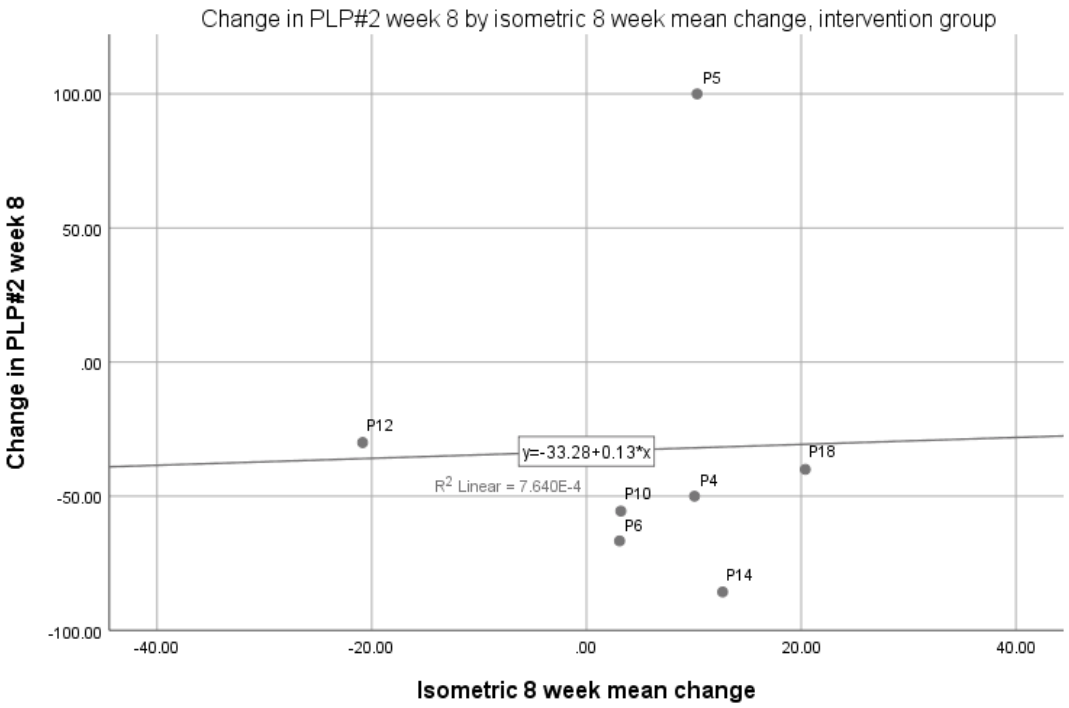


Figure 57 Change in PLP#2 week 8 by isometric 8 week mean change, intervention group

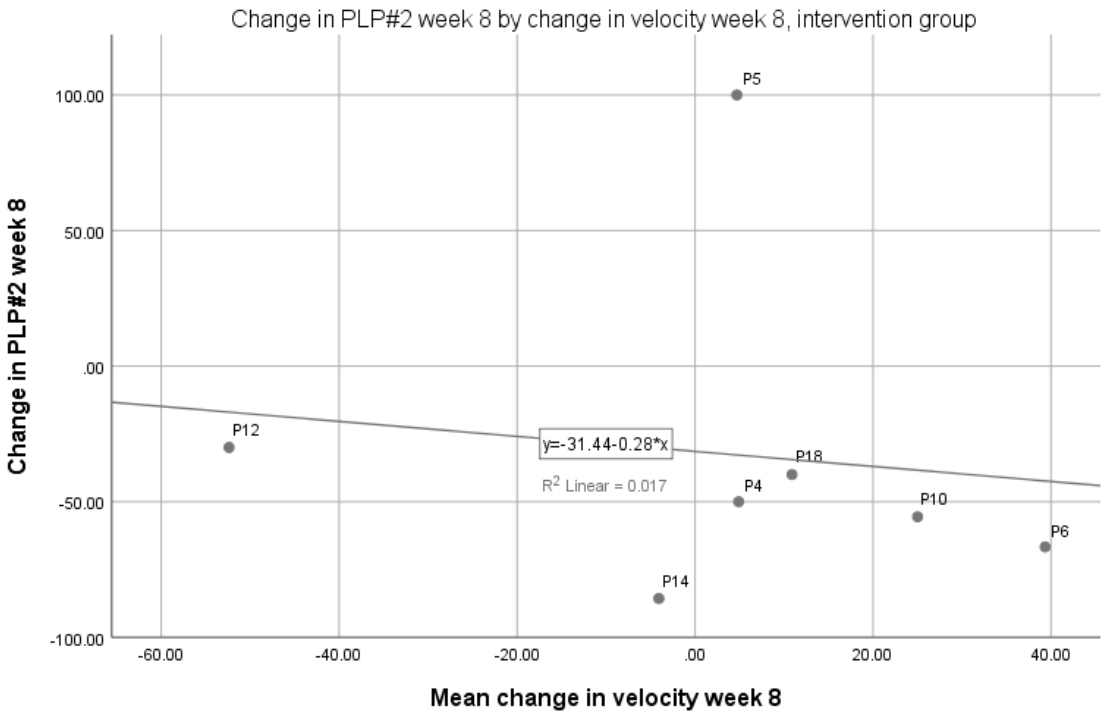


Figure 58 Change in PLP#2 week 8 by change velocity weeks 8, intervention group

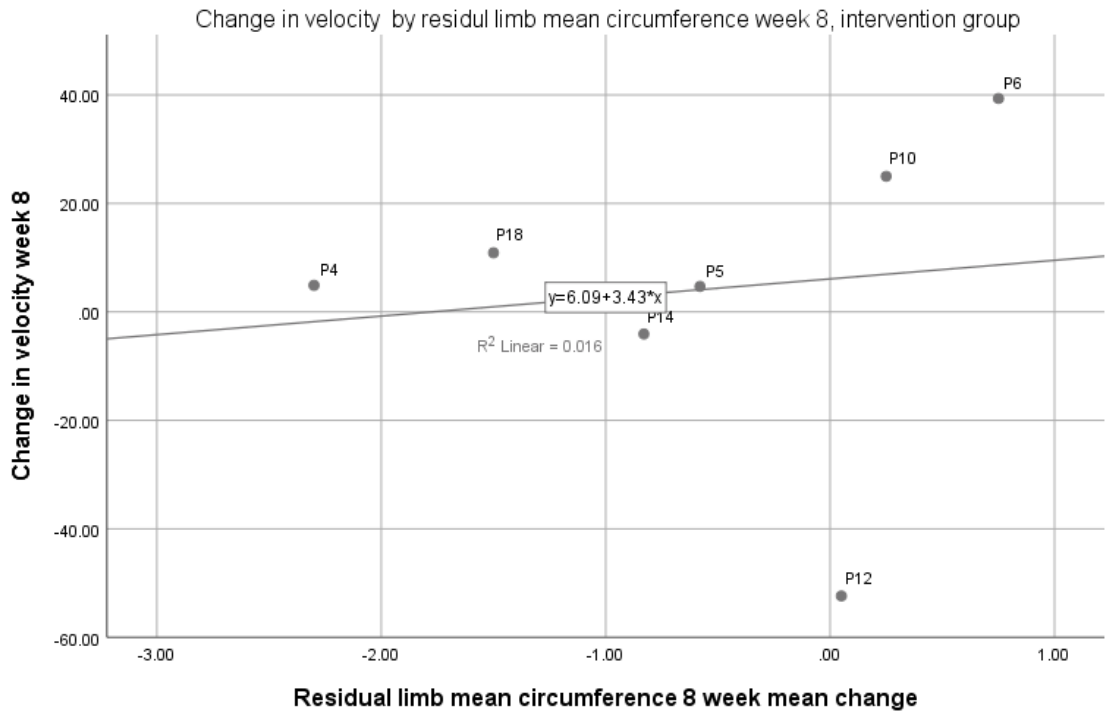


Figure 59 Change in velocity by residual limb mean circumference week 8, intervention group

5.0 DISCUSSION

5.1 SUMMARY

Among the amputee population, statistics show that transtibial amputations are the most prevalent [31]. Rising health care and durable medical costs are just a few of the obstacles the amputee population faces. Determining a safe, effective, low cost, at home intervention (modality) to restore or improve the health of the residual limb is a necessity. The purpose of this dissertation was twofold: 1) to explore the efficacy of an NMES intervention on amputee health related outcomes and 2) feasibility of at home use of the NMES intervention.

Subject retention in longitudinal studies can be very challenging. Retention in the control group was excellent (0%) while attrition was high in the intervention group (50%). It's important to realize however that our attrition was due in part to the detection of clinically significant residual limb size changes and socket discomfort reported by the participants induced by the NMES (n=3 or 30% of the subjects). Only two subjects dropped out for unrelated reasons (20%). Due to the small sample size of five participants left in the treatment group at week twelve, we found it not meaningful or accurate to apply an ANOVA to the treatment group. Therefore, we explored the data through descriptive and non-parametric statistics, group comparison box plots and intervention group comparison scatter plots.

Pre (baseline) and post (12 week) intervention mean and standard deviations were computed, along with mean changes and confidence intervals. We found that the standard deviations were large for many of the outcomes indicating that the subjects varied widely in their responsiveness to the intervention. The confidence intervals were also wide implying that the sample size was too small to draw any conclusive information on the benefits of NMES. The effect sizes ranged from small to large however some of them were not consistent with our main hypothesis. For example, isometric strength, showed a moderately strong effect size for the study (0.49) but the result was not consistent with our hypothesis (control group mean strength increased while intervention group remained the same). For this reason, we examined the individual effects in more detail to identify those who saw clinically relevant benefits from the NMES intervention.

Talbot et al performed a RCT study [159] (control, n=21 and NMES = 23) in military personnel with unilateral TTA. The study consisted of applying NMES to the quadriceps muscle for 12 weeks looking at outcome measures in knee extension and flexion strength, mobility and quality of life. The NMES protocol was similar to ours where they asked the participant to wear the NMES at home 5 days a week for 15 contractions. The study outcome measures were knee extension and flexion strength by use of a handheld dynamometer, mobility items; 2-minute walk test (2MWT), the timed up and go test (TUG), timed stair climb, a timed chair rise test, and a pain severity questionnaire and a pain interference questionnaire. Pain severity was measured using a 4-item subscale of the Brief Pain Inventory. Pain was assessed at its "worst," "least," "average," and "current" level. Scores on this scale range from 0 (no pain) to 10 (pain, as bad as one can imagine). A mean pain score was calculated from the four items. Pain interference was

measured as how pain hindered daily activities: general activities, walking, work, mood, enjoyment of life, relations with others, and sleep using the Brief Pain Inventory. Participants rated each item on a scale from 0-10 (0=does not interfere; 10=completely interferes). The interference score represents the mean of the seven items.

Of the 44 participants who enrolled in the study, 30 (68%) completed the study [159]. NMES compliance was monitored through a daily training log kept by the participants and an onboard data logger hidden in the NMES device. Adherence on the basis of participant's self-reported training log showed use of the intervention for approximately 50% of the recommended sessions whereas the NMEs device compliance monitor showed a 27% adherence rate [159]. A limitation to their device was that if it turned off before cycle completion, the data would not be recorded. This study had a 35% attrition rate for the intervention group and a 29% attrition rate for the control group. There was no mention of drop out related to the intervention working sooner than 12 weeks as we found in our study. One possible explanation could be that their participants were not as compliant as our participants. Our Intervention group participants had higher NMES compliance (4 days versus 2.5 days per week) and higher attrition (50% versus 35%).

No statistically significant differences were found between groups for any outcome measure in the Talbot et al. study. There was no mention if they looked at clinical significance. The data showed that there were increases in both extension and flexion strengths of both groups from baseline to week 12. The pain scores showed a decrease in scores for both groups was well. We had a similar result with our strength data, although we found a statistically significant decrease in the residual limb pain score.

5.1.1 Effects of NMES on Strength

To answer the first hypothesis, ***in comparison to a control group, NMES training will show greater increases in isometric and isokinetic knee extension strength post training when compared to baseline (pre-training) in the residual limb as measured using a Biodex 4 system***. We did not find any statistical significance increase from baseline to week 12, however we found a clinically significant increase from baseline to week 12 in both strength tests with two participants (P10 and P18) and also a clinical significance from baseline to week 24 in both strength tests with two participants (P5 and P18). As mentioned above, we found an effect size of 0.49 for isometric strength and an effect size of 0.08 for isokinetic strength. The confidence interval ranges for both of these variables were wide, indicating that the sample size is too small. Moreover, the power computed for both variables was low. The effects were also not consistent with our hypothesis. These findings suggest that we may need to refine our measurement techniques and conduct future testing with a much larger cohort.

5.1.2 Effects of NMES on Limb Size (Circumference and Volume)

To answer the second hypothesis, ***in comparison to a control group, NMES training will show greater increases in volume of the residual limb after (post) training when compared to***

baseline (pre-training) as measured using a hand held three-dimensional motion-tracking laser scanner Cad/Cam system. The residual limb mean size circumference measurements showed a slight decrease in residual limb size in both groups and the volume measurements showed very little change. Mean change scores indicate that there was not a difference in residual limb mean circumference, showing a small effect size of 0.15. For volume however, the effect size was 0.40 but this result was also not consistent with our hypothesis as it appears the control group increased slightly in mean volume while the intervention group remained stable. For two participants (P13 and P15), we saw a clinically significant increase in residual limb circumference size and withdrew them from the intervention at week 4 and had them return for the final follow up visit. Subject P4 had a clinically significant decrease in residual limb volume and was withdrawn from the intervention at week 8. Data shows that there was a lot of variability with the residual limb circumferences and volumes across subjects and notable outliers. To rule out confounding issues concerning weight gain or loss over the course of the study, we measured the Intervention group participants BMI over all time points. An average individual mean change decrease of .6% was found. This is approximately a 1.8 kg difference (approximately 4 lbs.). This is not likely enough of a difference to affect the residual limb volume.

A residual limb may be considered to have reached maturity between 12–18 months post-operative. Mature residual limbs are still subject to changes in residual limb volume. The amount of daily volume fluctuation is likely to vary greatly among amputees as a function of comorbidities, edema, prosthesis fit, activity level, environment, BMI, dietary habits (sodium/salt intake), medication, hormonal changes and menstrual cycle. Daily volume changes in mature

residual limbs are believed to be the result of three interrelated mechanisms: (1) pooling of blood in the venous compartment; (2) arterial vasodilatation; and (3) changes in the interstitial fluid volume. If the lymphatic system is compromised or out of balance the residual limb will change volume [57]. We didn't expect to see a decrease in limb volume or size because NMES typically increases muscle mass given the correct dosage. The factors contributing to the decrease in muscle mass or size may have been due to the muscle being over worked or we may have increased blood flow to stimulate the lymphatic system decreasing edema.

5.1.3 Effects of NMES on Pain

To answer the third hypothesis, ***in comparison to a control group, NMES training will show greater decreases in chronic residual limb pain, phantom limb pain and phantom sensation after (post) training when compared to baseline (pre-training) as measured by a pain questionnaire designed for amputees measured using a numeric rating scale.*** The NMES had a positive benefit in reducing residual limb pain in our TTA population. This is not too surprising as this finding is consistent with many studies using TENS and NMES in the literature. Presence of chronic limb pain was also an inclusion criterion in the study. Residual limb pain was the only statistically significant variable we found to decrease overtime in the intervention group. We also saw a clinically significant decrease from baseline to week 12 in the intervention group for phantom limb pain. We saw a slight increase of phantom limb pain at week 24 which was still found to be a clinically significant decrease compared baseline. Data from the

individual case studies showed that seven participants reported decreases in pain across the various pain outcomes across time. The larger effect sizes among the pain variables tells us that the intervention had a benefit to our intervention group.

5.1.4 Effects of NMES on Gait Variables

To answer the fourth hypothesis, ***in comparison to a control group, NMES training will show greater increases in velocity and step length and percentage in stance time on the amputated limb after (post) training when compared to baseline (pre-training) as measured using the GAITRite® system***. The majority (7/10) of the intervention group participants reported that they are fairly active. Two participants reported mobility issues with their sound leg due to current diabetes and past trauma, and one participant was being treated for neck pain due to a past car accident. We did not find a clinically significant increase in any of the gait parameters. Two intervention participants did show a clinically relevant increase over time in velocity and step length. Seven of the intervention participant's baseline scores were at or close to average scores found in literature (147) for the existing TTA population for velocity. The same seven plus another participant spent approximately 60% of their time during gait in the stance phase at baseline. The intervention group mean score decreased slightly possibly due to the effects of the intervention with prosthetic socket fit but was not found to be clinically significant. We did not see a clinically significant change in prosthetic step length between groups. On an individual basis, four participants (P4, P6, P10 and P18) showed clinically significant increases in step length over time.

5.1.5 NMES compliance, intensity and effect on outcomes

Most participants wore the NMES intervention four to seven times a week. We compared dosage (times the intervention was worn per week) but we were unable to draw any conclusive findings due to the small sample size. Participants who wore the NMES intervention four to seven times a week seemed to have a greater benefit in decreased pain and increased velocity.

The NMES intensity varied per participant. Some participants could only tolerate intensity of slightly over 20 at baseline while others maxed the device at 100 at baseline. Participant number 14 wore the NMES at the highest intensity levels at all three muscle groups.

Participant 14 showed clinically significant increases in isometric and isokinetic strengths at week 8, he also showed decreases in PLP, PLP CPGS across 4 week, 8 week, and 12 week time points. The data showed that participant 14 had a clinically significant slight increase in residual limb circumference size at week 4 and week 8 but showed a decrease in size over the remaining time points.

Participant 6 and 18 wore the intervention for all 12 weeks. Although they started the intensity low they slowly increased the intensity over time to be greater than baseline at week 12. These two participants experienced favorable results in various outcome measures. Participant 5 also wore the intervention all 12 weeks and showed an increase in intensity with the quadriceps and anterior tibialis muscles; data showed that her intensity level fluctuated with the gastrocnemius muscle. Participant 10 wore the intervention for 12 weeks. His data showed an

increase in intensity across time points with the quadriceps muscle, a slight fluctuation with the intensity with the anterior tibialis muscle and a steady intensity with the gastrocnemius muscle. Participant 6, 10 and 18 experienced numerous clinically significant changes during the NMES intervention period.

5.1.6 Relationships among the outcomes

We compared changes observed between various outcome measures at 8 weeks due to the larger sample size, n=7. We found three moderately strong relationships: the association between increased residual limb pain changes and velocity increases; the association between strength gains and increased velocity and the association between decreased residual limb pain changes and isometric strength gains. All these relationships occurred in the expected direction.

5.1.7 Feasibility:

The second purpose of the study was to establish the feasibility of conducting a randomized control trial with chronic TTA and an at home NMES intervention.

5.1.8 Process parameters:

5.1.8.1 Recruitment, consent and retention**:**

We found it somewhat difficult to recruit twenty participants in a timely manner. It took fifteen months to recruit twenty eligible participants. We exhausted all of our no cost option proposed resources. Additional options we could have implemented was to pay for advertising through the local newspaper, local television channel or use the Amputee Coalition of America (ACA) to advertise on their website and monthly magazine.

All of the control group participants completed all visits of the study. In the intervention group, we had two participants drop out of the study after the 8 week visit due to reasons out of our control. We had three participants stop the intervention due to clinically significant residual limb size change. These participants were followed at the three month follow up visit and performed all outcome measures at that visit.

Since we found the groups reported different pain scores at baseline, we could have controlled this better by stratification during randomization. We could have matched the participants based on their current pain level. Furthermore, we could have also looked at matching the participants on gender, age and length of the residual limb.

We could not control the events of the two intervention group participants dropping out of the study as one had an amputation of the toes on his sound side and the other participant fractured his femur on the sound side. Overall the participants were compliant with the intervention. For the participants who were not as compliant, it seems plausible that a form of

tele-rehabilitation approach which enables in-home training or monitoring of participants might present a suitable solution.

An exit questionnaire was administered to the intervention group participants on their perceived benefit from the NMES. Below is a table (Table 5) showing each question asked, the question, total score, and the participant's comments. All ten participants responded and completed the questionnaire. The response scale for each question varied from 0 (strongly disagree) to 5 (strongly agree). The results of the exit questionnaire suggested that the majority of participants perceived the NMES intervention to be useful in overall improvement of their involved limb and in regard to residual limb strength. The participants rated change in limb size as their second highest choice perceiving the NMES intervention to be useful in overall improvement of their residual limb. All participants found the NMES device was easy to use, seven participants would continue treatment at home and eight would recommend it to a fellow amputee. Six of the participants comments included that the NMES took their pain away, and one participant noticed an increase in phantom pain after not using the intervention. Four participants noticed a change in limb volume within the first 4 weeks. Two participants stated that they were going to ask their physician to write a prescription for a NMES device to use at home. One participant commented that they would recommend the NMES intervention if it can control the limb size.

The exit survey data overall suggested positive benefits of the NMES treatment however our quantitative data were not as strong in supporting our hypotheses. One reason for this could be that when pain is reduced, people feel better overall and can have an improved self-perception of physical status. This is similar to results found by Talbot et al, 2014 reporting that a clear

improvement was observed in self-perception of physical status as measured by the Medical Outcome Questionnaire (MOQ), but not in mental health.

Participants reported in our survey that they felt like they had more energy, stamina and vitality. In the future, we may want to include some outcome measures that may be more sensitive to capturing changes in these functions such as distance traveled with the two-minute walk test (2 MWT), timed up and go test (TUG), Amputee Mobility Predictor, timed sit to stand or stair climb tests. Participants were also telling us that their limbs felt firmer. Therefore, an ultrasound test may be useful for quantifying changes in quadriceps muscle mass and fatty tissue. We can also explore gait analysis with a gait lab looking at the trunk and upper body biomechanics in addition to the lower limbs.

Table 5 Exit Questionnaire.

Exit Interview Question	Group mean (SD)	Comments
1. After using the NMES intervention protocol I found it useful in overall improvement of your involved limb, in regards to **strength.**	3.00 (±1.85)	(P10)NMES built a lot of muscle up, (P18) noticed a decrease in my strength since not using it (P14) increase vitality and strength
2. After using the NMES intervention protocol I found it useful in overall improvement of your involved limb, in regards to **pain.**	2.44 (±2.03)	(P10)Frequently had pain prior to NMES, (P10)phantom pain went away for a good while after using NMES, (P18) noticed an increase in phantom pain after not using it.(P14) now rubs limb for up to 20 min post NMES (P6) took my pain away, no meds
3. After using the NMES intervention protocol I found it useful in overall improvement of your involved limb, in regards to **limb size.**	2.63 (±1.60)	(P10)believes he built up more muscle that gave him strength and could do more (P10) more strength and ale to do more, (P18) limb felt firmer, got rid of fattiness, (P14) notices limb was smaller near the bottom post intervention(P16) noticed size increase closer to knee and size decrease towards bottom of limb
4. After using the NMES intervention protocol I found it useful in overall improvement of your involved limb, in regards to **walking ability.**	2.63 (±1.77)	(P10)felt that he was walking faster and had more movement (p18) had more endurance during intervention use and was walking better compared to now, (P14) not really sure, muscles feel better when getting used
5. The health of my residual limb changed in a positive way.	2.63 (1.64)	(P14) developed a callous on limb after stopped intervention(P15) most definitely
6. The device was easy to use.	4.63 (±0.48)	
7. I would like to continue to use the NMES device at home on a continuous basis.	3.38 (±2.15)	(P10)Finding time at the same time of day is hard (p18) if used long term it could help with strength (P5) Iasked my physician for an Rx (P6) planned to ask physician for Rx(15) not sure as I saw a quick increase in limb size
8. I would recommend the NMES intervention to other amputees.	4.13 (±1.10)	(P10)Definitely (P14) feels it might help people not as mobile as him, if you don't use your muscles you will lose them(P13) if it can control the limb size and flucuations

5.1.8.2 Resource parameters:

We found that seven of the ten participants were compliant to the set protocol. We asked the participants to wear the NMES for 5 times a week in hopes that they would wear it 2-3 times per week. The group mean days wearing the intervention over the 12 week period was 4.10 to 4.37 times per week. The participant's log book and NMES data logger on the device reflect compliance. In order to ensure compliance, an in-person visit would be optimal to administer the NMES treatment.

5.1.8.3 Management parameters:

Thirty-two participants were screened through a telephone call and were given a face to face visit if they were deemed eligible. Informed consent was obtained by twenty-one participants, and the participant started the study the same day if possible. One of these participants failed to show for his initial baseline visit and was withdrawn from the study. All twenty participants who signed consent and underwent the screening procedures passed the criteria including a monofilament test and a visual contraction of the muscle while wearing the NMES device. We did our best to have the participants not share which group they were in with the blinded evaluators. Having blinded evaluators worked well, but there were a few occasions where we could not stop the participant from asking questions about the NMES intervention, batteries or electrode replacement in front of the blind evaluators. To prevent this from happening in future studies, the co-investigator could meet privately with the participant prior to the start of the testing session to answer all questions or concerns privately. Time of day the participant was tested and wore the intervention was documented in the log book. Participant use of logs books was consistent with data logger recordings.

5.1.8.4 Treatment parameters:

No adverse events were reported. The post intervention results only found a significant decrease in residual limb pain through a Mann Whitney U test and not with the primary outcome of strength. Individual case studies revealed clinically significant improvements with various outcome measures (see appendix A1).

5.1.9 Lessons learned

There were many lessons learned through this study, from randomization to equipment use, intervention use, collection of data, and controlling for attrition.

5.2 EQUIPMENT:

5.2.1 Biodex set up:

This was our first experience with testing this population of subjects with the Biodex machine. It was a lengthy process to learn how the machine operated; how to properly set up the machine to anatomically match the dynamometer to the subject's limb and torso, input subject data to create a file and learn how to read the data it produced. It was essential to document the chair height / length and seat height / angle for each subject and to zero out the rotational axis and seat axis. First, we discovered it was difficult to align the subject's residual limb on the Biodex leg section, therefore we had two custom plates fabricated at HERL mentioned previously. Once testing, we found that the subjects could have used a "warm up" period on a treadmill or walked in the hallway to get their muscles warmed for the Biodex exercises. Although we gave the participants practice time on the Biodex, it didn't seem like enough to warm up their limb. We could have revised the IRB to reflect this. We quickly learned that the extension of the knee extension piece of the Biodex was critical in producing a maximum torque readout. We found that the more we

telescoped the dynamometer arm, and or the longer the residual limb the more torque force was expected. We also were aware of proper alignment of the knee center and back rest adjustments which effected increased torque production.

In order to reduce any chance of skin abrasions on the residual limb from the Biodex straps, we had the subject wear their gel liner or we provided a gel liner during strength testing. We added an extra figure of eight strap to secure the residual limb, but a custom-made silicone backed pad with a non-stretch strap would have been ideal. Furthermore, to make the transtibial amputee subject more compatible with the knee extension attachment piece, an adjustable hard socket or posterior socket section to wear on the Biodex may provide more consistent torque measures due to the fact that the pad had too much give and the strap had too much stretch. This may decrease overall limb migration providing for more accurate results and indications (indicators) of change.

5.2.2 GAITRite® mat system

It was also our first experience working with the GAITRite® mat system. The main issues we had with this system were foot falls and turn around on the mat in the trials. We eventually eliminated the turnaround in our testing procedures. We did not find statistical significance on the three gait parameters tested however, to enhance future data collection, it would be ideal to document normal speed on a treadmill. We would then slowly increase the speed to increase natural speed and gait rhythm, then test participants on the GAITRite® mat after the treadmill exercise to see how they performed. This may allow us to see a change with a persons self-

selected walking speed and the subject's K-level may be improved with this exercise. In the future, we may want test other gait parameters, pressures on the foot and the center of pressure. We may also want to implement the Timed Up and Go (TUG) test as an outcome measure to evaluate the subject's performance.

5.2.3 Scanner measurements

We had a bit of a challenge always getting the Omega scanner to digitize the residual limb so the image appeared on the screen. We also had to be aware of the limb orientation during the scanning. We had to make sure that the knee extension was consistent, approximately 5-10 degrees, each time the residual limb was scanned. We also had to make sure the limb was in an optimal position to prevent distortion of the distal limb tissue. This is often difficult for the transtibial amputee to hold their limb in a set spot for a prolonged time. We quickly learned to position the subject in an optimal height chair for ease of scanning each subject's posterior aspect of the residual limb. In the future, we can fabricate a clear alignment piece that would not distort the limb but hold it in an optimal position or double check the knee extension measurement with a goniometer. Reflective markers need to be positioned in the same place at each visit, a photo of the residual limb would be the ideal way to document this.

5.2.4 Tape measure measurements

We had inconsistencies with the tape measure circumference measurements. Although all investigators were trained in proper measurement techniques, tension of the tape measure is a personal preference and technique as is difficult to control per individual. Results showed that the hand tape measure was pulled tighter than the measurements picked up from the scanner resulting in smaller measurements with the use of the tape measure. We had two evaluators take hand measurements to compare inter-rater reliability. If only one evaluator was present, we had the evaluator take the measurements three times to test intra-rater reliability.

5.2.5 NMES set up / wear:

One of the main goals to the study is to achieve proper compliance with intervention participants. To ensure compliance, we could have had the participant come in for a face to face visit to receive the intervention. This study was designed as an “at home” intervention to ease burden on the participants. For the most part participants were very compliant with the protocol at home. For future studies, if compliance becomes a problem, checking in on the person every other day to remind them to wear the device or setting a reminder on their phone to use the device would be options to consider.

Adherence to the time of day the person is tested needs to closely monitored. It would be ideal to have the person come in the same time of day at each visit and to wear the NMES at the

same time each day. In a future study it would be ideal to have all intervention subjects be fit with an i-fit® prosthetic socket which will allow for volume changes and to accommodate changes in the residual limb.

We mentioned earlier that compliance was measured by retrieving data from the NMES device which had a data logger storage feature. The subject was asked to bring the NMES to each visit to check the data logger, batteries and electrode pads. We compared the data from each device to the daily log book and have reported compliance with the overall results. Not all participants remembered to bring their log book and NMES device to each visit. We checked the device at the following visit and compared it to the log book for accuracy. On average all participants recorded their NMES use and intensity levels in the logbook which matched well with the NMES device readings.

We used a monofilament test to determine if the subject had loss of protected sensation (LOPS) of the limb. We tested ten areas on the limb, in which the subject had to pass the monofilament test with correctly identifying seven of the ten areas on the limb. All subjects we tested passed this inclusion criteria. This is the first step to validating this test to show that the monofilament test for a transtibial amputee can be standardized. In the future, we hope to devise a research question looking at this aspect of testing for LOPS and how it could correlate to liner / suspension selection. The next step may be to have the subject identify between a finger touch and a bristle from the monofilament test. We would like to explore if a monofilament test would give additional specificity to which liner / suspension to use, (3mm vs 6mm vs. 9mm, vs custom)

The single subject results show that Body Mass Index (BMI) may have a direct effect on how well the NMES intervention works. It is critical that BMI is monitored closely for each subject.

5.2.6 Attrition:

The study gave us a general idea of what attrition will look like for a future study. We will over recruit knowing that some will drop out. For the three participants that dropped out, revisions should have been added into the IRB and the OPERF study that state that the subject would still be followed in the study over each time point and would perform each outcome measure, although the participant would not be using the intervention from that time point through week 12.

5.3 STUDY LIMITATIONS

This study had a number of limitations. Limitations to the study involved attrition, small sample size, equipment and funding, sensitivity of the outcome measures to changes. We had two participants that were lost to follow up due to events out of our control. We withdrew another three participants for part of the study due to detecting a clinically significant change in residual limb mean circumference size. Due to attrition, our sample size decreased to five subjects finding it difficult to run the proposed ANOVA. The equipment involved in the study did not always

work properly or it was sometimes difficult to book the Pitt research room to test the subjects. We had problems with the Willow Wood scanner (calibrating properly), the Biodex (calibrating the dynamometer) and GAITRite® system mat (programming and executing due to old technology and computer) on several occasions, which delayed the testing time. We performed this study on a $5,000 budget funded by OPERF for a sample size n=12, therefore we had asked the SHRS for additional finding from the Research Development funds in order to pay for additional supplies and participant payment for the eight additional participants.

We also had limitations concerning our control group which included high variability in their demographic factors and that they had less pain than the intervention group at baseline. Also they were not given a placebo. The reason for this is that unlike in drug studies, where the difference between intervention and placebo is imperceptible, in rehabilitation science, it is often the case, as in this study, that the intervention was clearly apparent because it is large and visible. We felt that this was the best group to compare to initially (e.g. standard of living). If an amputee is not doing any additional treatment, the residual limb health is likely to decline, while NMES will maintain or improve deficits.

Our analysis is limited to look at baseline to 12-week data, although we did look at some 8 week data exploring compliance, and the effects of changes on the outcomes, more of this data could be analyzed in the future to identify trends.

We did not do intention treat because we had too much missing data and the imputation techniques would have led to results that would not be valid. The impact of missing data on quantitative research can be serious, leading to biased estimates of parameters, loss of information, decreased statistical power, increased standard errors, and weakened

generalizability of findings [180]. The missing data was a noted limitation of our research study. Jakobson et al., reported that if the proportions of missing data are very large (for example, more than 40%) on important variables, then trial results may only be considered as hypothesis generating research [181].

Not knowing the quality of the muscle is a limitation of the study. We did not have an objective measure of muscle quality in the study at baseline. Since our population was older and many years post amputation, the participants muscle is not going to respond like it would if it were a younger adult or sound limb. A study by Shrek, et al found that unilateral transtibial and transfemoral amputees experience a 93% to 117% difference between the sound and involved limb in muscle cross-sectional areas, and transtibial amputees had a between limb difference of 39% at the fat cross-sectional areas. Furthermore, thigh percent fat was significantly higher ($p<0.05$) in the amputated thigh for both populations [38].

The problem with a 'poor quality' muscle and NMES use is the ability for the muscle fibers to respond well to the electrical stimulation due to decreases in fiber size and number [182]. With aging, type II (high force) muscle fibers are more affected than type I muscle fibers. Autopsy studies reveal 25 % fewer muscle fibers in the medial vastus lateralis of older (72 years) than in younger (30 years) individuals. Moreover, biopsy studies also show a changing fiber type distribution with age where the percentage and area of type II fibers in the vastus lateralis is reduced. As fiber type distribution changes, so does the oxidative enzyme activity and muscle capillarization, which decrease. This in turn, changes the entire quality of the muscle [182]. Also, with age, the contractile characteristics of muscle fibers change. Muscle fibers transition to take on slow, type I characteristics of fatigue resistant and slow contraction speed. Muscle atrophy

has similar effects on the fiber composition as aging. A study by Helliwell et al, reported muscle fiber atrophy with ICU patients affecting both type I and type II fibers. Ninety-eight muscle biopsies were taken from 57 patients. The sequel biopsies found a mean daily decrease of 4% for type II fibers and 3% for type I fibers [183]. Absolute fiber atrophy was present in 12% of the biopsies, while 69% of the paired biopsies showed relative atrophy of the fibers in the second biopsy [183]. Atrophy was observed within the first 10 days after admission to ICU, and was present throughout the biopsy specimen, affecting either all fibers or predominantly type II fibers. The longer the person is sedentary, the decrease in fibers (increase in atrophy) occurs. The overall muscle quality, whether it be due to age or atrophy, has an effect on how well the NMES intervention will work. A possible reason that our participant 13 and 15 responded quickly to the NMES is that they were less than two years post amputation and may have had less atrophy than other subjects were further out from their amputation.

Future studies on NMES with amputees should consider including an objective measure of muscle quality, for example, DEXA or ultrasound which have been used in other studies to quantify gains in lean muscle mass [182].

Lastly, the quality of the muscle contractions could have affected our results and we were unable to ensure adequate contractions were occurring since the participants performed the intervention at home. However, the participants were very compliant and understood the protocol. After reviewing the data from intensity graphs, some participants could have increased the intensity who were already maxed at 100, since the participants did not complain about the NMES stimulation, they may have worn it to their toleration level. However, some participants

noted in the log book that they decreased the NMES intensity for that day dependent upon how their residual limb felt.

Like muscle quality, the intensity of muscle contraction should be measured if possible in future studies. One way to measure this possibly would be with the Biodex. The participant would be properly set up in the Biodex chair and we would apply the NMES to elicit a muscle contraction into full knee extension and test the isometric torque value on the Biodex. We would compare the torque values produced to other TTA in previous literature or torque produced without the NMES and solely a quadriceps muscle contraction.

5.3.1 Study Considerations

Residual limb volume is a key factor affecting socket fit and limb health. While it was an important variable to include in our study we found mixed results in response to the intervention. Although our group of participants were fairly active overall, stimulating the residual limb muscles may have changed blood flow to the limb and may have reduced edema while building muscle mass resulting in little to no change in volume. It's also possible the NMES may have increased muscle mass but reduced the transcutaneous fat layers resulting in little change in volume. It will be important in future studies to include a means to quantify edema and changes to muscle and fat mass. A study by Shrek et al, 2010 measured muscle and fat between the amputated limb and sound limb with the use of a computed tomography (CT) scan. The downfall

to this method is that a CT scan utilizes X-rays and is a stationary machine [38]. A more cost effective, radiation free and portable method is an ultrasound which uses high-frequency sound waves for imaging. Studies have shown that ultrasound has been used to measure the cross-sectional area of the quadriceps muscle successfully to determine muscle size and muscle quality [184]. Furthermore, a study by Bochkezanian et al., used an extended-field-of-view ultrasound imaging protocol to measure increases in quadriceps muscle cross-sectional area after high-intensity knee extension NMES strength training. The study found that high-intensity NMES strength training induced substantial increases ($p=0.04$) in evoked tetanic knee extensor torque and quadriceps cross-sectional area [185].

Length of time since amputation, age, and the amount of atrophy experienced are factors that affect muscle quality [186]. The greater the time since amputation the greater the amount of muscle atrophy expected to occur in the residual limb in the absence of any intervention (e.g. PT, strength training, electrical stimulation). The length of time since amputation varied widely in our study (e.g. mean average year since amputation was 7.88 (STD=7.18)) which likely translated to people having varying degrees of muscle quality. The average age of the participant was 52.8 years old (STD = 13.95). Sarcopenia is defined as the age-associated loss of skeletal muscle mass and function. The causes of sarcopenia are multifactorial and can include disuse, altered endocrine function, chronic diseases, inflammation, insulin resistance, and nutritional deficiencies [187-190]. The effects of sarcopenia may be amplified in someone with a transtibial amputation.

5.4 RECOMMENDATIONS FOR FUTURE RESEARCH

To restructure and plan a new study we need a larger sample size. Randomization for this study would need to be implemented with a stratified protocol. We may have learned more if this first pilot study on NMES had been a single cohort study and a A-B-A study design (e.g. monitor the group for a period of time first, then introduce the intervention then monitor the group again for a carryover effects). This design was proposed initially in our grant application to OPERF but reviewers asked to revise the design to include a control group. In future studies, the control group could be given a sham NMES device. In a random control trial study by Berman et al, acupuncture treatment was given to one group and the other group received a credible sham acupuncture. In addition, they added a nonpharmacological treatment (education) as a second control group. The outcome measures researched were pain, function and patient global assessment. While the participants in the true acupuncture group were more likely to correctly guess their treatment, this masking procedure was reasonably successful in blinding participants in the sham control group since most participants believed that they were receiving true acupuncture throughout the study. The study reported that differential awareness of group membership may have contributed to the positive results found. The between-group masking differences may have reflected the differential pain and function improvements due to the treatments themselves because real acupuncture was benefiting its recipients, they, in turn, assumed that they were receiving real rather than sham treatment [191]. The study also found that because of the educational control group's excessive attrition rate, coupled with the fact that its participants were not blinded to group membership, they felt that the true versus sham

acupuncture contrasts are the more valid comparisons. While pain among participants who were receiving true acupuncture decreased more than in the sham group at all of the post baseline assessments, this difference was not statistically significant. However, the true acupuncture group's improvement in function from baseline was significantly greater than that of the sham control group with almost 40% improvement from baseline [191]. Perhaps in our future work we could look at adding volitional exercise or a NMES sham to the control group role.

Prior to the study, we anticipated that the proposed NMES therapy would increase muscle mass but were unable to measure the changes in the muscles directly. In order to measure the muscle mass, we would need to use a DEXA, ultrasound or computed tomography (CT) scan, that offers cross sectional images, to determine if we see muscle or edema / fluid in the residual limb and thigh. This will tell us the quality of the muscle tissue and measure the muscle size and density. We would then assess changes in muscle mass in response to the dosage of NMES.

There are many factors that should be examined in future work such as NMES dosage, reliability of Biodex testing with or without the prosthesis, a more efficient way to measure the limb with an upgraded laser technology, the use of a gait lab for gait analysis and a pain survey that focused on better defining residual limb and phantom pain. Because we lost subjects to changes in the residual limb we would want to incorporate a comfortable adjustable fitting socket into the protocol to be worn instead of temporarily replacing their current socket to see if we could retain more subjects in study.

To recruit a large enough sample size to determine statistical significance and to control for compliance better in future related studies, the study could be carried out in a setting where amputees may frequent. Life Pittsburgh is a local facility that offers all-inclusive treatments and

resources for people over 55 years old whose health issues interfere with their daily lives. Life Pittsburgh offers many health options such as medical care, physical therapy treatment, occupational therapy treatment and social needs therefore making it an optimal place to recruit or perform study duties.

Although our study looked at NMES as an at home treatment by itself, adding exercise to the NMES treatment may have compounding benefits. Takano et al in 2010 [192] found that Hybrid training (HYBT) method utilizing combined electrical stimulation and voluntary muscle contraction caused significant changes in muscle torque and cross-sectional area concerning knee extensors for elderly people. Furthermore, Wigerstad-Lossing et al [193], found that electrical stimulation in combination with volitional exercise can limit muscle weakness and muscle wasting after knee ligament surgery. Piva et al, found that patients who completed the NMES treatment and volitional exercise program increased their lean muscle mass, muscle strength and physical function [13].

It would also be interesting to explore the relationship between subject sociodemographic data and changes in outcome measures such as years since amputation, gender, length of the limb, and age of the participant and body mass index.

We would also want to consider in redesigning the study to modify the IRB to allow money to be paid for follow up interviews/visits if the subject had dropped out of the study. This will allow the investigator to gather additional data and clarify information from the participant about their personal experiences with the intervention

5.5 CONCLUSION

This study was inconclusive for determining the benefit of NMES with TTAs. Although we found that the NMES intervention decreased residual limb pain it's unclear if NMES should be recommended to a TTA for the shear purpose of reducing pain when there are other electrical stimulation protocols (e.g. TENS) that have produced similar results among amputees. However, with a refined protocol and larger cohort of TTAs it's possible that NMES may demonstrate more conclusive results as the participants perceived greater benefit from the NMES with other outcomes than what the data showed.

The results of this study demonstrated feasibility of at home NMES use for unilateral transtibial amputees. The information gained through this study has profound merit for academicians as well as rehabilitation science researchers. This knowledge is extremely useful in identifying effective treatment strategies. The development of a novel home-based training program may lead to a new low-cost treatment that assists individuals with amputation to maintain residual limb volume, improve strength, improve gait parameters and manage chronic and phantom pain.

APPENDIX A

APPENDIX A1:

Individual case study

Participant 4

Participant 5

Participant 6

Participant 10

Participant 12

Participant 13

Participant 14

Participant 15

Participant 16

Participant 18

Individual case studies

Ten participants were tested in the intervention group. A summary for each individual on a case by case basis with demographic information and compliance over time are listed below. The yellow shaded boxes show a clinically significant difference for the listed outcome at a specific time point.

Subject 4:

Gender: Male
Ethnicity: Caucasian
Age: 54
Weight: 203 Lbs.
Height: 6'2"
BMI: 27.5
Cause of amputation: complications due to diabetes
Side of amputation: right
Years since amputation: 11.2
Length of limb: 15 cm

Table 6 Compliance percentage over time subject 4

Subject 4		0-4 weeks	4-8 weeks	8-12 weeks
Data Logger	Days per week	7	4.5	NA
	Number of sessions	81	54	NA
	Percentage compliant	135%	90%	NA

Compliance: During the first four weeks of treatment, the subject wore the NMES treatment seven times per week with 135% compliance rate; and at his eight week visit he wore the NMES intervention on average four and a half times a week with a 90% compliance rate.

Strength: At the subject's four-week visit, the subject's isometric strength increased from baseline over 40%, and his isokinetic strength remained close to the same as the baseline torque. At the eight-week visit, the subject had an increase of 19.65% of isometric strength from baseline to 8 weeks and also shown an increase in isokinetic strength of 19.93% from baseline to eight-week visit.

Limb size: At the subject's four-week visit, the subject's limb volume shown a slight decrease in size, however it was not significant enough to warrant a new prosthetic socket or discontinuation of the NMES treatment. We discussed sock management with the subject and he agreed to add socks as needed. At the eight-week visit, we saw a clinically significant change of the residual limb volume and advised the subject to stop the intervention and followed him for twelve weeks to the final time point. Upon evaluation of his skin, it appears that he was developing an ulcer on the anterior patella tendon area of the residual limb. He complained of pain residual limb pain as well. We listened to the patient and reviewed on the scanner measurements. We saw a greater than 5% decrease in volume on the eight-week visit.

Pain: At the week four visit, the subject reported that his residual limb pain decreased by 57% although his phantom sensation increased by 68%, we feel this was due to the decreased changes of the limb volume. At the eight-week visit, the subject's pain level remained continuous with a decrease of 57 % in residual limb pain and a decrease of 50% from baseline

for phantom limb pain. The subject's phantom sensation improved slightly from week four, reporting a 26.32% increase from baseline to week eight.

Gait: At baseline, the subject's velocity was almost normal compared to the average velocity of transtibial amputees. Therefore, we did not see a clinically significant increase in velocity for either week four or week eight but did see a small increase of 7.84% and 4.9% respectively which increased the subject's velocity to the normal walking speed for found in literature for transtibial amputees. We also saw a decrease in percentage of stance on the amputated side by 22%, this may be because of the increase he reported with phantom pain.

At the eight-week visit, we found clinical significance with the subject's strength, residual limb and phantom limb pain and limb size. Besides noting the clinically significant decrease change in size at the week eight visit, we decided to discontinue the intervention at week eight as he was having pistoning problems in his prosthetic socket which caused the beginning of an abrasion on his residual limb. Prior to his twelve-week visit, the subject called and said that he had fractured his femur on the sound side. We planned to follow up with him for the final visit, but he was unable to weight bear to complete the final visit in the allotted time frame.

Table 7 Participant 4 data

Participant 4

Outcome Measure	*Baseline (0 weeks)*	*0-4 weeks*	*Percentage change*	*0-8 weeks*	*Percentage change*	*0-12 weeks*	*Percentage change*	*12 week-24 week*	*Percentage change 12 to 24 weeks*	Percentage change 24 week compared to baseline
Isometric knee extension strength 60°/sec (Nm/Kg)	16.64	23.31	40.08%	19.91	19.65%	NA	NA	NA	NA	NA
Isokinetic knee extension strength 60°/sec (Nm/Kg)	16.81	16.98	1.01%	20.16	19.93%	NA	NA	NA	NA	NA
Residial Limb Pain (0-10 scale)	7	3	-57.14%	3	-57.14%	NA	NA	NA	NA	NA
Phantom Limb Pain (0-10 scale)	4	3	-25.00%	2	-50.00%	NA	NA	NA	NA	NA
Phantom Sensation (0-72 scale)	19	32	68.42%	24	26.32%	NA	NA	NA	NA	NA
Chronic Pain Grade Scale (RLP)	1	1	0.00%	1	0.00%	NA	NA	NA	NA	NA
Chronic Pain Grade Scale (PLP)	1	1	0.00%	1	0.00%	NA	NA	NA	NA	NA
Velocity (m/s)	1.02	1.1	7.84%	1.07	4.90%	NA	NA	NA	NA	NA
Percentage in stance on amputated side	57.8	45.07	-22.02%	50.5	-12.63%	NA	NA	NA	NA	NA
Step length (m) on amputated side	0.46	0.9	95.65%	0.7	52.17%	NA	NA	NA	NA	NA
Residual limb circumference at tibial tubercle(TT) (cm) on amputated side	36.04	35.9	-0.39%	34.6	-4.00%	NA	NA	NA	NA	NA
Residual limb 1" below TT (cm)	35.24	34.7	-1.53%	33.2	-5.79%	NA	NA	NA	NA	NA
Residual limb2" below TT (cm)	34.93	33.3	-4.67%	31	-11.25%	NA	NA	NA	NA	NA
Residual limb 3" below TT (cm)	33.02	31.3	-5.21%	27.2	-17.63%	NA	NA	NA	NA	NA
Residual limb mean circumference (cm)	34.81	33.8	-2.90%	31.5	-9.51%	NA	NA	NA	NA	NA
Volume (cm^3)	1024.19	989.78	-3.36%	971.75	-5.12%	NA	NA	NA	NA	NA
Intensity of NMES Quadriceps Muscle	62	74	19.35%	70	12.90%	NA	NA	NA	NA	NA
Intensity of NMES Anterior Tibialis Muscle	82	87	6.10%	76	-7.32%	NA	NA	NA	NA	NA
Intensity of NMES Gastrocnemius Muscle	76	80	5.26%	76	0.00%	NA	NA	NA	NA	NA
Number of Sessions / Compliance %		81 / 135%		54 /90%		NA				

Subject 5:

Gender: Female
Ethnicity: African American
Age: 42
Weight: 249 Lbs.
Height: 5'7"
BMI: 41.1
Cause of amputation: Trauma
Side of amputation: Right
Years since amputation: 26.2
Length of limb: 15.9 cm

Table 8 Compliance percentage over time subject 5

Subject 5		0-4 weeks	4-8 weeks	8-12 weeks
Data Logger	Days per week	1.5	.83	1
	Number of sessions	15	10	12
	Percentage compliant	25%	16.67%	20%

Compliance: During the first four weeks of NMES intervention, the subject wore the NMES treatment on average one and a half times a week with a 25% compliance rate; and at the eight-week visit she wore the NMES intervention an average of .83 times out of the week with 16.67% compliance rate. At the twelve-week visit the subject wore the NMES intervention one day out of the week with a 20% compliance rate. The subject reports that she was sick with the

flu for most of the time between the eight weeks and twelve-week timeframe, so she only wore the intervention four times over the four-week time frame.

Strength: During the four-week visit, the subject's isometric strength increased 17.25% from baseline. The subject's isokinetic strength increased 30.24%. At the eight-week visit, the subject's isometric strength increased 28.57% from baseline and the subject's isokinetic strength increased 40.35% from baseline. At the twelve-week visit, the subject's isometric strength decreased 27.47% from baseline and the subject's isokinetic strength increased 13.76% from baseline. At the final visit, the subject's isometric strength increased 102.73% from week twelve and 47% from the baseline visit. The subject's isokinetic strength increased 48.91% at the week twelve visit. She only wore the NMES treatment one-time a week during the eight weeks and twelve week time points.

Limb size: Clinically significant changes were seen at the eight-week visit. The subject's limb decreased in size and she reported that she had to add two or three-ply socks throughout the rest of the study. We saw a 6.55% decrease in circumference three inches distal to the tibial tubercle using the hand scanner. At the twelve-week visit, we saw a clinically significant decrease of 8.74% in size at the tibial tubercle. During the final visit the measurements reported to be very similar to her baseline measurements, concluding that her residual limb returned to its baseline size.

Pain: The subjects residual limb pain decreased overtime showing a clinical significance at week eight and again at the final visit. The subject's phantom limb pain increased over the time she wore the intervention then decreased from week twelve to the final visit. The subject's phantom limb sensation showed a clinical significant decreased of 62.5% at week eight and

remained decreased though the final visit with a decrease of 64.29% from week 12 to the final visit. The subject's chronic grade score for residual limb pain decreased over 66%.

Gait: We did not see a clinically significant change in the gait parameters until week twelve. The subject's velocity increased 26.56% from baseline to week twelve. At the final visit her velocity reverted back to levels close to baseline through the eight-week visit.

Although this subject did not wear the intervention as directed throughout the study, she reported that she felt that the intervention helped her and was going to ask her Physical Medicine and Rehabilitation (PM&R) physician for a prescription for a NMES device. It appears that her strength may have plateaued between 8-12 weeks but was the highest at the final visit.

Table 9 Participant 5 data

Participant 5

Outcome Measure	*Baseline (0 weeks)*	*0-4 weeks*	*Percentage change*	*0-8 weeks*	*Percentage change*	*0-12 weeks*	*Percentage change*	*12 week-24 week*	*Percentage change 12 to 24 weeks*	Percentage change 24 week compared to baseline
Isometric knee extension strength 60°/sec (Nm/Kg)	9.1	10.67	17.25%	11.7	28.57%	6.6	-27.47%	13.38	102.73%	47.03%
Isokinetic knee extension strength 60°/sec (Nm/Kg)	8.5	11.07	30.24%	11.93	40.35%	9.67	13.76%	14.4	48.91%	69.41%
Residial Limb Pain (0-10 scale)	8	6	-25.00%	2	-75.00%	7	-12.50%	5	-28.57%	-37.50%
Phantom Limb Pain (0-10 scale)	3	6	100.00%	6	100.00%	14	366.67%	5	-64.29%	66.67%
Phantom Sensation (0-72 scale)	16	18	12.50%	6	-62.50%	14	-12.50%	5	-64.29%	-68.75%
Chronic Pain Grade Scale (RLP)	3	3	0.00%	1	-66.67%	1	-66.67%	1	0.00%	-66.67%
Chronic Pain Grade Scale (PLP)	1	2	100.00%	1	0.00%	1	0.00%	1	0.00%	0.00%
Velocity (m/s)	0.64	0.7	9.37%	0.67	4.69%	0.81	26.56%	0.68	-16.05%	6.25%
Percentage in stance on amputated side	48.56	49.5	1.94%	46.7	-3.83%	49.5	1.94%	47.2	-4.65%	-2.80%
Step length (m) on amputated side	0.73	0.69	-5.48%	0.77	5.48%	0.71	-2.74%	0.71	0.00%	-2.74%
Residual limb circumference at tibial tubercle(TT) (cm) on amputated side	49.2	48.9	-0.61%	47.8	-2.85%	44.9	-8.74%	49.6	10.47%	0.81%
Residual limb 1" below TT (cm)	43.74	43.3	-1.01%	44.2	1.05%	44	0.59%	43	-2.27%	-1.69%
Residual limb2" below TT (cm)	43.2	42.4	-1.85%	41.9	-3.01%	42.6	-1.39%	43	0.94%	-0.46%
Residual limb 3" below TT (cm)	41.2	40.1	-2.67%	38.5	-6.55%	41	-0.49%	41.9	2.20%	1.70%
Residual limb mean circumference (cm)	44.34	43.68	-1.49%	43.1	-2.80%	43.13	-2.73%	47.63	10.43%	7.42%
Volume (cm^3)	113.9	109.2	-4.13%	118	3.60%	114.6	0.61%	111	-3.14%	-2.55%
Intensity of NMES Quadriceps Muscle	69	68	-1.45%	68	-1.45%	75	8.70%	NA	NA	NA
Intensity of NMES Anterior Tibialis Muscle	60	57	-5.00%	66	10.00%	79	31.67%	NA	NA	NA
Intensity of NMES Gastrocnemius Muscle	100	87	-13.00%	89	-11.00%	100	0.00%	NA	NA	NA
Number of Sessions / Compliance %		15 /25%		10 / 16.67%		12 / 20%				

Subject 6:

Gender: Female
Ethnicity: Caucasian
Age: 60
Weight: 136 lbs.
Height: 5'1"
BMI: 27.1
Cause of amputation: Trauma
Side of amputation: left
Years since amputation: 11.3
Length of limb: 17 cm

Table 10 Compliance percentage over time subject 6

Subject 6		0-4 weeks	4-8 weeks	8-12 weeks
Data Logger	Days per week	5.25	6.5	6.25
	Number of sessions	63	78	75
	Percentage compliant	105%	130%	125%

Compliance: During the first four weeks of NMES intervention, the subject wore the NMES treatment for 5.25 times a week with a 105% compliance rate; and at the eight week visit she wore the NMES intervention 6.5 times out of the week with a 130% compliance rate. During the twelve week visit the subject wore the NMES intervention 6.25 times a week with a 125% compliance rate.

Strength: We did not see an increase in strength until the subject's eight-week visit. At the eight-week visit, the subject's isometric strength increased 36.15% from baseline visit. The

subject's isokinetic strength increased 9.65%. At the week twelve visit, the subject's isometric strength increased 15.29% from baseline visit and the subject's isokinetic strength decreased 12.75% from baseline visit. At the final visit, the subject's isometric strength increased 24.72% from week twelve and the subject's isokinetic strength decreased 5.48% from week twelve. It appears that her strength plateaued between 8-12 weeks but was the highest at the 8 week visit.

Limb size: At the week four visit, the scanner measurements revealed one area, three inches below the tibial tubercle showed clinically significant changes of 4.88%. This subject has a longer residual limb then the other subjects and the three-inch area was the area of her gastrocnemius muscle which showed an increase in size. The subject reported that her socket felt a little tight but not bothersome. At the next two visits, we saw an increase in size or a trend towards increase with a clinically significant change in all scanner measurements. We asked the subject to discontinue the NMES but she was so pleased with the results and did not need to take her pain medication that she continued to use the NMES against our recommendation. At the final visit, the residual limb decreased in size slightly less than the week twelve measurements. The subject had no complaints of the socket fitting loose or reported that she had to wear additional sock ply. We also saw an increase in her residual limb volume at week eight of 3.22%.

Pain: The subjects residual limb pain decreased overtime showing a clinically significance at each time point. The subject's phantom limb pain decreased over the time she wore the intervention then increased from week twelve to the final visit. The subject's phantom limb sensation showed a clinical significant decrease at week twelve and remained decreased though the final visit.

Gait: We saw a clinically significant change in velocity which carried over to the final visit. The subject was already close to normal percentage in stance on the amputated side at the baseline visit at 58% but improved 20.69% to 70% at week eight. The subject's step length increased 90% at week 4 and remained stable at an 83.33% increase throughout the study.

This subject was very compliant with the intervention and daily log book. She reports that she did not have to take her pain medication after the four week visit and she discontinued Neurontin at night after the eight week visit. She reported at the final visit that she only took ibuprofen as needed. She reports several days of wearing her prosthesis for over 10 hours a day. She went to Disney with her grandchildren and reported a blister, she continued the use of the NMES and the blister was healed in seven days. She reported that she still walked on her prosthesis during the day. Subject reported at about week eleven she was having difficulty fitting into prosthesis. The subject eventually had a new socket fabricated due to the increase in volume from the NMES. She reports that she wants her PMR physician to write her a prescription for a NMES device for home use.

Table 11 Participant 6 data

Participant 6

Outcome Measure	*Baseline (0 weeks)*	*0-4 weeks*	*Percentage change*	*0-8 weeks*	*Percentage change*	*0-12 weeks*	*Percentage change*	*12 week-24 week*	Percentage change 12 to 24 weeks	Percentage change 24 week compared to baseline
Isometric knee extension strength 60°/sec (Nm/Kg)	10.07	9.7	-3.67%	13.71	36.15%	11.61	15.29%	14.48	24.72%	43.79%
Isokinetic knee extension strength 60°/sec (Nm/Kg)	10.67	8.77	-17.81%	11.7	9.65%	9.31	-12.75%	8.8	-5.48%	-17.53%
Residial Limb Pain (0-10 scale)	6	4	-33.33%	4	-33.33%	4	-33.33%	3	-25.00%	-50.00%
Phantom Limb Pain (0-10 scale)	6	3	-50.00%	2	-66.67%	1	-83.33%	3	200.00%	-50.00%
Phantom Sensation (0-72 scale)	24	24	0.00%	36	50.00%	4	-83.33%	2	-50.00%	-91.67%
Chronic Pain Grade Scale (RLP)	1	1	0.00%	1	0.00%	1	0.00%	1	0.00%	0.00%
Chronic Pain Grade Scale (PLP)	2	1	-50.00%	1	-50.00%	1	-50.00%	1	0.00%	-50.00%
Velocity (m/s)	0.61	0.94	54.10%	0.85	39.34%	0.96	57.38%	1	4.17%	63.93%
Percentage in stance on amputated side	58	57.1	-1.55%	70	20.69%	58	0.00%	57.13	-1.50%	-1.50%
Step length (m) on amputated side	0.3	0.57	90.00%	0.55	83.33%	0.55	83.33%	0.59	7.27%	96.67%
Residual limb circumference at tibial tubercle(TT) (cm) on amputated side	26.2	26.8	2.29%	27.8	6.11%	27.4	4.58%	27.2	-0.73%	3.82%
Residual limb 1" below TT (cm)	26.3	26.8	1.90%	26.8	1.90%	27.8	5.70%	27.1	-2.52%	3.04%
Residual limb2" below TT (cm)	25.4	25.9	1.97%	26.8	5.51%	26.9	5.91%	26.9	0.00%	5.91%
Residual limb 3" below TT (cm)	23.17	24.3	4.88%	25.4	9.62%	26.5	14.37%	25.3	-4.53%	9.19%
Residual limb mean circumference (cm)	25.27	25.95	2.69%	26.7	5.66%	27.15	7.44%	26.63	-1.92%	5.38%
Volume (cm^3)	712.84	665.31	-6.67%	735.78	3.22%	712.84	0.00%	658.76	-7.59%	-7.59%
Intensity of NMES Quadriceps Muscle	35.00	50.00	42.86%	54.00	54.29%	54.00	54.29%	NA	NA	NA
Intensity of NMES Anterior Tibialis Muscle	35.00	42.00	20.00%	45.00	28.57%	42.00	20.00%	NA	NA	NA
Intensity of NMES Gastrocnemius Muscle	36.00	44.00	22.22%	44.00	22.22%	43.00	19.44%	NA	NA	NA
Number of Sessions / Compliance %		63 /105%		78 /130%	75 /125%					

Subject 10:

Gender: Male
Ethnicity: Caucasian
Age: 65
Weight: 192 lbs.
Height: 5’8”
BMI: 30.8
Cause of amputation: Diabetes
Side of amputation: Right
Years since amputation: 2.33
Length of limb: 13.5 cm

Table 12 Compliance percentage over time subject 10

Subject 10		0-4 weeks	4-8 weeks	8-12 weeks
Data Logger	Days per week	6	6	5.25
	Number of sessions	72	72	63
	Percentage compliant	120%	120%	105%

Compliance: During the first 4 weeks of treatment, the subject wore the NMES treatment an average of 6 times per week with a 120% compliance rate; and during the eight week visit he wore the NMES intervention on average 6 days a week with a 120% compliance rate, and on the twelve week visit the subject wore the NMES on average 5.25 times a week with a 105% compliance rate.

Strength: We did not see an increase in strength until the subject's eight-week visit. At the eight-week visit, the subject's isometric strength increased 35.66% from the baseline visit and the subject's isokinetic strength increased 8.05%. At the week twelve visit, the subject's isometric strength increased 19.12% from the baseline visit and the subject's isokinetic strength decreased 16.48% from the baseline visit. At the final visit, the subject's isometric strength decreased 3.51% from week twelve and the subject's isokinetic strength decreased 32.11% from week twelve. It appears that his strength plateaued between 8-12 weeks.

Limb size: Subject seen at baseline visit, initial measurements were taken and states that he wears a three-ply sock fit with his gel liner. At the week four visit, the scanner measurements revealed a decrease in size at all four circumferences, three of the measurements showed a clinically significant reduction between 3% - 5.83%. Subject reports that he adds two or three ply of sock throughout the day as needed. Since the prosthesis was still fitting adequately, we decided to monitor the subject's limb through to the week eight visit. At the week eight visit we still saw a decrease in size but not as significant as it appeared the limb was gaining size. This continued to week twelve where we saw a clinically significant (8.73%) increase in size compared to baseline at the tibial tubercle level. At the follow up three-month visit, the residual limb decreased in size to where the subject reported wearing a five ply to nine ply sock at the end of the day. The subject's volume decreased by 6.55% and remained decreasing through the 24 week visit.

Pain: The subject's residual limb pain decreased over both four weeks and eight-week time points. At the twelve-week time point, the subject reported the same degree of pain as baseline. At the three-month visit, with no intervention, the subject reported a 25% increase in

residual limb pain from week twelve. The subject reported a slight fluctuation of phantom limb pain over the time points, however at week twelve the subject reported a 77% decrease in phantom limb pain from baseline. At the final visit, the subject reported phantom limb pain increased from week twelve, however, from baseline it remained clinically significantly lower. The subject reported phantom sensation increased over all time points, the highest being an 88% increase at week eight during the intervention.

Gait: The subject's baseline velocity was close to normal for the transtibial amputation population. Over the four weeks and eight-week time points the subject's velocity increased with a clinically significant change of over 25%. His velocity plateaued at week four, then decreased close to baseline values at week twelve and the final visit. The subject's percentage in stance on the amputated side was close to normal at baseline at 57.93% and did not clinically significantly increase but decreased 12.53% at week twelve. This may correlate with the increase phantom limb sensation which was reported as a 44% increase from baseline. We saw a clinically significant increase in the subject's step length over the four weeks and eight-week time points. At the final visit his step length decreased close to the baseline visit measure.

Table 13 Participant 10 data

Participant 10

Outcome measure	Baseline (0 weeks)	0-4 weeks	*Percentage change*	0-8 weeks	*Percentage change*	0-12 weeks	Percentage change	12 week-24 week	Percentage change 12 to 24 weeks	Percentage change 24 week compared to baseline
Isometric knee extension strength 60°/sec (Nm/Kg)	12.45	11.99	-3.69%	16.89	35.66%	14.83	19.12%	14.31	-3.51%	14.94%
Isokinetic knee extension strength 60°/sec (Nm/Kg)	13.29	12.48	-6.09%	14.36	8.05%	15.48	16.48%	10.51	-32.11%	-20.92%
Residial Limb Pain (0-10 scale)	8	7	-12.50%	4	-50.00%	8	0.00%	10	25.00%	25.00%
Phantom Limb Pain (0-10 scale)	9	10	11.11%	4	-55.56%	2	-77.78%	5	150.00%	-44.44%
Phantom Sensation (0-72 scale)	25	35	40.00%	47	88.00%	36	44.00%	53	47.22%	112.00%
Chronic Pain Grade Scale (RLP)	2	2	0.00%	1	-50.00%	2	0.00%	2	0.00%	0.00%
Chronic Pain Grade Scale (PLP)	2	2	0.00%	1	-50.00%	1	-50.00%	1	0.00%	-50.00%
Velocity (m/s)	1.08	1.38	27.78%	1.35	25.00%	1.05	-2.78%	1.05	0.00%	-2.78%
Percentage in stance on amputated side	57.93	56.6	-2.30%	59.9	3.40%	50.67	-12.53%	60.9	20.19%	5.13%
Step length (m) on amputated side	0.59	0.68	15.25%	0.81	37.29%	0.6	1.69%	0.58	-3.33%	-1.69%
Residual limb circumference at tibial tubercle(TT) (cm) on amputated side	33.2	32.2	-3.01%	32.6	-1.81%	36.1	8.73%	32.7	-9.42%	-1.51%
Residual limb 1" below TT (cm)	34.3	32.3	-5.83%	32.6	-4.96%	34.6	0.87%	32.2	-6.94%	-6.12%
Residual limb2" below TT (cm)	33	32.5	-1.52%	33	0.00%	32.4	-1.82%	32.4	0.00%	-1.82%
Residual limb 3" below TT (cm)	33.4	32.3	-3.29%	32.1	-3.89%	32.4	-2.99%	31.3	-3.40%	-6.29%
Residual limb mean circumference (cm)	33.48	32.33	-3.43%	32.58	-2.69%	33.88	1.19%	32.15	-5.11%	-3.97%
Volume (cm^3)	775.11	724.31	-6.55%	740.70	-4.44%	758.72	-2.11%	748.89	-1.30%	-3.38%
Intensity of NMES Quadriceps Muscle	100.00	96.00	-4.00%	100.00	0.00%	100.00	0.00%	NA	NA	NA
Intensity of NMES Anterior Tibialis Muscle	100.00	95.00	-5.00%	100.00	0.00%	100.00	0.00%	NA	NA	NA
Intensity of NMES Gastrocnemius Muscle	85.00	86.00	1.18%	85.00	0.00%	85.00	0.00%	NA	NA	NA
Number of Sessions / Compliance %		72 / 120%		72 / 120%	63/ 105%					

Subject 12:

Gender: Male
Ethnicity: Caucasian
Age: 46
Weight: 250 lbs.
Height: 6’4”
BMI: 32.1
Cause of amputation: Trauma
Side of amputation: Right
Years since amputation: 1.08
Length of limb: 14 cm

Table 14 Compliance percentage over time subject 12

Subject 12		0-4 weeks	4-8 weeks	8-12 weeks
Data Logger	Days per week	1.25	2.25	NA
	Number of sessions	15	27	NA
	Percentage compliant	25%	45%	NA

Compliance: During the first 4 weeks of treatment, the subject wore the NMES treatment 1.25 times per week with a 25% compliance rate; and during the eight-week visit he wore the NMES intervention 2.25 times a week with a 45% compliance rate. The subject was dropped from the study due to amputation of the forefoot on the sound side in early May. He did not return for the twelve week or final three month visit. It was apparent that the condition of his sound limb affected his overall strength, pain, gait and NMES device compliance. We followed

up with patient over the phone to get clarity on compliance and prosthetic socket fit. Subject reports that he also has type 2 diabetes.

Strength: Over the course of the last two visits, the subject's isometric strength was clinically significantly decreased by 49.46%. The subject's isokinetic strength was clinically significantly decreased by 46.18%.

Limb size: The scanner measurements showed that the subject's volume did not show a clinically significant volume change until week eight at the three inch below TT level. We saw a 3.51% size decrease at the 3" level. However, results show that his volume decreased 3% at week4. We feel that the leg position while scanning (may have had too much flexion at the knee) may have distorted the measurements.

Pain: His residual limb pain increased slightly over the time points, 16.67%, however his phantom limb pain decreased 30% over the two time points (week 4 and week 8). The subject's phantom sensation increased slightly at week eight; 5.56%.

Gait: The subject's velocity increased at the week four visit by 12.70% however, at the eight week visit the velocity decreased 52.38% from the baseline visit. This was most likely due to the problems with the toes on the sound side. The subject's percentage in stance on the amputated side was above average for an amputee at baseline and increased 43.93% at week eight, we believe this is due to the problems with the sound foot. The subject's step length on the amputated side clinically significantly increased at week four by 14.29%. However, at week eight the subject's step length on the amputated side decreased 38.78% from baseline. These are below than normal values for a TTA.

Table 15 Participant 12 data

Participant 12

Outcome Measure	*Baseline (0 weeks)*	*0-4 weeks*	*Percentage change*	*0-8 weeks*	*Percentage change*	*0-12 weeks*	*Percentage change*	*12 week-24 week*	*Percentage change 12 to 24 weeks*	Percentage change 24 week compared to baseline
Isometric knee extension strength 60°/sec (Nm/Kg)	15.71	10.87	-30.81%	7.94	-49.46%	NA	NA	NA	NA	NA
Isokinetic knee extension strength 60°/sec (Nm/Kg)	15.05	13.1	-12.96%	8.1	-46.18%	NA	NA	NA	NA	NA
Residial Limb Pain (0-10 scale)	6	7	16.67%	7	16.67%	NA	NA	NA	NA	NA
Phantom Limb Pain (0-10 scale)	10	7	-30.00%	7	-30.00%	NA	NA	NA	NA	NA
Phantom Sensation (0-72 scale)	18	18	0.00%	19	5.56%	NA	NA	NA	NA	NA
Chronic Pain Grade Scale (RLP)	4	4	0.00%	4	0.00%	NA	NA	NA	NA	NA
Chronic Pain Grade Scale (PLP)	4	4	0.00%	4	0.00%	NA	NA	NA	NA	NA
Velocity (m/s)	0.63	0.71	12.70%	0.3	-52.38%	NA	NA	NA	NA	NA
Percentage in stance on amputated side	47.08	42.39	-9.96%	67.76	43.93%	NA	NA	NA	NA	NA
Step length (m) on amputated side	0.49	0.56	14.29%	0.3	-38.78%	NA	NA	NA	NA	NA
Residual limb circumference at tibial tubercle(TT) (cm) on amputated side	41	40.4	-1.46%	39.8	-2.93%	NA	NA	NA	NA	NA
Residual limb 1" below TT (cm)	39.5	39	-1.27%	39	-1.27%	NA	NA	NA	NA	NA
Residual limb2" below TT (cm)	39.1	38.7	-1.02%	40.1	2.56%	NA	NA	NA	NA	NA
Residual limb 3" below TT (cm)	39.9	39.1	-2.01%	38.5	-3.51%	NA	NA	NA	NA	NA
Residual limb mean circumference (cm)	39.88	39.3	-1.45%	39.35	-1.33%	NA	NA	NA	NA	NA
Volume (cm^3)	1391.26	1433.87	3.06%	1397.82	0.47%	NA	NA	NA	NA	NA
Intensity of NMES Quadriceps Muscle	54.00	53.00	-1.85%	55.00	1.85%	NA	NA	NA	NA	NA
Intensity of NMES Anterior Tibialis Muscle	81.00	94.00	16.05%	100.00	23.46%	NA	NA	NA	NA	NA
Intensity of NMES Gastrocnemius Muscle	54.00	51.00	-5.56%	53.00	-1.85%	NA	NA	NA	NA	NA
Number of Sessions / Compliance %		15 / 25%		27 / 45%		NA				

Subject 13:

Gender: Male
Ethnicity: Caucasian
Age: 20
Weight: 245 lbs.
Height: 6'1"
BMI: 34.1
Cause of amputation: Infection
Side of amputation: Left
Years since amputation: 3.5
Length of limb: 15.5 cm

Table 16 Compliance percentage over time subject 13

Subject 13		0-4 weeks	4-8 weeks	8-12 weeks
Data Logger	Days per week	4.83	NA	NA
	Number of sessions	58	NA	NA
	Percentage compliant	97%	NA	NA

Compliance: During the first four weeks of treatment, the subject wore the NMES treatment on average 4.83 times per week with a 97% compliance rate. We saw a clinically significant difference in limb size, withdrew the intervention at week four, monitored the subject's progress and asked him to return in three months for the final follow up visit.

Strength: The subject's baseline strength was the highest out of all intervention group subjects. At the week four visit we saw a clinically significant decrease in strength of 30.50%

from the baseline visit. At week four we also saw a decrease in isokinetic strength of 23.78% from the baseline visit. This could the result of his residual limb pain level increasing 50% at the four week visit from baseline. The subject was followed at a three-month time interval from the four week visit due to a change in limb size and subject complaints of pain on the residual limb due to socket fit. At the three-month visit, the subject strength's was less than the baseline strength.

Limb size: We saw a clinically significant change at week four from baseline in three out of four scanner measurements. Since we detected the change in size, we discontinued the intervention and followed the subject at the three-month time point where is maintained close to the same size as week four in which we saw a carryover effect. The subject had a clinically significant change in volume increasing 3.4% at week 24 from baseline.

Pain: The subject's residual limb pain increased 50% at the week four visit. The subject complained of pain on the back of the residual limb. He also complained of pimples / bumps on the posterior aspect of his limb near the edge of the socket backwall. He said it was not from the NMES pads but a result of the prosthetic socket becoming too small.

Gait: The subject's velocity remained constant during the week four visit. His velocity was higher than the average for a transtibial amputee and close to average for an able- bodied person. His percentage in stance decreased 18.32% which is understandable as we saw a clinically significant increase in his residual limb pain. His step length on the amputated side increased slightly but was not found to be clinically significant.

Table 17 Participant 13 data

Participant 13

Outcome Measure	Baseline (0 weeks)	0-4 weeks	*Percentage change*	0-8 weeks	*Percentage change*	0-12 weeks	Percentage change	12 week-24 week	Percentage change 12 to 24 weeks	Percentage change 24 week compared to baseline
Isometric knee extension strength 60°/sec (Nm/Kg)	26.13	18.16	-30.50%	NA	NA	NA	NA	12.32	NA	-52.85%
Isokinetic knee extension strength 60°/sec (Nm/Kg)	22.37	17.05	-23.78%	NA	NA	NA	NA	10.44	NA	-53.33%
Residial Limb Pain (0-10 scale)	4	6	50.00%	NA	NA	NA	NA	4	NA	0.00%
Phantom Limb Pain (0-10 scale)	3	3	0.00%	NA	NA	NA	NA	0	NA	-100.00%
Phantom Sensation (0-72 scale)	25	19	-24.00%	NA	NA	NA	NA	7	NA	-72.00%
Chronic Pain Grade Scale (RLP)	1	2	100.00%	NA	NA	NA	NA	0	NA	-100.00%
Chronic Grade Pain Scale (PLP)	1	0	-100.00%	NA	NA	NA	NA	0	NA	-100.00%
Velocity (m/s)	1.27	1.26	-0.79%	NA	NA	NA	NA	1.49	NA	17.32%
Percentage in stance on amputated side	70.03	57.2	-18.32%	NA	NA	NA	NA	59.4	NA	-15.18%
Step length (m) on amputated side	0.69	0.75	8.70%	NA	NA	NA	NA	0.8	NA	15.94%
Residual limb circumference at tibial tubercle(TT) (cm) on amputated side	37.2	38.1	2.42%	NA	NA	NA	NA	37.7	NA	1.34%
Residual limb 1" below TT (cm)	35	36.5	4.29%	NA	NA	NA	NA	36.2	NA	3.43%
Residual limb2" below TT (cm)	33.8	35.5	5.03%	NA	NA	NA	NA	35.1	NA	3.85%
Residual limb 3" below TT (cm)	33.2	35.9	8.13%	NA	NA	NA	NA	35.8	NA	7.83%
Residual limb mean circumference (cm)	34.75	36.5	5.04%	NA	NA	NA	NA	35.93	NA	3.40%
Volume (cm^3)	1273.27	1299.49	2.06%	NA	NA	NA	NA	1325.18	NA	4.08%
Intensity of NMES Quadriceps Muscle	24.00	41.00	70.83%	NA	NA	NA	NA	NA	NA	NA
Intensity of NMES Anterior Tibialis Muscle	100.00	61.00	-39.00%	NA	NA	NA	NA	NA	NA	NA
Intensity of NMES Gastrocnemius Muscle	64.00	78.00	21.88%	NA	NA	NA	NA	NA	NA	NA
Number of Sessions / Compliance %		58 / 97%		NA		NA				

Subject 14:

Gender: Male
Ethnicity: Caucasian
Age: 56
Weight: 180 lbs.
Height: 5’7”
BMI: 29.7
Cause of amputation: Diabetes
Side of amputation: Right
Years since amputation: 1.58
Length of limb: 14.5 cm

Table 18 Compliance percentage over time subject 14

Subject 14		0-4 weeks	4-8 weeks	8-12 weeks
Data Logger	Days per week	7	4.91	7
	Number of sessions	90	59	90
	Percentage compliant	150%	98.3%	150%

Compliance: During the first four weeks of treatment, the subject wore the NMES treatment seven days per week with 150% compliance. On week eight we saw a compliance rate of 98.3% and on week twelve we saw a 150% compliance rate. Subject reports that he stopped applying the NMES, between week 6 and week 7, to the anterior tibialis muscle for approximately 5 days due to a reoccurring bursa formation. This was drained, and he continued the intervention on the anterior tibialis muscle.

Strength: We did not see a clinically significant increase in either the isometric or isokinetic strength until the week eight visit. It appears that his strength plateaued between the week eight and week twelve visits as the isometric strength decreased 46.50% from baseline at week twelve and the isokinetic strength decreased by 14.88% at week twelve.

Limb size: We did not see a consistent clinically significant increase or decrease of $\pm$5% with the subject's limb size with the scanner measurements. We did see a clinically significant limb size decrease at the 3" below the tibial tubercle level at week eight and week twelve. We believe this was due to the fluid filled bursa he experienced, per notes in log book, between week four and week six which was drained during week seven. Data showed that the subject's overall volume remained stable over all time points.

Pain: The subject report no residual limb pain throughout all time points during the study. At the week four and week eight time points, the subject reported an 85.71% decrease in phantom limb pain and a continuous decrease in phantom limb sensation across the four week, eight week and twelve week time points. At the final visit he reported an increase in phantom sensation but maintained the same phantom limb pain level compared to week twelve. The data show that the NMES decreased the subject's phantom limb pain and phantom sensation from to 12 weeks with a carryover effect maintaining a low phantom limb pain score at the final week 24 visit and a phantom limb sensation score less than baseline at the final week 24 visit.

Gait: There was no clinically significant change in velocity throughout the time points. We saw a slight decrease in percentage in stance on the amputated side at the week four visit. There was no clinically significant change in step length on the amputated side throughout the time points.

Table 19 Participant 14 data

Participant 14

Outcome Measure	*Baseline (0 weeks)*	*0-4 weeks*	*Percentage change*	*0-8 weeks*	*Percentage change*	*0-12 weeks*	*Percentage change*	*12 week-24 week*	*Percentage change 12 to 24 weeks*	Percentage change 24 week compared to baseline
Isometric knee extension strength 60°/sec (Nm/Kg)	15.14	13.99	-7.60%	17.92	18.36%	8.1	-46.50%	15.1	86.42%	-0.26%
Isokinetic knee extension strength 60°/sec (Nm/Kg)	17.54	14.56	-16.99%	21.77	24.12%	14.93	-14.88%	7.37	-50.64%	-57.98%
Residial Limb Pain (0-10 scale)	0	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0.00%
Phantom Limb Pain (0-10 scale)	7	1	-85.71%	1	-85.71%	2	-71.43%	2	0.00%	-71.43%
Phantom Sensation (0-72 scale)	19	9	-52.63%	7	-63.16%	5	-73.68%	14	180.00%	-26.32%
Chronic Pain Grade Scale (RLP)	0	0	0.00%	0	0.00%	2	200.00%	0	0.00%	0.00%
Chronic Pain Grade Scale (PLP)	1	0	-100.00%	0	-100.00%	0	-100.00%	0	-100.00%	-100.00%
Velocity (m/s)	0.98	0.9	-8.16%	0.94	-4.08%	0.91	-7.14%	0.88	-3.30%	-10.20%
Percentage in stance on amputated side	59.06	54.56	-7.62%	61.67	4.42%	61.63	4.35%	61.33	-0.49%	3.84%
Step length (m) on amputated side	0.6	0.61	1.67%	0.62	3.33%	0.59	-1.67%	0.61	3.39%	1.67%
Residual limb circumference at tibial tubercle(TT) (cm) on amputated side	30.9	31.25	1.13%	32	3.56%	30.8	-0.32%	30.5	-0.97%	-1.29%
Residual limb 1" below TT (cm)	30.3	31.6	4.29%	30.8	1.65%	30	-0.99%	29.4	-2.00%	-2.97%
Residual limb2" below TT (cm)	30.5	31.6	3.61%	29.8	-2.30%	29.7	-2.62%	28.5	-4.04%	-6.56%
Residual limb 3" below TT (cm)	30.3	30	-0.99%	28.5	-5.94%	26.7	-11.88%	26.7	0.00%	-11.88%
Residual limb mean circumference (cm)	30.5	31.11	2.00%	30.28	-0.72%	29.3	-3.93%	28.78	-1.77%	-5.64%
Volume (cm^3)	716.11	724.31	1.14%	714.48	-0.23%	712.84	-0.46%	714.48	0.23%	-0.23%
Intensity of NMES Quadriceps Muscle	70.00	76.00	8.57%	90.00	28.57%	100.00	42.86%	NA	NA	NA
Intensity of NMES Anterior Tibialis Muscle	100.00	100.00	0.00%	100.00	0.00%	100.00	0.00%	NA	NA	NA
Intensity of NMES Gastrocnemius Muscle	100.00	100.00	0.00%	100.00	0.00%	100.00	0.00%	NA	NA	NA
Number of Sessions / Compliance %		90 / 150%		59 / 98.3%		90/150%				

Subject 15:

Gender: Male
Ethnicity: Caucasian
Age: 53
Weight: 169 lbs.
Height: 5’71/2”
BMI: 27.9
Cause of amputation: vascular
Side of amputation: Right
Years since amputation: 1.67
Length of limb: 15 cm

Table 20 Compliance percentage over time subject 15

Subject 15		0-4 weeks	4-8 weeks	8-12 weeks
Data Logger	Days per week	1.91	NA	NA
	Number of sessions	23	NA	NA
	Percentage compliant	38%	NA	NA

Compliance: During the first 1 week of treatment, the data logger on the NMES device reports that subject wore the NMES treatment 1.91 times a week (38% compliance). He reported that he discontinued the use of the NMES intervention as he felt tightness and pain on the limb due to the socket fit. He returned to us to complete the four week visit before monitoring him through to his three month final visit.

Strength: We saw a clinically significant decrease in both isometric (19.41%) and isokinetic (18.54%) strength at the week four visit. His strength measures maintained at the same level at the three-month visit.

Limb size: We saw a clinically significant increase of 3.48% in size at the tibial tubercle lever during the scanner measurements. We also saw a clinically significant increase of 3.70% at the 1” below the tibial tubercle level. The scanner measurements showed that the residual limb atrophied back to smaller than the baseline measurements at week 24.

Pain: There was no clinically significant change in any of the three pain outcomes from the questionnaires; however, he verbally reported residual limb pain from the prosthetic socket fit at the week four visit.

Gait: There were no clinically significant change in any of the three gait parameters.

Table 21 Participant 15 data

Participant 15

Outcome Measure	*Baseline (0 weeks)*	*0-4 weeks*	*Percentage change*	*0-8 weeks*	*Percentage change*	*0-12 weeks*	*Percentage change*	*12 week-24 week*	Percentage change 12 to 24 weeks	Percentage change 24 week compared to baseline
Isometric knee extension strength 60°/sec (Nm/Kg)	18.03	14.53	-19.41%	NA	NA	NA	NA	14.46	NA	-19.80%
Isokinetic knee extension strength 60°/sec (Nm/Kg)	17.1	13.93	-18.54%	NA	NA	NA	NA	13.94	NA	-18.48%
Residial Limb Pain (0-10 scale)	3	3	0.00%	NA	NA	NA	NA	2	NA	-33.33%
Phantom Limb Pain (0-10 scale)	7	7	0.00%	NA	NA	NA	NA	9	NA	28.57%
Phantom Sensation (0-72 scale)	12	12	0.00%	NA	NA	NA	NA	10	NA	-16.67%
Chronic Pain Grade Scale (RLP)	1	1	0.00%	NA	NA	NA	NA	0	NA	-100.00%
Chronic Pain Grade Scale (PLP)	1	1	0.00%	NA	NA	NA	NA	3	NA	200.00%
Velocity (m/s)	0.9	0.92	2.22%	NA	NA	NA	NA	0.94	NA	4.44%
Percentage in stance on amputated side	59.6	59.76	0.27%	NA	NA	NA	NA	60.1	NA	0.84%
Step length (m) on amputated side	0.57	0.58	1.75%	NA	NA	NA	NA	0.59	NA	3.51%
Residual limb circumference at tibial tubercle(TT) (cm) on amputated side	31.6	32.7	3.48%	NA	NA	NA	NA	31	NA	-1.90%
Residual limb 1" below TT (cm)	29.7	30.8	3.70%	NA	NA	NA	NA	29.3	NA	-1.35%
Residual limb2" below TT (cm)	28.3	28.8	1.77%	NA	NA	NA	NA	26.7	NA	-5.65%
Residual limb 3" below TT (cm)	27.1	26.8	-1.11%	NA	NA	NA	NA	20.3	NA	-25.09%
Residual limb mean circumference (cm)	29.18	29.78	2.06%	NA	NA	NA	NA	26.98	NA	-7.54%
Volume (cm^3)	740.70	743.97	0.44%	NA	NA	NA	NA	791.50	NA	6.86%
Intensity of NMES Quadriceps Muscle	31.00	32.00	3.23%	NA	NA	NA	NA	NA	NA	NA
Intensity of NMES Anterior Tibialis Muscle	23.00	24.00	4.35%	NA	NA	NA	NA	NA	NA	NA
Intensity of NMES Gastrocnemius Muscle	32.00	38.00	18.75%	NA	NA	NA	NA	NA	NA	NA
Number of Sessions / Compliance %		23/ 38%		NA		NA				

Subject 16:

Gender: Male
Ethnicity: Caucasian
Age: 42
Weight: 157 lbs.
Height: 6’2”
BMI: 21.3
Cause of amputation: Trauma
Side of amputation: Left
Years since amputation: 17.5
Length of limb: 12.7 cm

Table 22 Compliance percentage over time subject 16

Subject 16		0-4 weeks	4-8 weeks	8-12 weeks
Data Logger	Days per week	5	NA	NA
	Number of sessions	60	NA	NA
	Percentage compliant	100%	NA	NA

Compliance: During the first four weeks of NMES treatment, the subject wore the NMES treatment five days per week with a 100% compliance rate. After his week four visit the subject had neck surgery to repair two bulging discs in his neck. Participant reports that he had cervical myelopathy caused from a past car accident. Symptoms of cervical myelopathy may include incoordination in the hands, a heavy feeling in the legs, or numbness and tingling in the legs. It is

generally a slowly progressive condition. He was followed at a three-month final visit from the four week visit date.

Strength: We saw a large clinically significant decrease in isometric and isokinetic strength at the four-week visit. This decrease in isometric strength remained at the final week 24 visit and was less than the baseline torque. However, his isokinetic strength increased slightly (12%) at the week 24 visit.

Limb size: The scanner measurements reported a clinically significant decrease at the tibial tubercle level and 3” below the tibial tubercle level and a trend towards clinical significance at the 1” and 2” level below the tibial tubercle. At the final three-month visit, data showed measurements were close to baseline.

Pain: At the four week visit the subject reported a 40% decrease in residual limb pain; a 100% decrease in phantom limb pain and an 80% decrease in phantom sensation. At the final three-month visit, the subject’s residual limb pain increase 40% from baseline but the phantom limb pain and phantom sensation remained less than the baseline score. He reports that he was only taking a small dose of Tramadol, which is a narcotic-like pain reliever.

Gait: The subject shown a decrease in all three gait parameters at the week four visit. We believe this is due to his neck pain which affected his overall strength and gait. At the final three-month visit, the subject returned using a cane for stability. This was accounted for with the gait rite software.

Table 23 Participant 16 data

Participant 16

Outcome Measure	*Baseline (0 weeks)*	*0-4 weeks*	*Percentage change*	*0-8 weeks*	*Percentage change*	*0-12 weeks*	*Percentage change*	*12 week-24 week*	Percentage change 12 to 24 weeks	Percentage change 24 week compared to baseline
Isometric knee extension strength 60°/sec (Nm/Kg)	11.7	5.13	-56.15%	NA	NA	NA	NA	6.8	NA	-41.88%
Isokinetic knee extension strength 60°/sec (Nm/Kg)	10.49	6.9	-34.22%	NA	NA	NA	NA	11.76	NA	12.11%
Residial Limb Pain (0-10 scale)	5	3	-40.00%	NA	NA	NA	NA	7	NA	40.00%
Phantom Limb Pain (0-10 scale)	6	0	-100.00%	NA	NA	NA	NA	1	NA	-83.33%
Phantom Sensation (0-72 scale)	60	12	-80.00%	NA	NA	NA	NA	8	NA	-86.67%
Chronic Pain Grade Scale (RLP)	2	0	-100.00%	NA	NA	NA	NA	1	NA	-50.00%
Chronic Pain Grade Scale (PLP)	3	0	-100.00%	NA	NA	NA	NA	0	NA	-100.00%
Velocity (m/s)	0.94	0.38	-59.57%	NA	NA	NA	NA	0.52	NA	-44.68%
Percentage in stance on amputated side	64.8	53.27	-17.79%	NA	NA	NA	NA	67.55	NA	4.24%
Step length (m) on amputated side	0.65	0.57	-12.31%	NA	NA	NA	NA	0.52	NA	-20.00%
Residual limb circumference at tibial tubercle(TT) (cm) on amputated side	32.8	31.3	-4.57%	NA	NA	NA	NA	32.9	NA	0.30%
Residual limb 1" below TT (cm)	30.9	30.1	-2.59%	NA	NA	NA	NA	31	NA	0.32%
Residual limb2" below TT (cm)	29.5	28.7	-2.71%	NA	NA	NA	NA	29.1	NA	-1.36%
Residual limb 3" below TT (cm)	27.4	23.7	-13.50%	NA	NA	NA	NA	26.6	NA	-2.92%
Residual limb mean circumference (cm)	30.15	28.45	-5.64%	NA	NA	NA	NA	29.9		-0.83%
Volume (cm^3)	770.19	807.88	4.89%	NA	NA	NA	NA	784.94	NA	1.91%
Intensity of NMES Quadriceps Muscle	72.00	62.00	-13.89%	NA	NA	NA	NA	NA	NA	NA
Intensity of NMES Anterior Tibialis Muscle	77.00	77.00	0.00%	NA	NA	NA	NA	NA	NA	NA
Intensity of NMES Gastrocnemius Muscle	69.00	72.00	4.35%	NA	NA	NA	NA	NA	NA	NA
Number of Sessions / Compliance %		60 / 100%		NA		NA				

Subject 18:

Gender: Female
Ethnicity: Caucasian
Age: 46
Weight: 191 lbs.
Height: 5’3”
BMI: 35.7
Cause of amputation: Trauma
Side of amputation: Left
Years since amputation: 16.58
Length of limb: 12.7

Table 24 Compliance percentage over time subject 18

Subject 18		0-4 weeks	4-8 weeks	8-12 weeks
Data Logger	Days per week	4	5.42	1
	Number of sessions	48	65	13
	Percentage compliant	80%	108%	22%

Compliance: During the first four weeks of NMES intervention, the subject wore the NMES treatment four times a week with an 80% compliance rate; and at the eight week visit she wore the NMES intervention 5.42 times a week with a 108% compliance rate. During the twelve week visit she wore the NMES intervention one time a week with a 22% compliance rate. The subject reports that she had gum surgery between week 8 and week 12, so she did not wear NMES as instructed 5 times a week.

Strength: We saw a large clinically significant increase (75.51%) in isometric strength at the week four visit. We saw a large clinically significant increase (87.92%) in isokinetic strength at the week four visit. At the eight-week visit, the subject's isometric strength increased 156.01% from baseline. The subject's isokinetic strength increased 113.93% from baseline to week eight. At the week twelve visit, the subject's isometric strength increased 112.47% from baseline and the subject's isokinetic strength increased 83.05% from baseline. At the final visit, the subject's isometric strength increased 42.69% from week 12 and the subject's isokinetic strength increased 24.66% from week twelve. This showed a carryover effect with the subject testing the strongest at the three-month visit.

Limb size: We did not see any clinically statistical changes in residual limb size until week eight. The subject's residual limb decreased in size over 6% in three of the four circumferences. The values maintained at about the same level; this could be due to lower compliance rate of NMES during week eight and week twelve. The subject had no complaints of the socket fitting loose or reported that she had to wear additional sock ply. At the final visit the residual limb increased in size approximately 2% from the week twelve measurements but remained smaller than or close to baseline measurements. The volume measurements show an increase in limb volume at week eight and week twelve which carried over to the final three month visit.

Pain: The subject's residual limb pain scores decreased over all time points compared to baseline. The subject's phantom limb pain decreased over the week four, week eight and week twelve time points compared to baseline showing clinical significance. The subject's phantom limb sensation showed a decrease over week four, week eight and week twelve time points from

baseline but was not clinically significant. There was a 100% decrease in the chronic pain grade scale for residual limb pain at weeks four, eight, twelve and twenty-four. There was a decrease in the chronic pain grade scale for phantom limb pain at week eight continuing through to week twenty-four.

Gait: We saw a clinically significant change in velocity in week four and week twelve which carried over to the final visit. The subject had already achieved normal percentage in stance on the amputated side at the baseline visit at 62.56% and maintained close to normal parameters throughout the study. The subject's step length also showed a clinically significant improvement over week four and week eight visits; trending towards clinical significance at week twelve.

Table 25 Participant 18 data

Participant 18

Outcome Measure	*Baseline (0 weeks)*	*0-4 weeks*	*Percentage change*	*0-8 weeks*	*Percentage change*	*0-12 weeks*	*Percentage change*	*12 week - 24 week*	*Percentage change 12 to 24 weeks*	Percentage change 24 week compared to baseline
Isometric knee extension strength 60°/sec (Nm/Kg)	4.41	7.74	75.51%	11.29	156.01%	9.37	112.47%	13.37	42.69%	203.17%
Isokinetic knee extension strength 60°/sec (Nm/Kg)	5.96	11.2	87.92%	12.75	113.93%	10.91	83.05%	13.6	24.66%	128.19%
Residial Limb Pain (0-10 scale)	5	3	-40.00%	3	-40.00%	2	-60.00%	3	50.00%	-40.00%
Phantom Limb Pain (0-10 scale)	5	4	-20.00%	3	-40.00%	3	-40.00%	4	33.33%	-20.00%
Phantom Sensation (0-72 scale)	16	14	-12.50%	12	-25.00%	14	-12.50%	16	14.29%	0.00%
Chronic Pain Grade Scale (RLP)	1	0	-100.00%	0	-100.00%	0	-100.00%	0	0.00%	-100.00%
Chronic Pain Grade Scale (PLP)	1	1	0.00%	0	-100.00%	0	-100.00%	0	0.00%	-100.00%
Velocity (m/s)	1.01	1.23	21.78%	1.12	10.89%	1.18	16.83%	1.19	0.85%	17.82%
Percentage in stance on amputated side	62.56	60.55	-3.21%	58.8	-6.01%	61.45	-1.77%	60.9	-0.90%	-2.65%
Step length (m) on amputated side	0.54	0.6	11.11%	0.6	11.11%	0.59	9.26%	0.58	-1.69%	7.41%
Residual limb circumference at tibial tubercle(TT) (cm) on amputated side	34.4	34.3	-0.29%	32	-6.98%	32.2	-6.40%	33	2.48%	-4.07%
Residual limb 1" below TT (cm)	36.6	36.2	-1.09%	34.4	-6.01%	34.7	-5.19%	35.4	2.02%	-3.28%
Residual limb2" below TT (cm)	34.4	34	-1.16%	32.3	-6.10%	32.2	-6.40%	32.9	2.17%	-4.36%
Residual limb 3" below TT (cm)	28.3	27.5	-2.83%	27.3	-3.53%	28	-1.06%	28.6	2.14%	1.06%
Residual limb mean circumference (cm)	33.43	33	-1.29%	31.5	-5.77%	31.78	-4.94%	32.33	1.73%	-3.29%
Volume (cm^3)	717.75	704.64	-1.83%	714.48	-0.46%	716.11	-0.23%	724.31	1.14%	0.91%
Intensity of NMES Quadriceps Muscle	40.00	48.00	20.00%	50.00	25.00%	50.00	25.00%	NA	NA	NA
Intensity of NMES Anterior Tibialis Muscle	35.00	42.00	20.00%	51.00	45.71%	53.00	51.43%	NA	NA	NA
Intensity of NMES Gastrocnemius Muscle	42.00	45.00	7.14%	50.00	19.05%	52.00	23.81%	NA	NA	NA
Number of Sessions / Compliance %		48 / 80%		65 / 108%		13 / 22%				

APPENDIX A2:

Forms, questionnaires and surveys used in study:

Demographic Survey

NMES Checklist

NMES Use handout

Monofilament test

NMES Data Use collection form

Pain Questionnaire

Biodex Intake Forms

3D Scanner Intake Form

Gait Data Form

Lower Extremity Prosthetics Form

Intervention Group Daily Log Form

Control Group Daily Log Form

Exit Questionnaire

Subject ID: _____

NMES Study Demographic Survey

Initial Survey

Subject ID: _____

PART I: Demographics

Date of Birth: _____

Age: _____

Gender: ❑ Male ❑ Female

Ethnic Origin:

❑ Black or

❑ Hispanic or Latino

❑ Two or more races

❑Asian

❑American Indian or Alaskan Native

❑ White or Caucasian

❑ Native Hawaiian or other Pacific Islander

Are you a US Veteran? ❑ Yes, I am a veteran ❑ No, I am not a veteran ❑ No, I am active duty

If yes, what was your rank at time of discharge or injury?

❑ Enlisted ❑ Warrant Officer ❑ Officer

What is the highest level of formal education you have completed?

❑ 8th grade or less
❑ 9th through 11th grade
❑ High school diploma or GED
❑ Associates Degree
❑ Bachelors Degree
❑ Masters Degree
❑ Doctorate (including PhD, MD, JD, etc)
Other: _____

Please indicate your marital status:

❑ Single (a person who has never married)

❑ Single, but living with a partner as if married

❑ Married (a person who is legally married)

Other: _____

❑ Divorced (a person who is legally divorced)

❑ Separated (legal separation or living apart from married partner)

❑ Widowed

Subject ID: _____

\

Please identify your primary occupational, educational or training status.

- ❑ Working, part-time or full-time, including military (gainfully and legally employed)
- ❑ Homemaker
- ❑ On-the-job training
- ❑ Not employed by choice
- ❑ Not employed due to disability
- ❑ Retired
- ❑ Student
- ❑ Unemployed, unable to find a job

Other: _____

Subject ID: _____

PART II: Injury/Diagnosis Information

What is the cause of your amputation?

❑ Trauma

❑ Vascular

❑ Diabetes related
❑ Malignancy/Cancer
Other: _____

Which side is your amputation?

❑ LEFT LIMB ❑ RIGHT LIMB

Date of amputation:_____/_____/_____

Do you have diabetes? Yes ❑ No ❑

If yes, do you have; ❑ Type 1 (juvenile onset/insulin dependent)
or ❑ Type 2 (adult onset/non-insulin dependent)

-

What year (approximate) were you diagnosed with diabetes? _____

Do you wear a prosthetic limb? Yes ❑ No ❑

Do you use any walking aids (e.g. stick, crutches or frame)? Yes ❑ No ❑

If yes, which and how many? (e.g. one/two sticks) _____

Can you walk 50 meters (150 feet) without stopping? Yes ❑ No ❑

Have you recently sustained any injury to your amputation stump? Yes ❑ No ❑

Subject ID: _____

Do you have any of the following diagnoses?

❑ Traumatic Brain Injury ❑ Muscular Dystrophy ❑ Cerebral Palsy
❑ Spina Bifida ❑ Multiple Sclerosis ❑ None
Other: _____

Do you have any of the following medical conditions at the present time?

❑ Asthma, emphysema, or chronic bronchitis
❑ Arthritis or rheumatism
❑ Cancer, diagnosed in the past 3 years
❑ Diabetes
❑ Digestive problems (such as ulcer, colitis, or gallbladder disease)
❑ Kidney disease
❑ Liver problems (such as cirrhosis)
❑ Stroke
❑ Heart trouble (such as angina, congestive heart failure, or coronary artery disease)
❑ None of the above

Subject ID: _____

PART III: Assistive Technology

Do you use a wheelchair? _____If no, skip the next two questions.

What type of wheelchair do you use most often?

❑ Manual ❑ Power ❑ Power Assist

What is the make (brand) of your primary wheelchair?

❑Action/Invacare ❑ Everest & Jennings ❑ Permobil ❑ Pride
❑Sunrise/Quickie ❑ TiLite Other:

Do you use any of the following assistive devices? (check all that apply)

❑ Walker ❑ Cane ❑ Scooter
❑ Standard crutches ❑ Forearm Crutches ❑ Lower limb orthoses (brace)
❑ Lower limb prosthesis ❑ Upper limb prosthesis ❑ None of the above

What is your one, primary means of mobility? (use > 40 hours per week)

❑ Wheelchair ❑ Cane ❑ Scooter
❑ Standard crutches ❑ Forearm Crutches ❑ Lower limb orthoses (brace)
❑ Lower limb prosthesis ❑ Walker

PART IV: Community

How many hours per week do you work for pay?_____

Is this amount satisfactory to you or would you like to be doing more or less?

❑ More ❑ Less ❑ Same ❑ Don't know

How important is working for pay to you?

❑ Most Important ❑ Very Important ❑ Moderately Important
❑ A Little Bit Important ❑ Not At All Important ❑ Don't Know

Subject ID: _____

How many hours a week do you go to school/training? _____

Is this amount satisfactory to you or would you like to be doing more or less?

❑ More ❑ Less ❑ Same ❑ Don't know

How important is going to school/training to you?

❑ Most Important ❑ Very Important ❑ Moderately Important
❑ A Little Bit Important ❑ Not At All Important ❑ Don't Know

How much of an effect do you believe participation in sports has on your ability to engage and succeed in education?

❑ No effect ❑ A little effect ❑ Some effect ❑ A moderate effect ❑ A large effect

How many hours a week do you do volunteer work? _____

Is this amount satisfactory to you or would you like to be doing more or less?

❑ More ❑ Less ❑ Same ❑ Don't know

How important is doing volunteer work to you?

❑ Most Important ❑ Very Important ❑ Moderately Important
❑ A Little Bit Important ❑ Not At All Important ❑ Don't Know

How much of an effect do you believe participation in sports has on your ability to seek out and engage in volunteer work?

❑ No effect ❑ A little effect ❑ Some effect ❑ A moderate effect ❑ A large effect

Thank you for your answers

Subject ID: _____

NMES Checklist

ID #_____	**Date:** _____
Timeline:	**Time:** _____
❑ **Baseline**	

1. Issued NMES unit Yes ❑ No❑
2. Issued 3 sets of electrode pads Yes ❑ No❑
3. Instructed participant on pad placement Yes ❑ No❑
4. Gave participant a photo of pad placement on residual limb Yes ❑ No❑
5. Instructed participant on settings Yes ❑ No❑
   a. Frequency setting _____
   b. Amplitude setting _____
   c. Time on interval_____
   d. Time off interval_____
6. Instruct participant on wear schedule Yes ❑ No❑
7. Instructed participant on how to remove and store electrodes
   Yes ❑ No❑
8. Gave participant instructional handout Yes ❑ No❑

Subject ID: _____

"Functional Electrical Stimulation Use in Trans-Tibial Amputations"

Product Instructional Guide for the EMPI Continuum

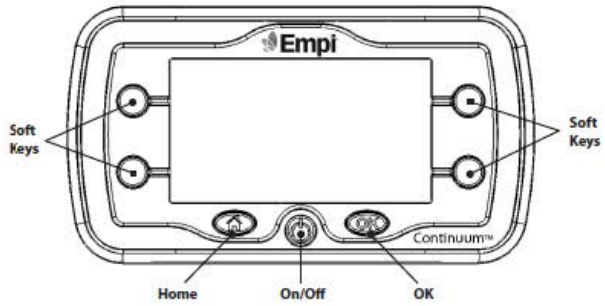
This handout will serve as a guide to remind you how to use and apply the FES device

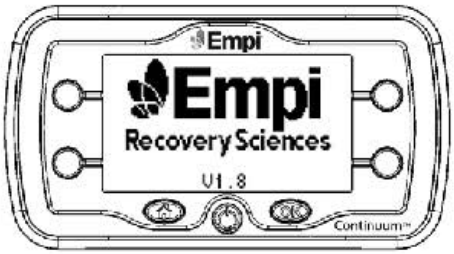
A. **Use of device, programming and adjusting stimulation**
B. **Electrode pad placement and precautions**
C. **Wear schedule**
D. **Pad removal & storage**
E. **Maintenance, Cleaning, Storage, Misc.**
F. **Contact information**

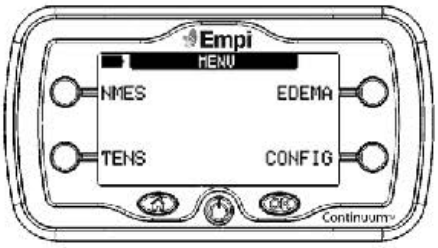
A. Use of device Subject ID: _____

You will be given two FES devices.

1. Press the ON/ OFF button located on the bottom row, middle button. You should first see the Empi logo along with software version number displayed on the LCD for four seconds before displaying a therapy options menu as seen below. The device is now ready to function.

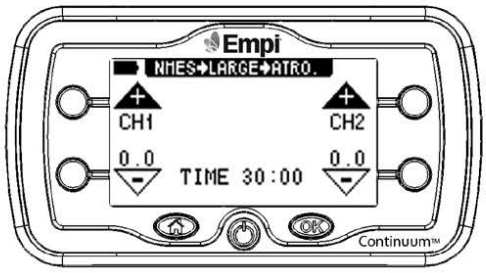






2. The unit has previously been configured for operation for NMES by the investigator.

Subject ID: _____



3. Press the soft key next to the channel 1 UP arrow. This is will ramp up the level of stimulation for Channel 1. You should see a dot next to the channel output on the LCD screen when output is active.

4. Repeat this procedure for channel 2 on the right if using two channels. You can only ramp intensity on an active channel and can only ramp one channel at a time.

5. If the device has operated as expected, turn it off by pressing the ON/OFF button located on the bottom row, middle button.

6. If the LCD does not display any information at startup, check the battery compartment. See page 4 for instructions.

7. If after changing batteries, the device still does not respond, call the contact person in this brochure.

Programming and adjusting stimulation

1. Connect the lead wires to the device. If only one lead wire will be used, plug it into the Channel 1 output channel.

CAUTION: Ensure the device is OFF before connecting the lead wires.

2. Power on the device by pressing the ON/OFF button

3. Press the type of treatment option desired from the main options menu.

4. Select the desired localized treatment type.

5. Select and/or modify the related parameter values for the treatment type and then press

OK to lock them in.

6. Begin the treatment by pressing the intensity buttons found to both the left and right of the screen. Press the Up arrows to increase intensity on channel 1 or 2 and press the Down arrows to decrease intensity on channel 1 or 2.

7. Treatment can be ended by pressing the OFF button for at least 1sec.

8. Store electrodes for future use. See instructions on page 12 for electrode storage and maintenance.

9. The Continuum device locks the intensity increase buttons to prevent accidental increases in intensity. Subject ID: _____
This safety feature is activated after 20 seconds of unchanged intensity.

10. To unlock the device, press either intensity Down button. You can now increase the intensity.

Battery and belt clip use

Changing the Batteries

Change BOTH of the AA batteries when:

- The low battery indicator is visible on the LCD
- The Low Battery message displays
- If the device will not turn on.

Turn off the device to replace batteries. Open the battery door by placing your thumb on the notch and the bottom back side of the unit and pushing up while pulling out. When replacing the batteries, be sure the battery polarity (+ and -) markings match the markings on the device. Use AA alkaline or rechargeable NiMH batteries. The life of the battery is dependent on the program and amplitude (intensity) being used. The battery use indicater may not show 100% power when a fully charged rechargable battery is used; but the battery life is still full.

NOTE: Do not overcharge batteries. Batteries should remain in the charger for the recommended time.

Remove the batteries from the charger once charge is complete. See the Instruction for Use for the battery charger for recommended charging time.

To Replace the Battery Cover

1. Insert the small lip at the base of the cover.
2. Insert the top of the cover and press until it clicks securely in place.

Using the Belt Clip Subject ID: _____

This unit has a simple belt clip that fits comfortably over a belt or waistband. To attach the belt clip, simply face the back of the device toward you and slide the device, with a gentle pull of the clip tab. When in place, you should feel a slight tension holding it in place.

Using the Kick Stand

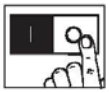
This unit has a simple, pull-out Kick Stand for use in patient monitoring. To use the kick stand, turn the device to the back side. Placing your finger on the recessed tab, pull the stand out for use.

B. Electrode Pad placement

The electrodes will be placed on the following locations: medial and lateral quadriceps (front thight), anterior tibialis (front shin) and the gastrocnemius / soleus muscle (calf). The FES settings will be individualized and optimized for eliciting muscle contraction of the residual limb while maintaining a comfortable level of stimulation. We will begin by setting the FES in a range of 20-50 Hz. The FES unit will be set for a 1:2 ratio of on/off time. **A photo of your electrode set up will be provided to you at the first visit.**

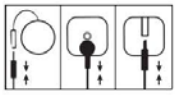
Electrode Setup

Please follow photo provided to you at baseline visit.



1. Make sure unit is turned off and lead wires are disconnected before and after treatment.

2. Clean electrode application area with soap and water. Rinse and dry. Electrode should only be applied to intact, clean skin (e.g., not over open wounds, lesions, infected, or inflamed areas).



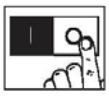
Subject ID: _____

3. With electrodes still on liner, connect lead wire from unit electrode connector. No bare metal should be visible.

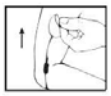
4. Remove electrode from liner by grasping the edge of the electrode and peeling it off the liner. Retain liner for storage.

5. Place the electrode on exact skin location by applying the center of the electrode first and smoothing down to electrode edges.

6. Attach lead wire to unit and begin treatment.



7. After treatment, turn unit off.



8. Remove electrode from skin by peeling electrode edge.

Precautions

9. DO NOT place electrodes on broken skin. If skin irritation develops, discontinue use. Consult physician. Replace electrodes when they do not adhere or when treatment becomes uncomfortable.

10. DO NOT use unit while driving or operating machinery. Subject ID: _____

11. For single patient use only. These electrodes may be repositioned up to several times on the same patient.

12. Stimulation should not be applied to transcerebrally or over the anterior neck region.

13. Keep electrodes separated during treatment.

14. DO NOT remove electrode by pulling on the lead wire.

15. DO NOT exceed 0.1 Watts/cm^2.

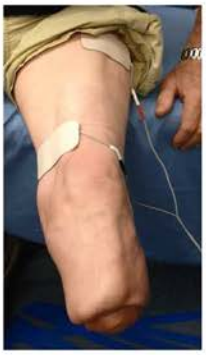
16. Using stimulation electrodes that are too small or incorrectly applied could result in discomfort or skin burns.

C. Wear Schedule Subject ID: _____

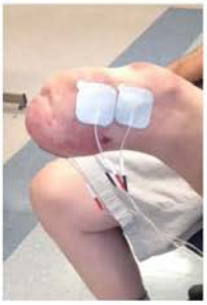
Please follow these guidelines to "wean into" wearing the device. Increasing the wear time too quickly may result in complications.

Please use the FES device as follows:

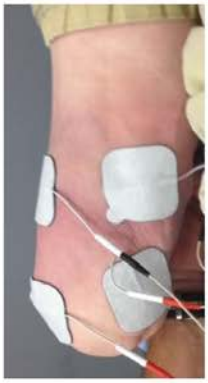
15 contractions on the quadriceps muscle



15 contractions on the anterior tibia muscle



15 contractions on the gastrocnemius muscle

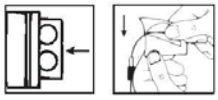


Wear the device every day without your prosthesis on. Try to wear the FES at the same time of day each time you wear it. At minimum, wear the FES device at least 3 days per week.
The intensity may be turned up as tolerated. Document the intensity on the daily log book reading every time the FES device is worn.

D. Pad Removal & Storage

Re-Application and Storage of

1. If adhesive becomes over-saturated, allow electrode to air dry in a refrigerator with adhesive side up until gel regains tack.
2. If the electrode gel appears dry, add a few drops of water to the electrode gel. Let rest to regain tack and apply to skin.
3. If electrode accumulates dirt/dust in the adhesive, the impedance increases, usually leading to increased heat dissipation at the electrode
which can lead to skin burns. Inspect the electrode for dust and dirt accumulation before reuse. Feel the electrode to make sure it is still tacky. If the adhesive has accumulates too much dirt/dust, it will no longer adhere. Replace electrode when it does not adhere.
4. Between uses (on the same patient), return electrode to liner and store in resealable bag in a cool place out of direct sunlight.



NOTE: The life of the electrode varies depending on skin conditions, skin preparation, type of stimulation, storage, and climate.

E. Maintenance,Cleaning,Storage, Misc.

Maintenance

Under normal conditions, the device does not require periodic maintenance, calibration or testing.

Cleaning

Use a damp cloth with mild soap to clean the exterior of the device and lead wires. Use of other cleaning solutions may damage these items. Never immerse the device in liquids.

Storage

To store the stimulator for an extended time (more than 30 days), remove the batteries and store the device in a cool, dry place.

Tripping – Care should be used to avoid tripping on lead wires, especially when the foot switch is utilized.

Damage From Liquids – Do not immerse the device in water or other liquids. Water or liquids could cause malfunction of internal components of the system, causing a risk of injury to the patient.

Uncomfortable Stimulation – If the stimulation levels are uncomfortable or become uncomfortable, reduce the intensity to a comfortable level. Contact your clinician if the problem persists.

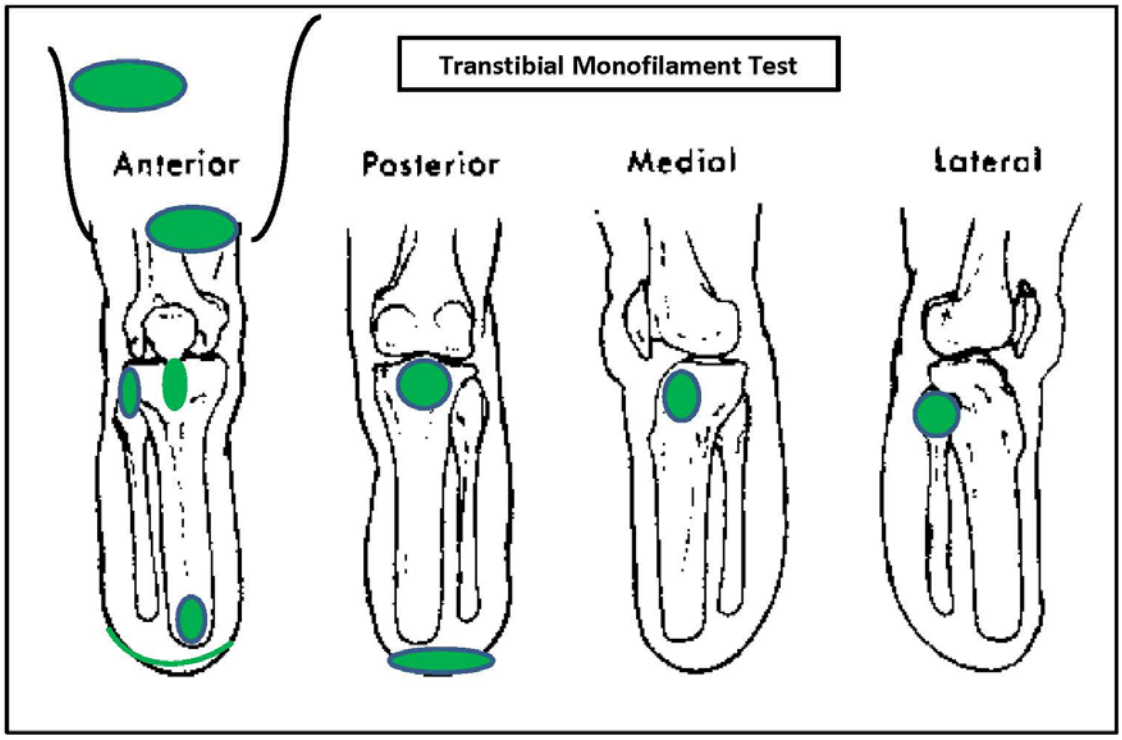
Skin Reactions – On rare occasions, therapy can result in transient skin reactions such as rash, inflammation, irritation, or burns. These skin reactions may be the result of individual sensitivity to the condition of the skin at the onset of treatment, reaction to the materials in the electrodes, or a poor connection between the electrodes and the patient's skin. Advise the patient of this possibility before starting treatment. If a visible skin reaction does occur, instruct the patient to discontinue the treatment and consult the prescribing physician or licensed practitioner.

Electromagnetic Compatibility – Care must be taken when operating this device adjacent to or stacked with other equipment. Potential electromagnetic or other interference could occur to this or other equipment. Try to minimize this interference by not using other equipment in conjunction with it. (i.e. cell phone, etc.). The Empi Continuum should not be used adjacent to or stacked with other equipment and if adjacent or stacked use is necessary, the Empi Continuum should be observed to verify normal operation in the configuration in which it will be used.

Accessories – Use only accessories that are specifically designed for this device. Do not use the accessories manufactured by other companies on this device

F. Contact Information

Please contact Sara Peterson with any questions or concerns, day or night.
Phone: 412-383-6757
Email: speterso@pitt.edu



Yes	No		Yes	No		Yes	No		Yes	No	
		Patella tendon			Popliteal			Medial flare			Fibular head
		Lateral flare			Distal end						
		Incision site									
		Anterior tibial crest									
		Anterior distal thigh									
		Anterior proximal thigh									

Subject ID _____

Subject ID: _____

NMES use data from device

ID:	DATE:	Height:	Weight: BMI:		
	Session				
	Baseline	4 weeks	8 weeks	12 weeks	3 month follow up
Number of sessions					
Total hours used					
Average time					
Channel 1 intensity					
Channel 2 intensity					

Pain Questionnaire Subject ID: _____

ID #: _____Date:_____

RESIDUAL LIMB PAIN

Residual Limb Pain (RLP): Residual limb pain is defined as painful sensations in the residual portion of the limb. The residual limb is defined as the portion of the residual limb that he still intact after amputation. (i.e. stump)

1. How would you rate your **residual limb pain** on a 0 to 10 scale at the present time; that is right now, where 0 is "no pain" and 10 is "pain as bad as could be"?

No pain **Pain as bad as could be**

0 1 2 3 4 5 6 7 8 9 10

2. Please rate your **residual limb pain** you experienced in the past month on a scale of 0-10 (0 being no pain and 10 being the worst pain possible)

No pain **Pain as bad as could be**

0 1 2 3 4 5 6 7 8 9 10

3. In the past month, on the average, how intense was your **residual limb pain** rated on a 0-10 scale where 0 is "no pain" and 10 is "pain as bad as could be"? (That is your usual pain at times you were experiencing pain)

No pain **Pain as bad as could be**

0 1 2 3 4 5 6 7 8 9 10

4. In the past month, how much has your **residual limb pain** interfered with your daily activities rated on a 0 to 10 scale where 0 is "no interference' and 10 is "unable to carry on any activities"?

No interference **Unable to carry on any activities**

0 1 2 3 4 5 6 7 8 9 10

5. In the past month, how much has **residual limb pain** changed your ability to take part in recreational, social and family activities where 0 is "no change" and 10 is "extreme change"?

No change **Extreme change**

0 1 2 3 4 5 6 7 8 9 10

6. In the past month, how much has **residual limb pain** changed your ability to work (including housework) where 0 is "no change" and 10 is "extreme change"?

No change **Extreme change**

0 1 2 3 4 5 6 7 8 9 10

7. About how many days in the last month have you been kept from your usual activities

(work, school or housework) because of **residual limb pain**? _____ **Days**

ID #: _____ Subject ID: _____

PHANTOM LIMB PAIN

Phantom Limb Pain (PLP): Phantom limb pain is defined as painful sensations felt in the missing portion of the amputated limb.

1. How would you rate your **phantom limb pain** on a 0 to 10 scale at the present time; that is right now, where 0 is "no pain" and 10 is "pain as bad as could be"?

No pain **Pain as bad as could be**

0 1 2 3 4 5 6 7 8 9 10

2. Please rate your **phantom limb pain** you experienced in the past month on a scale of 0-10 (0 being no pain and 10 being the worst pain possible)

No pain **Pain as bad as could be**

0 1 2 3 4 5 6 7 8 9 10

3. In the past month, on the average, how intense was your **phantom limb pain** rated on a 0-10 scale where 0 is "no pain" and 10 is "pain as bad as could be"? (That is your usual pain at times you were experiencing pain)

No pain **Pain as bad as could be**

0 1 2 3 4 5 6 7 8 9 10

4. In the past month, how much has your **phantom limb pain** interfered with your daily activities rated on a 0 to 10 scale where 0 is "no interference' and 10 is "unable to carry on any activities"?

No interference **Unable to carry on any activities**

0 1 2 3 4 5 6 7 8 9 10

5. In the past month, how much has your **phantom limb pain** changed your ability to take part in recreational, social and family activities where 0 is "no change" and 10 is "extreme change"?

No change **Extreme change**

0 1 2 3 4 5 6 7 8 9 10

6. In the past month, how much has **phantom limb pain** changed your ability to work (including housework) where 0 is "no change" and 10 is "extreme change"?

No change **Extreme change**

0 1 2 3 4 5 6 7 8 9 10

7. About how many days in the last month have you been kept from your usual activities

(work, school or housework) because of **phantom limb pain?** _____ **Days**

ID # _____ Subject ID: _____

PHANTOM LIMB SENSATION

Phantom Limb Sensations: Phantom limb sensations are non-painful sensations felt as originating from the portion of the amputated limb that is missing. Check the column to indicate the level of your pain for each word, or leave blank if it does not apply to you.

	Mild	Moderate	Severe
1 Throbbing	____	____	____
2 Shooting	____	____	____
3 Stabbing	____	____	____
4 Sharp	____	____	____
5 Cramping	____	____	____
6 Gnawing	____	____	____
7 Hot-burning	____	____	____
8 Aching	____	____	____
9 Heavy	____	____	____
10 Tender	____	____	____
11 Splitting	____	____	____
12 Stinging	____	____	____
13 Cutting	____	____	____
14 Piercing	____	____	____
15 Radiating	____	____	____
16 Tight	____	____	____
17 Nagging	____	____	____
18 Squeezing	____	____	____
19 Tingling	____	____	____
20 Shocking	____	____	____
21 Tiring-Exhausting	____	____	____
22 Sickening	____	____	____
23 Fearful	____	____	____
24 Cruel-Punishing	____	____	____

Subject ID: _____

ID #: _____

Are you currently using any techniques or medication to control the pain?

Yes ❑ No ❑

If yes, name the technique or medication. Please list.

	Times administered:				
Technique or Medication:	**Daily**	**1x week**	**2-3x week**	**4-5x week**	**1-2x month**

Have you experienced a change in health since your last visit?

Yes ❑ No ❑ If yes, explain_____

Have you had a change in medications since your last visit?

Yes ❑ No ❑ If yes, explain_____

Have you had any change in activity since your last visit?

Yes ❑ No ❑ If yes, explain_____

Thank you very much for taking the time to participate in the study. If you would like to leave any comments please write them below.

_____

_____

Subject ID: _____

Biodex intake form: Isometric

ID:	DATE:	Height:	Weight:		
Biodex lever arm setting: Right side: _____ Left side: _____	**Timeline:** ❑Baseline ❑ 4 weeks ❑ 8 weeks ❑ 12 weeks ❑ 3 month follow up				
	Peak Torque				
Extension: Isometric	Rep 1	Rep 2	Rep 3		
Residual Limb 60° knee flexion					

Biodex intake form: Isokinetic Subject ID: _____

ID:	DATE:	Height:	Weight: BMI:		
Amputated side: Right side: _____ Left side: _____	**Timeline:** ❑Baseline ❑ 4 weeks ❑ 8 weeks ❑ 12 weeks ❑ 3 month follow up				
	Peak Torque				
Extension 60°/second velocity:	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Residual Limb / set 1					
Residual Limb / set 2					
Residual Limb / set 3					

3D Scanner Intake Form Subject ID: _____

ID #_____ **Date:** _____

Timeline:

❑ **Baseline** ❑ **4 weeks** ❑ **8 weeks** ❑**12 weeks** ❑**3 month follow up**

Marks will be placed on the residual limb at the tibial tuberosity, fibular head and anterior distal end of the tibia.

Mass _____ Volume_____

5 cm Proximal to the proximal edge of the patella _____

Tibial tuberosity circumference _____

2.5 cm below tibial tuberosity _____

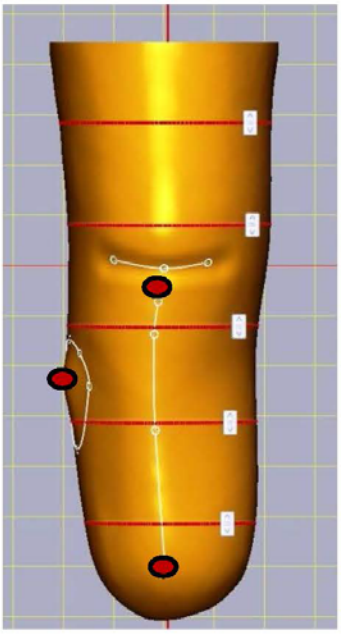
5 cm below tibial tuberosity _____

7.5 cm below tibial tuberosity _____

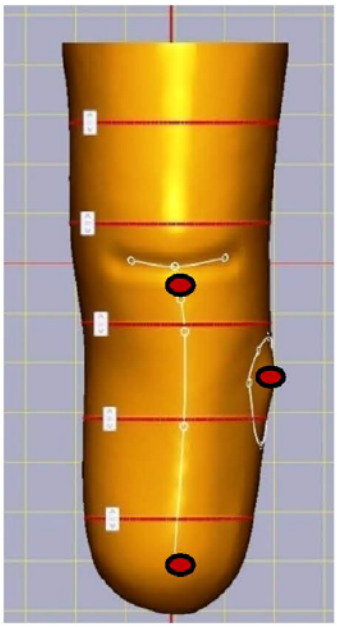
Tibial tubercle to anterior distal end of tibia _____

Tibial tubercle to end of residual limb _____

Right limb

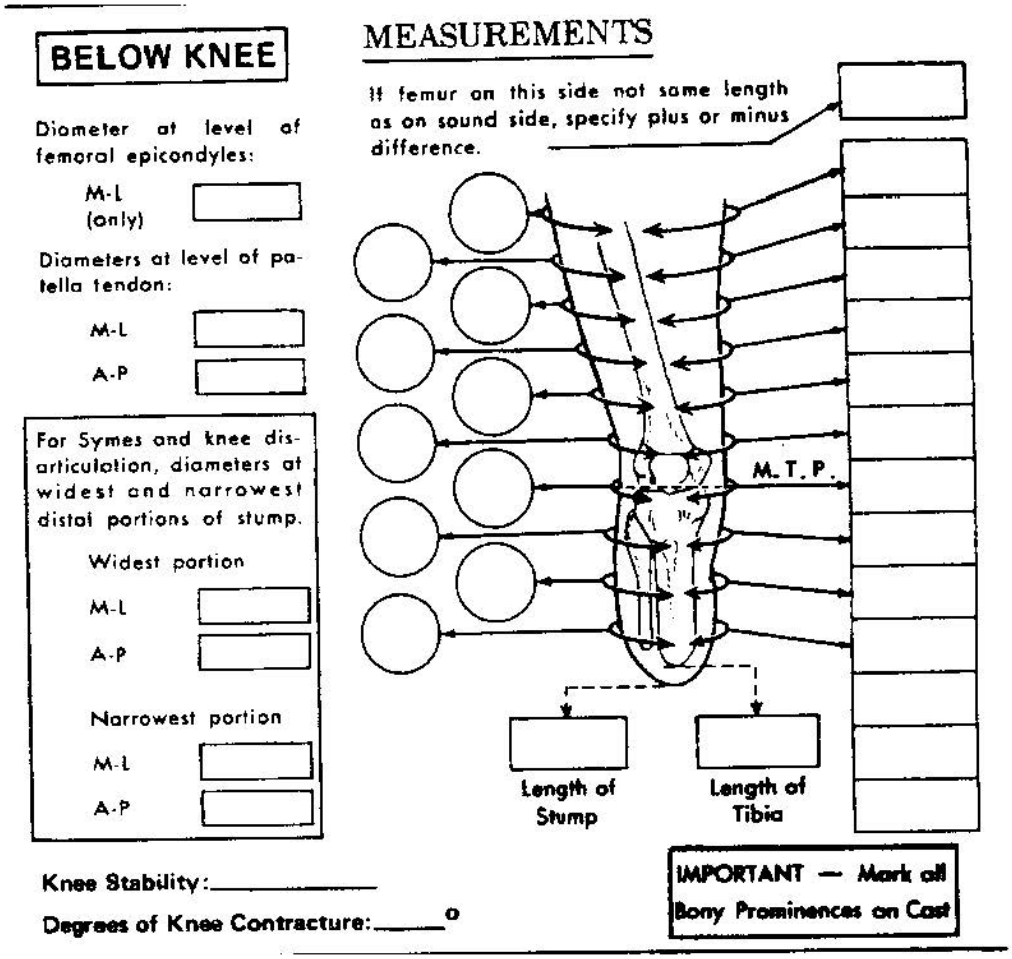


Left limb



Gait data sheet

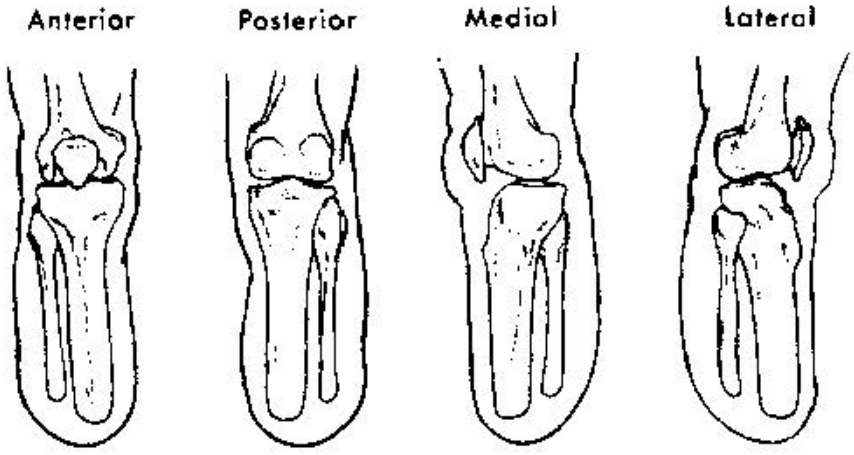
ID:	DATE:	Height:	Weight: BMI:		
	Session				
	Baseline	4 weeks	8 weeks	12 weeks	3 month follow up
Velocity					
Trial 1					
Trial 2					
Trail 3					
Average					
Step length					
Trial 1					
Trail 2					
Trial 3					
Average					
Percentage in stance time					
Trial 1					
Trial 2					
Trial 3					
Average					



Show Location of residual limb Details

A=Abrasion
B=Boil or skin infection
Bu=Bursa
Bs=Bone Spur
D=Discoloration
E=Edema
I=Irritation
M=Muscle bunching
P=Pressure Point
R= Redundant tissue
S=Scar
T=Trigger point

BELOW KNEE



Limb Shape:_____ Distal Padding:_____

Subcutaneous Tissue: Heavy ☐
Light ☐
Average ☐

Distal Pressure Tolerance: None ☐
Slight ☐
Good ☐

Condition of Thigh Musculature: ☐ Atrophy
☐ Normal

30

Condition of Stump Musculature: ☐ Atrophy

☐ Normal

Knee Stability: _____

Range of Knee Motion: _____

Condition of Cut Bones:

Tibia:_____

Fibula:_____

Remarks:_____

_____

Signature Date

Intervention Group Daily Log Book. Please answer these questions each day.

DATE: ID:	Comments
Please record FES setting: **Quadriceps (Thigh)** Channel 1 _____Hz _____pulse intensity_____ **Anterior Tibialis (Front below knee)** Channel 1 _____Hz _____pulse intensity_____ **Gastrocnemius (Back below knee)** Channel 1 _____Hz _____pulse intensity_____ Channel 2 _____Hz _____pulse intensity_____	
Did you experience a change in your health or medications today? Please circle YES or NO	
What was your activity level today? Please make a hash mark describing your activity level. **Sedentary** **Highly active** |----------|	
Did you experience any changes in your limb's condition? Please circle YES or NO	
How many hours did you wear your prosthesis today? Please circle one. 0 < 1 hour 1-3 hours 3-6 hours 6-10 hours 10+ hours	
Did you experience any limb **pain** with your prosthesis today? YES or NO What was your average **pain** level today? **No pain** **Extreme pain** |----------| 1 10	
How many minutes did you wear the FES device today? Please record specific time. 0 minutes <5 minutes 5-10 minutes 15 minutes other:_____	
Did you experience redness or irritation that lasted more than 20 minutes after using the FES today? Please circle YES or NO If Yes, please comment.	
Did you experience any limb **discomfort** with your prosthesis today? YES or NO What was your average **discomfort** level today? **No discomfort** **Extreme discomfort** |----------| 1 10	

Control Group Daily Log Book. Please answer these questions each day.

DATE: ID:	COMMENTS:
Did you experience a change in your health or medications today? Please circle YES or NO If Yes, please comment.	
What was your activity level today? Please make a hash mark describing your activity level. **Sedentary** **Highly active** I----------I	
Did you experience any changes in your limb's condition? Please circle YES or NO If Yes, please comment.	
How many hours did you wear your prosthesis today? Please circle one. 0 < 1 hour 1-3 hours 3-6 hours 6-10 hours 10+ hours	
Did you experience any limb **pain** with your prosthesis today? YES or NO What was your average pain level today? **No pain** **Extreme pain** I----------I	
Did you experience any limb **discomfort** with your prosthesis today? YES or NO What was your average pain level today? **No discomfort** **Extreme discomfort** I----------I	

NMES Use in Transtibial Amputations

Exit Telephone Questionnaire

1. After using the NMES intervention protocol I found it useful in overall improvement of your involved limb, in regards to **strength.**

   Strongly Disagree Strongly Agree

   0 1 2 3 4 5

   Comment:_____

2. After using the NMES intervention protocol I found it useful in overall improvement of your involved limb, in regards to **pain.**

   Strongly Disagree Strongly Agree

   0 1 2 3 4 5

   Comment:_____

3. After using the NMES intervention protocol I found it useful in overall improvement of your involved limb, in regards to **limb size.**

   Strongly Disagree Strongly Agree

   0 1 2 3 4 5

   Comment:_____

4. After using the NMES intervention protocol I found it useful in overall improvement of your involved limb, in regards to **walking ability.**

   Strongly Disagree Strongly Agree

   0 1 2 3 4 5

   Comment:_____

5. The health of my residual limb changed in a positive way.

Strongly Disagree					Strongly Agree
0	1	2	3	4	5

Comment:_____

6. The device was easy to use.

Strongly Disagree					Strongly Agree
0	1	2	3	4	5

Comment:_____

7. I would like to continue to use the NMES device at home on a continuous basis.

Strongly Disagree					Strongly Agree
0	1	2	3	4	5

Comment:_____

8. I would recommend the NMES intervention to other amputees.

Strongly Disagree					Strongly Agree
0	1	2	3	4	5

Comment:_____

BILIOGRAPHY

1. Deyo, R.A., et al., *A controlled trial of transcutaneous electrical nerve stimulation (TENS) and exercise for chronic low back pain.* N Engl J Med, 1990. **322**(23): p. 1627-34.

2. Sluka, K.A. and D. Walsh, *Transcutaneous electrical nerve stimulation: basic science mechanisms and clinical effectiveness.* J Pain, 2003. **4**(3): p. 109-21.

3. Doucet, B.M., A. Lam, and L. Griffin, *Neuromuscular electrical stimulation for skeletal muscle function.* Yale J Biol Med, 2012. **85**(2): p. 201-15.

4. Valenti, F., *[NEUROMUSCULAR ELECTROSTIMULATION IN CLINICAL PRACTICE].* Acta Anaesthesiol, 1964. **15**: p. 227-45.

5. Kegel, B., et al., *Effects of isometric muscle training on residual limb volume, strength, and gait of below-knee amputees.* Phys Ther, 1981. **61**(10): p. 1419-26.

6. Renstrom, P., et al., *Thigh muscle atrophy in below-knee amputees.* Scand J Rehabil Med Suppl, 1983. **9**: p. 150-62.

7. Isakov, E., et al., *Stump length as related to atrophy and strength of the thigh muscles in trans-tibial amputees.* Prosthet Orthot Int, 1996. **20**(2): p. 96-100.

8. Raya, M.A., et al., *Impairment variables predicting activity limitation in individuals with lower limb amputation.* Prosthet Orthot Int, 2010. **34**(1): p. 73-84.

9. Vaz, M.A., et al., *Neuromuscular electrical stimulation (NMES) reduces structural and functional losses of quadriceps muscle and improves health status in patients with knee osteoarthritis.* J Orthop Res, 2013. **31**(4): p. 511-6.

10. Bruce-Brand, R.A., et al., *Effects of home-based resistance training and neuromuscular electrical stimulation in knee osteoarthritis: a randomized controlled trial.* BMC Musculoskelet Disord, 2012. **13**: p. 118.

11. Stein, C., et al., *Effects of Electrical Stimulation in Spastic Muscles After Stroke: Systematic Review and Meta-Analysis of Randomized Controlled Trials.* Stroke, 2015. **46**(8): p. 2197-205.

12. Stackhouse, S.K., et al., *Neuromuscular electrical stimulation versus volitional isometric strength training in children with spastic diplegic cerebral palsy: a preliminary study.* Neurorehabil Neural Repair, 2007. **21**(6): p. 475-85.

13. Piva, S.R., et al., *Neuromuscular electrical stimulation and volitional exercise for individuals with rheumatoid arthritis: a multiple-patient case report.* Phys Ther, 2007. **87**(8): p. 1064-77.

14. Snyder-Mackler, L., et al., *Strength of the quadriceps femoris muscle and functional recovery after reconstruction of the anterior cruciate ligament. A prospective, randomized clinical trial of electrical stimulation.* J Bone Joint Surg Am, 1995. **77**(8): p. 1166-73.

15. Fitzgerald, G.K., S.R. Piva, and J.J. Irrgang, *A modified neuromuscular electrical stimulation protocol for quadriceps strength training following anterior cruciate ligament reconstruction.* J Orthop Sports Phys Ther, 2003. **33**(9): p. 492-501.

16. Yang, Y.S., et al., *Biomechanical analysis of functional electrical stimulation on trunk musculature during wheelchair propulsion.* Neurorehabil Neural Repair, 2009. **23**(7): p. 717-25.

17. Kim, K.M., et al., *Effects of neuromuscular electrical stimulation after anterior cruciate ligament reconstruction on quadriceps strength, function, and patient-oriented outcomes: a systematic review.* J Orthop Sports Phys Ther, 2010. **40**(7): p. 383-91.

18. Walls, R.J., et al., *Effects of preoperative neuromuscular electrical stimulation on quadriceps strength and functional recovery in total knee arthroplasty. A pilot study.* BMC Musculoskelet Disord, 2010. **11**: p. 119.

19. Ephraim, P.L., et al., *Phantom pain, residual limb pain, and back pain in amputees: results of a national survey.* Arch Phys Med Rehabil, 2005. **86**(10): p. 1910-9.

20. Houghton, A.D., et al., *Phantom pain: natural history and association with rehabilitation.* Ann R Coll Surg Engl, 1994. **76**(1): p. 22-5.

21. Nikolajsen, L., *Postamputation pain: studies on mechanisms.* Dan Med J, 2012. **59**(10): p. B4527.

22. Mulvey, M.R., et al., *Transcutaneous electrical nerve stimulation for phantom pain and stump pain in adult amputees.* Pain Pract, 2013. **13**(4): p. 289-96.

23. Finsen, V., et al., *Transcutaneous electrical nerve stimulation after major amputation.* J Bone Joint Surg Br, 1988. **70**(1): p. 109-12.

24. Neder, J.A., et al., *Home based neuromuscular electrical stimulation as a new rehabilitative strategy for severely disabled patients with chronic obstructive pulmonary disease (COPD).* Thorax, 2002. **57**(4): p. 333-7.

25. Quittan, M., et al., *Strength improvement of knee extensor muscles in patients with chronic heart failure by neuromuscular electrical stimulation.* Artif Organs, 1999. **23**(5): p. 432-5.

26. Iwatsu, K., et al., *Feasibility of neuromuscular electrical stimulation immediately after cardiovascular surgery.* Arch Phys Med Rehabil, 2015. **96**(1): p. 63-8.

27. Vieira, P.J., et al., *Neuromuscular electrical stimulation improves clinical and physiological function in COPD patients.* Respir Med, 2014. **108**(4): p. 609-20.

28. Laufer, Y., H. Shtraker, and M. Elboim Gabyzon, *The effects of exercise and neuromuscular electrical stimulation in subjects with knee osteoarthritis: a 3-month follow-up study.* Clin Interv Aging, 2014. **9**: p. 1153-61.

29. Snyder-Mackler, L., et al., *Electrical stimulation of the thigh muscles after reconstruction of the anterior cruciate ligament. Effects of electrically elicited contraction of the quadriceps femoris and hamstring muscles on gait and on strength of the thigh muscles.* J Bone Joint Surg Am, 1991. **73**(7): p. 1025-36.

30. Michael, J., Bowker, JH. (Eds.), *Atlas of amputations and limb deficiencies: surgical, prosthetic, and rehabilitation principles*. Vol. 3. 2004, Rosemont, IL: American Academy of Orthopaedic Surgeons.

31. Spires, M., Kelly, BM., Davis, AJ. , *Prosthetic Restoration and Rehabilitation of the Upper and Lower Extremity*2013: Demos Medical Publishing.

32. Gray, H., *Anatomy of the Human Body*1918, Philadelphia: Lea & Febiger.

33. Pasquina, P., Cooper, R A. , *Care of the combat amputee*2009: Government Printing Office.

34. Katsamouris, A., et al., *Transcutaneous oxygen tension in selection of amputation level.* Am J Surg, 1984. **147**(4): p. 510-7.

35. Karanfilian, R.G., et al., *The value of laser Doppler velocimetry and transcutaneous oxygen tension determination in predicting healing of ischemic forefoot ulcerations and amputations in diabetic and nondiabetic patients.* J Vasc Surg, 1986. **4**(5): p. 511-6.

36. Brown, B.J., et al., *Ertl below-knee amputation using a vascularized fibular strut in a nontrauma elderly population: a case series.* Ann Plast Surg, 2014. **73**(2): p. 196-201.

37. Jaegers, S.M., J.H. Arendzen, and H.J. de Jongh, *Changes in hip muscles after above-knee amputation.* Clin Orthop Relat Res, 1995(319): p. 276-84.

38. Sherk, V.D., M.G. Bemben, and D.A. Bemben, *Interlimb Muscle and Fat Comparisons in Persons With Lower-Limb Amputation.* Archives of Physical Medicine and Rehabilitation, 2010. **91**(7): p. 1077-1081.

39. Gailey, R., et al., *Review of secondary physical conditions associated with lower-limb amputation and long-term prosthesis use.* J Rehabil Res Dev, 2008. **45**(1): p. 15-29.

40. Eisert, O. and O.W. Tester, *Dynamic exercises for lower extremity amputees.* Arch Phys Med Rehabil, 1954. **35**(11): p. 695-704.

41. Kohler, F., et al., *Developing Core Sets for persons following amputation based on the International Classification of Functioning, Disability and Health as a way to specify functioning.* Prosthet Orthot Int, 2009. **33**(2): p. 117-29.

42. Murphy, E.F. and A.B. Wilson, Jr., *Anatomical and physiological considerations in below-knee prosthetics.* Artif Limbs, 1962. **6**: p. 4-15.

43. Fraisse, N., et al., *[Muscles of the below-knee amputees].* Ann Readapt Med Phys, 2008. **51**(3): p. 218-27.

44. Portincasa, A., et al., *Microsurgical treatment of injury to peripheral nerves in upper and lower limbs: a critical review of the last 8 years.* Microsurgery, 2007. **27**(5): p. 455-62.

45. Centomo, H., et al., *Differences in the coordination of agonist and antagonist muscle groups in below-knee amputee and able-bodied children during dynamic exercise.* J Electromyogr Kinesiol, 2008. **18**(3): p. 487-94.

46. Sadeghi, H., P. Allard, and P.M. Duhaime, *Muscle power compensatory mechanisms in below-knee amputee gait.* Am J Phys Med Rehabil, 2001. **80**(1): p. 25-32.

47. Smith, D., Michael, JW., Bowker, JH, Perry, J, *Atlas of amputations and limb deficiencies: surgical, prosthetic, and rehabilitation principles* 3rd ed2004, Rosemont, IL American Academy of Orthopaedic Surgeons. 367–384.

48. Zachariah, S.G., et al., *Shape and volume change in the transtibial residuum over the short term: preliminary investigation of six subjects.* J Rehabil Res Dev, 2004. **41**(5): p. 683-94.

49. Renstrom, P., G. Grimby, and E. Larsson, *Thigh muscle strength in below-knee amputees.* Scand J Rehabil Med Suppl, 1983. **9**: p. 163-73.

50. Sanders, J.E., et al., *Amputee socks: how does sock ply relate to sock thickness?* Prosthet Orthot Int, 2012. **36**(1): p. 77-86.

51. Sanders, J.E., et al., *Clinical utility of in-socket residual limb volume change measurement: case study results.* Prosthet Orthot Int, 2009. **33**(4): p. 378-90.

52. Cagle, J.C., et al., *Amputee socks: thickness of multiple socks.* Prosthet Orthot Int, 2014. **38**(5): p. 405-12.

53. Gholizadeh, H., et al., *Transtibial prosthetic socket pistoning: static evaluation of Seal-In((R)) X5 and Dermo((R)) Liner using motion analysis system.* Clin Biomech (Bristol, Avon), 2012. **27**(1): p. 34-9.

54. Eshraghi, A., et al., *Pistoning assessment in lower limb prosthetic sockets.* Prosthet Orthot Int, 2012. **36**(1): p. 15-24.

55. Rommers, G.M., et al., *A study of technical changes to lower limb prostheses after initial fitting.* Prosthet Orthot Int, 2000. **24**(1): p. 28-38.

56. Goswami, J., et al., *Walking in a vacuum-assisted socket shifts the stump fluid balance.* Prosthet Orthot Int, 2003. **27**(2): p. 107-13.

57. Sanders, J.E. and S. Fatone, *Residual limb volume change: systematic review of measurement and management.* J Rehabil Res Dev, 2011. **48**(8): p. 949-86.

58. Beil, T.L. and G.M. Street, *Comparison of interface pressures with pin and suction suspension systems.* J Rehabil Res Dev, 2004. **41**(6A): p. 821-8.

59. Klute, G.K., et al., *Vacuum-assisted socket suspension compared with pin suspension for lower extremity amputees: effect on fit, activity, and limb volume.* Arch Phys Med Rehabil, 2011. **92**(10): p. 1570-5.

60. Gorman, W.P., K.R. Davis, and R. Donnelly, *ABC of arterial and venous disease. Swollen lower limb-1: general assessment and deep vein thrombosis.* BMJ, 2000. **320**(7247): p. 1453-6.

61. Ciocon, J.O., B.B. Fernandez, and D.G. Ciocon, *Leg edema: clinical clues to the differential diagnosis.* Geriatrics, 1993. **48**(5): p. 34-40, 45.

62. Stewart, P., *Medications that Cause and Worsen Edema,* in *LymphLink*2009, National Lymphedema Network San Francisco, CA. p. 9-10.

63. Ruschhaupt WF, I., *Peripheral vascular diseases.* , in *The swollen limb*, G.R. Young JR, Olin JW, Bartolomew JR Editor 1991, Mosby Yearbook St. Louis. p. 639-50.

64. Williams, S.A., G. Rayman, and J.E. Tooke, *Dependent oedema and attenuation of postural vasoconstriction associated with nifedipine therapy for hypertension in diabetic patients.* Eur J Clin Pharmacol, 1989. **37**(4): p. 333-5.

65. Hyman, D.A. and P.R. Cohen, *Stasis dermatitis as a complication of recurrent levofloxacin-associated bilateral leg edema.* Dermatol Online J, 2013. **19**(11): p. 20399.

66. V., K., *Drugs and Lymphoedema: Those which may cause edema or make lymphedema worse.*, in *LymphLink*2012, National Lymphedema Network: San Francsico, CA. p. 1-3.

67. Cosmo, P., et al., *Effects of transcutaneous nerve stimulation on the microcirculation in chronic leg ulcers.* Scand J Plast Reconstr Surg Hand Surg, 2000. **34**(1): p. 61-4.

68. IO, W.M., et al., *Effect of neuromuscular electrical stimulation on foot/ankle volume during standing.* Med Sci Sports Exerc, 2003. **35**(4): p. 630-4.

69. Levine, S.P., et al., *Blood flow in the gluteus maximus of seated individuals during electrical muscle stimulation.* Arch Phys Med Rehabil, 1990. **71**(9): p. 682-6.

70. *Gait Analysis: Normal and Pathological Function.* Journal of Sports Science & Medicine, 2010. **9**(2): p. 353-353.

71. *Lower-Limb Prosthetics and Orthotics: Clinical Concepts.* Journal of Sports Science & Medicine, 2011. **10**(1): p. 235-235.

72. Esquenazi, A., *Pain management post amputation. Pain Management in Rehabilitation,* in *Pain Management in Rehabilitation,* M. Dr. Trilok Monga, Martin Grabois, Editor 2002, Demos Medical Publishing: New York, NY. p. 191-202.

73. Carlson, J.M., *Functional Limitations From Pain Caused by Repetitive Loading on the Skin: A Review and Discussion for Practitioners, With New Data for Limiting Friction Loads.* Jpo Journal of Prosthetics and Orthotics, 2006. **18**(4): p. 93-103.

74. Legro, M.W., et al., *Issues of importance reported by persons with lower limb amputations and prostheses.* J Rehabil Res Dev, 1999. **36**(3): p. 155-63.

75. Hsu, E. and S.P. Cohen, *Postamputation pain: epidemiology, mechanisms, and treatment.* J Pain Res, 2013. **6**: p. 121-36.

76. Ehde, D.M., et al., *Chronic phantom sensations, phantom pain, residual limb pain, and other regional pain after lower limb amputation.* Archives of Physical Medicine and Rehabilitation, 2000. **81**(8): p. 1039-1044.

77. Knotkova, H., et al., *Current and future options for the management of phantom-limb pain.* J Pain Res, 2012. **5**: p. 39-49.

78. Sherman, R.A., C.J. Sherman, and N.G. Gall, *A survey of current phantom limb pain treatment in the United States.* PAIN, 1980. **8**(1): p. 85-99.

79. Hazelgrove, J.F. and P.D. Rogers, *Phantom limb pain--a complication of lower extremity wound management.* Int J Low Extrem Wounds, 2002. **1**(2): p. 112-24.

80. Behr, J., et al., *Pain and pain-related interference in adults with lower-limb amputation: comparison of knee-disarticulation, transtibial, and transfemoral surgical sites.* J Rehabil Res Dev, 2009. **46**(7): p. 963.

81. Flor, H., *Phantom-limb pain: characteristics, causes, and treatment.* Lancet Neurol, 2002. **1**(3): p. 182-9.

82. Rakel, B. and J.O. Barr, *Physical modalities in chronic pain management.* Nurs Clin North Am, 2003. **38**(3): p. 477-94.

83. Seidel, S., et al., *[Mirror therapy for phantom limb pain--a systematic review]*. Wien Klin Wochenschr, 2009. **121**(13-14): p. 440-4.

84. Marasco, P.D., et al., *Robotic touch shifts perception of embodiment to a prosthesis in targeted reinnervation amputees.* Brain, 2011. **134**(3): p. 747-758.

85. Kuiken, T.A., et al., *Targeted muscle reinnervation for the upper and lower extremity.* Techniques in Orthopaedics, 2017. **32**(2): p. 109-116.

86. Julien, R.M., *A primer of drug action: a concise, nontechnical guide to the actions, uses, and side effects of psychoactive drugs.*2001, New York: WH Freeman and Company.

87. McCormick, Z., et al., *Phantom limb pain: a systematic neuroanatomical-based review of pharmacologic treatment.* Pain Med, 2014. **15**(2): p. 292-305.

88. Chabal, C., et al., *Long-term transcutaneous electrical nerve stimulation (TENS) use: impact on medication utilization and physical therapy costs.* Clin J Pain, 1998. **14**(1): p. 66-73.

89. Ezendam, D., R.M. Bongers, and M.J. Jannink, *Systematic review of the effectiveness of mirror therapy in upper extremity function.* Disabil Rehabil, 2009. **31**(26): p. 2135-49.

90. Trevelyan, E.G., W.A. Turner, and N. Robinson, *Acupuncture for the treatment of phantom limb pain in lower limb amputees: study protocol for a randomized controlled feasibility trial.* Trials, 2015. **16**: p. 158.

91. Covey, S.J., & Muonio, J. , *Flow Constraint and Loading Rate Effects on Prosthetic Liner Material and Human Tissue Mechanical Response.* JPO: Journal of Prosthetics and Orthotics, 2000. **12**(1): p. 15-32.

92. Kahle, J., *Conventional and Hydrostatic Transtibial Interface Comparison.* JPO: Journal of Prosthetics and Orthotics,, 1999. **11**(2): p. 85-91.

93. Schuch, C., Wilson, AB, *The USe of Surlyn and Polpropylene in Flexible Brim Socket Designs for Below-knww Prostheses.* Clinical Prosthetics and Orthotics, 1986. **10**(3): p. 105-110.

94. Desantana, J.M., K.A. Sluka, and G.R. Lauretti, *High and low frequency TENS reduce postoperative pain intensity after laparoscopic tubal ligation: a randomized controlled trial.* Clin J Pain, 2009. **25**(1): p. 12-9.

95. Giummarra, M.J. and G.L. Moseley, *Phantom limb pain and bodily awareness: current concepts and future directions.* Curr Opin Anaesthesiol, 2011. **24**(5): p. 524-31.

96. Mangold, S., et al., *Transcutaneous functional electrical stimulation for grasping in subjects with cervical spinal cord injury.* Spinal Cord, 2005. **43**(1): p. 1-13.

97. Smans, J., H.H. Korsten, and J.A. Blom, *Optimal surface electrode positioning for reliable train of four muscle relaxation monitoring.* Int J Clin Monit Comput, 1996. **13**(1): p. 9-20.

98. Mulvey, M.R., et al., *Transcutaneous electrical nerve stimulation (TENS) for phantom pain and stump pain following amputation in adults.* Cochrane Database Syst Rev, 2010(5): p. CD007264.

99. Ying, K.N. and A. While, *Pain relief in osteoarthritis and rheumatoid arthritis: TENS.* Br J Community Nurs, 2007. **12**(8): p. 364-71.

100. Mannheimer, C. and C.A. Carlsson, *The analgesic effect of transcutaneous electrical nerve stimulation (TNS) in patients with rheumatoid arthritis. A comparative study of different pulse patterns.* PAIN, 1979. **6**(3): p. 329-34.

101. Law, P.P., G.L. Cheing, and A.Y. Tsui, *Does transcutaneous electrical nerve stimulation improve the physical performance of people with knee osteoarthritis?* J Clin Rheumatol, 2004. **10**(6): p. 295-9.

102. Cheing, G.L. and C.W. Hui-Chan, *Would the addition of TENS to exercise training produce better physical performance outcomes in people with knee osteoarthritis than either intervention alone?* Clin Rehabil, 2004. **18**(5): p. 487-97.

103. Borghi, B., M. D'Addabbo, and R. Borghi, *Can neural blocks prevent phantom limb pain?* Pain Manag, 2014. **4**(4): p. 261-6.

104. Abrahams, M.S., et al., *Ultrasound guidance compared with electrical neurostimulation for peripheral nerve block: a systematic review and meta-analysis of randomized controlled trials.* Br J Anaesth, 2009. **102**(3): p. 408-17.

105. Jeon, Y. and B.K. Huh, *Spinal cord stimulation for chronic pain.* Ann Acad Med Singapore, 2009. **38**(11): p. 998-1003.

106. Krainick, J.U., U. Thoden, and T. Riechert, *Pain reduction in amputees by long-term spinal cord stimulation. Long-term follow-up study over 5 years.* J Neurosurg, 1980. **52**(3): p. 346-50.

107. Ducic, I., et al., *The role of peripheral nerve surgery in the treatment of chronic pain associated with amputation stumps.* Plast Reconstr Surg, 2008. **121**(3): p. 908-14; discussion 915-7.

108. Cheesborough, J.E., et al., *Targeted muscle reinnervation in the initial management of traumatic upper extremity amputation injury.* Hand (N Y), 2014. **9**(2): p. 253-7.

109. Meuwly, C., et al., *Anesthetic influence on occurrence and treatment of the trigemino-cardiac reflex: a systematic literature review.* Medicine (Baltimore), 2015. **94**(18): p. e807.

110. Ferris, A.E., et al., *Evaluation of a powered ankle-foot prosthetic system during walking.* Arch Phys Med Rehabil, 2012. **93**(11): p. 1911-8.

111. Boone, D.A., et al., *Influence of malalignment on socket reaction moments during gait in amputees with transtibial prostheses.* Gait Posture, 2013. **37**(4): p. 620-6.

112. Yu, W.W., et al., *Non-linear analysis of body responses to functional electrical stimulation on hemiplegic subjects.* Proc Inst Mech Eng H, 2009. **223**(6): p. 653-62.

113. Belanger, M., et al., *Electrical stimulation: can it increase muscle strength and reverse osteopenia in spinal cord injured individuals?* Arch Phys Med Rehabil, 2000. **81**(8): p. 1090-8.

114. Monaghan, B., B. Caulfield, and D.P. O'Mathuna, *Surface neuromuscular electrical stimulation for quadriceps strengthening pre and post total knee replacement.* Cochrane Database Syst Rev, 2010(1): p. CD007177.

115. Moore, S.R. and J. Shurman, *Combined neuromuscular electrical stimulation and transcutaneous electrical nerve stimulation for treatment of chronic back pain: a double-blind, repeated measures comparison.* Arch Phys Med Rehabil, 1997. **78**(1): p. 55-60.
116. Greve, J.M., et al., *Functional electrical stimulation (FES): muscle histochemical analysis.* Paraplegia, 1993. **31**(12): p. 764-70.

117. de Oliveira Melo, M., F.A. Aragao, and M.A. Vaz, *Neuromuscular electrical stimulation for muscle strengthening in elderly with knee osteoarthritis - a systematic review.* Complement Ther Clin Pract, 2013. **19**(1): p. 27-31.

118. Thakral, G., et al., *Electrical stimulation to accelerate wound healing.* Diabet Foot Ankle, 2013. **4**.

119. Peters, E.J., et al., *Electric stimulation as an adjunct to heal diabetic foot ulcers: a randomized clinical trial.* Arch Phys Med Rehabil, 2001. **82**(6): p. 721-5.

120. Broderick, B.J., et al., *Comparative lower limb hemodynamics using neuromuscular electrical stimulation (NMES) versus intermittent pneumatic compression (IPC).* Physiol Meas, 2014. **35**(9): p. 1849-59.

121. Gaines, J.M., E.J. Metter, and L.A. Talbot, *The effect of neuromuscular electrical stimulation on arthritis knee pain in older adults with osteoarthritis of the knee1.* Applied Nursing Research, 2004. **17**(3): p. 201-206.

122. Imoto, A.M., et al., *Is neuromuscular electrical stimulation effective for improving pain, function and activities of daily living of knee osteoarthritis patients? A randomized clinical trial.* Sao Paulo Med J, 2013. **131**(2): p. 80-7.

123. Demircioglu, D.T., et al., *The effect of neuromuscular electrical stimulation on functional status and quality of life after knee arthroplasty: a randomized controlled study.* J Phys Ther Sci, 2015. **27**(8): p. 2501-6.

124. Vance, C.G., et al., *Using TENS for pain control: the state of the evidence.* Pain Manag, 2014. **4**(3): p. 197-209.

125. Melzack, R. and P.D. Wall, *Pain mechanisms: a new theory.* Science, 1965. **150**(3699): p. 971-9.

126. DeSantana, J.M., et al., *Effectiveness of Transcutaneous Electrical Nerve Stimulation for Treatment of Hyperalgesia and Pain.* Curr Rheumatol Rep, 2008. **10**(6): p. 492-9.

127. Gaines, J.M., E.J. Metter, and L.A. Talbot, *The effect of neuromuscular electrical stimulation on arthritis knee pain in older adults with osteoarthritis of the knee.* Appl Nurs Res, 2004. **17**(3): p. 201-6.

128. Ziegler-Graham, K., et al., *Estimating the prevalence of limb loss in the United States: 2005 to 2050.* Arch Phys Med Rehabil, 2008. **89**(3): p. 422-9.

129. Staff, P.A.P.N., *Fact Sheet: Pain management and the amputee*, N.L.L.I. Center, Editor 2006, Amputee Coalition of America: Knoxville, TN. p. 1-11.

130. Santos, J., et al., *Tapentadol for chronic musculoskeletal pain in adults.* Cochrane Database Syst Rev, 2015. **5**: p. CD009923.

131. Dorn, C., et al., *The Effect of the Modified Lateral Suprascapular Block on Shoulder Function in Patients With Chronic Shoulder Pain.* Anesth Pain Med, 2015. **5**(6): p. e31640.

132. Branchini, M., et al., *Fascial Manipulation(R) for chronic aspecific low back pain: a single blinded randomized controlled trial.* F1000Res, 2015. **4**: p. 1208.

133. Kosasih, J.B. and M.B. Silver-Thorn, *Sensory changes in adults with unilateral transtibial amputation.* J Rehabil Res Dev, 1998. **35**(1): p. 85-90.

134. Dee, K.L., Z. , *Correct determination of body mass index in people with lower limb amputation.* , in *ARSA-Advanced Research in Scientific Areas*2012.

135. Himes, J.H., *New equation to estimate body mass index in amputees.* J Am Diet Assoc, 1995. **95**(6): p. 646.

136. Piva, S.R., et al., *Association between Disability and Psychological Factors and Dose of Neuromuscular Electrical Stimulation in Subjects with Rheumatoid Arthritis.* Physiother Pract Res, 2013. **34**(2): p. 57-66.

137. Stevens-Lapsley, J.E., et al., *Impact of body mass index on functional performance after total knee arthroplasty.* J Arthroplasty, 2010. **25**(7): p. 1104-9.

138. Lewek, M.D., K.S. Rudolph, and L. Snyder-Mackler, *Quadriceps femoris muscle weakness and activation failure in patients with symptomatic knee osteoarthritis.* J Orthop Res, 2004. **22**(1): p. 110-5.

139. Hays, R.D., C.D. Sherbourne, and R.M. Mazel, *User's manual for the medical outcomes study (MOS) core measures of health-related quality of life*1995, Santa Monica, CA: Rand Corp.

140. Raichle, K.A., et al., *The reliability and validity of pain interference measures in persons with spinal cord injury.* J Pain, 2006. **7**(3): p. 179-86.

141. Melzack, R., *The short-form McGill Pain Questionnaire.* PAIN, 1987. **30**(2): p. 191-7.

142. Von Korff, M., et al., *Grading the severity of chronic pain.* PAIN, 1992. **50**(2): p. 133-49.

143. Harbo, T., J. Brincks, and H. Andersen, *Maximal isokinetic and isometric muscle strength of major muscle groups related to age, body mass, height, and sex in 178 healthy subjects.* Eur J Appl Physiol, 2012. **112**(1): p. 267-75.

144. Pincivero, D.M., S.M. Lephart, and R.A. Karunakara, *Reliability and precision of isokinetic strength and muscular endurance for the quadriceps and hamstrings.* Int J Sports Med, 1997. **18**(2): p. 113-7.

145. Wilk, K.E., et al., *The relationship between subjective knee scores, isokinetic testing, and functional testing in the ACL-reconstructed knee.* J Orthop Sports Phys Ther, 1994. **20**(2): p. 60-73.

146. Spendiff, O., N. Longford, and E. Winter, *Effects of fatigue on the torque-velocity relation in muscle.* Br J Sports Med, 2002. **36**(6): p. 431-5.

147. Lund, H., et al., *Learning effect of isokinetic measurements in healthy subjects, and reliability and comparability of Biodex and Lido dynamometers.* Clin Physiol Funct Imaging, 2005. **25**(2): p. 75-82.

148. Corio, F., Troiano R., Magel, J., *The Effects of Spinal Stabilization Exercises on the Spatial and Temporal Parameters of Gait in Individuals With Lower Limb Loss.* Journal of Prosthtics & Orthotics, 2010. **22**(4): p. 230-236.

149. Robinson, J.L., G.L. Smidt, and J.S. Arora, *Accelerographic, temporal, and distance gait factors in below-knee amputees.* Phys Ther, 1977. **57**(8): p. 898-904.

150. Bateni, H., & Olney, S. J. , *Kinematic and kinetic variations of below-knee amputee gait.* JPO: Journal of Prosthetics and Orthotics, 2002. **14**(1): p. 2-10.

151. Herzig, D., N.A. Maffiuletti, and P. Eser, *The Application of Neuromuscular Electrical Stimulation Training in Various Non-neurologic Patient Populations: A Narrative Review.* PM R, 2015. **7**(11): p. 1167-78.

152. Gobbo, M., et al., *Muscle motor point identification is essential for optimizing neuromuscular electrical stimulation use.* J Neuroeng Rehabil, 2014. **11**: p. 17.

153. Botter, A., et al., *Atlas of the muscle motor points for the lower limb: implications for electrical stimulation procedures and electrode positioning.* Eur J Appl Physiol, 2011. **111**(10): p. 2461-71.

154. Delitto, A., et al., *A study of discomfort with electrical stimulation.* Phys Ther, 1992. **72**(6): p. 410-21; discussion on 421-4.

155. Klopsteg, P.E., et al., *Human limbs and their substitutes; presenting results of engineering and medical studies of the human extremities and application of the data to the design and fitting of artificial limbs and to the care and training of amputees [by] Paul E. Klopsteg, Philip D. Wilson, et al*1954, New York: McGraw-Hill.

156. McDonough, A.L., et al., *The validity and reliability of the GAITRite system's measurements: A preliminary evaluation.* Arch Phys Med Rehabil, 2001. **82**(3): p. 419-25.

157. McCleary, R.W. and J.C. Andersen, *Test-retest reliability of reciprocal isokinetic knee extension and flexion peak torque measurements.* J Athl Train, 1992. **27**(4): p. 362-5.

158. de Boer-Wilzing, V.G., et al., *Variation in results of volume measurements of stumps of lower-limb amputees: a comparison of 4 methods.* Arch Phys Med Rehabil, 2011. **92**(6): p. 941-6.

159. Talbot, L.A., et al., *A home-based protocol of electrical muscle stimulation for quadriceps muscle strength in older adults with osteoarthritis of the knee.* J Rheumatol, 2003. **30**(7): p. 1571-8.

160. Caggiano, E., et al., *Effects of electrical stimulation or voluntary contraction for strengthening the quadriceps femoris muscles in an aged male population.* J Orthop Sports Phys Ther, 1994. **20**(1): p. 22-8.

161. Lilja, M., P. Hoffmann, and T. Oberg, *Morphological changes during early trans-tibial prosthetic fitting.* Prosthet Orthot Int, 1998. **22**(2): p. 115-22.

162. Fernie, G.R. and P.J. Holliday, *Volume fluctuations in the residual limbs of lower limb amputees.* Arch Phys Med Rehabil, 1982. **63**(4): p. 162-5.

163. Stratford, P.W.S., G. , *FEATURE ARTICLES-The reliability, consistency, and clinical application of a numeric pain rating scale.* Physiotherapy Canada, 2001. **53**(2): p. 88-91.

164. Childs, J.D., S.R. Piva, and J.M. Fritz, *Responsiveness of the numeric pain rating scale in patients with low back pain.* Spine (Phila Pa 1976), 2005. **30**(11): p. 1331-4.

165. Farrar, J.T., et al., *Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale.* PAIN, 2001. **94**(2): p. 149-58.

166. Cherian, J.J., et al., *The effects of various physical non-operative modalities on the pain in osteoarthritis of the knee.* Bone Joint J, 2016. **98-B**(1 Suppl A): p. 89-94.

167. Williamson, A. and B. Hoggart, *Pain: a review of three commonly used pain rating scales.* J Clin Nurs, 2005. **14**(7): p. 798-804.

168. Collen, F.M., D.T. Wade, and C.M. Bradshaw, *Mobility after stroke: reliability of measures of impairment and disability.* Int Disabil Stud, 1990. **12**(1): p. 6-9.

169. Bohannon, R.W., *Comfortable and maximum walking speed of adults aged 20-79 years: reference values and determinants.* Age Ageing, 1997. **26**(1): p. 15-9.

170. Skinner, H.B. and D.J. Effeney, *Gait analysis in amputees.* Am J Phys Med, 1985. **64**(2): p. 82-9.

171. Waters, R.L., et al., *Energy cost of walking of amputees: the influence of level of amputation.* J Bone Joint Surg Am, 1976. **58**(1): p. 42-6.

172. Huang, C.T., et al., *Amputation: energy cost of ambulation.* Arch Phys Med Rehabil, 1979. **60**(1): p. 18-24.

173. Musselman, K.E., et al., *Training of walking skills overground and on the treadmill: case series on individuals with incomplete spinal cord injury.* Phys Ther, 2009. **89**(6): p. 601-11.

174. Tilson, J.K., et al., *Meaningful gait speed improvement during the first 60 days poststroke: minimal clinically important difference.* Phys Ther, 2010. **90**(2): p. 196-208.

175. Lam, T., V.K. Noonan, and J.J. Eng, *A systematic review of functional ambulation outcome measures in spinal cord injury.* Spinal Cord, 2008. **46**(4): p. 246-54.

176. Smidt, G.L., *Gait in Rehabilitation*1990, New York: Churchill Livingstone.

177. Breakey, J., *Gait of unilateral below-knee amputees.* . Orthot Prosthet, 1976. **30**: p. 17-24.

178. Delitto, A., et al., *Electrical stimulation versus voluntary exercise in strengthening thigh musculature after anterior cruciate ligament surgery.* Phys Ther, 1988. **68**(5): p. 660-3.

179. Pantovic, M., et al., *Effects of Neuromuscular Electrical Stimulation and Resistance Training on Knee Extensor/Flexor Muscles.* Coll Antropol, 2015. **39 Suppl 1**: p. 153-7.

180. Dong, Y. and C.-Y.J. Peng, *Principled missing data methods for researchers.* SpringerPlus, 2013. **2**(1): p. 222.

181. Biesecker, L.G., *Hypothesis-generating research and predictive medicine.* Genome research, 2013. **23**(7): p. 1051-1053.

182. Fragala, M.S., A.M. Kenny, and G.A. Kuchel, *Muscle quality in aging: a multi-dimensional approach to muscle functioning with applications for treatment.* Sports Medicine, 2015. **45**(5): p. 641-658.

183. Helliwell, T., et al., *Muscle fibre atrophy in critically ill patients is associated with the loss of myosin filaments and the presence of lysosomal enzymes and ubiquitin.* Neuropathology and applied neurobiology, 1998. **24**(6): p. 507-517.

184. Fukumoto, Y., et al., *Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons.* Eur J Appl Physiol, 2012. **112**(4): p. 1519-1525.

185. Bochkezanian, V., et al., *Effects of Neuromuscular Electrical Stimulation in People with Spinal Cord Injury.* Med Sci Sports Exerc, 2018.

186. Reimers, C.D., T. Harder, and H. Saxe, *Age-related muscle atrophy does not affect all muscles and can partly be compensated by physical activity: An ultrasound study1*. Journal of the neurological sciences, 1998. **159**(1): p. 60-66.

187. Nilwik, R., et al., *The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size.* Experimental gerontology, 2013. **48**(5): p. 492-498.

188. Paddon-Jones, D., et al., *Role of dietary protein in the sarcopenia of aging–*. The American journal of clinical nutrition, 2008. **87**(5): p. 1562S-1566S.

189. Sayer, A.A., et al., *The developmental origins of sarcopenia.* The Journal of Nutrition Health and Aging, 2008. **12**(7): p. 427.

190. Thompson, D., *Aging and sarcopenia.* Journal of Musculoskeletal and Neuronal Interactions, 2007. **7**(4): p. 344.

191. Berman, B.M., et al., *Effectiveness of acupuncture as adjunctive therapy in osteoarthritis of the knee: a randomized, controlled trial.* Annals of internal medicine, 2004. **141**(12): p. 901-910.

192. Takano, Y., et al., *Increasing muscle strength and mass of thigh in elderly people with the hybrid-training method of electrical stimulation and volitional contraction.* The Tohoku journal of experimental medicine, 2010. **221**(1): p. 77-85.

193. Wigerstad-Lossing, I., et al., *Effects of electrical muscle stimulation combined with voluntary contractions after knee ligament surgery.* Med Sci Sports Exerc, 1988. **20**(1): p. 93-98.