T.R.A.N.S.I.T. ELECTRICAL STIMULATION TO IMPROVE
MUSCLE QUALITY IN OLDER INDIVIDUALS:
A CASE SERIES

A Thesis
Presented to
The Honors Tutorial College
Ohio University

In Partial Fulfillment
of the Requirements for Graduation
from the Honors Tutorial College
with the degree of
Bachelor of Science in Biological Sciences

By
Eric Leach
April 2016
This thesis has been approved by

The Honors Tutorial College and the Department of Biological Sciences

Dr. David Russ, PT, Ph.D.
Associate Professor, Physical Therapy
Thesis Advisor

Dr. Soichi Tanda, Ph.D.
Honors Tutorial College, DOS
Biological Sciences

Dr. Jeremy Webster, Ph.D.
Dean, Honors Tutorial College
Acknowledgements

Completion of this project would not have been attainable without the instruction and guidance from Dr. Russ. Dr. Russ served as my tutor for two years and helped me develop academically and professionally. I appreciate the time he spent working with me.

The people at the Ohio Musculoskeletal and Neurological Institute were essential for this project. Specifically, Dr. Clark, Dr. Amano, Dr. Rush, Dr. Law, Ms. Hart, and Mr. Frempong-Boadu provided direct assistance. Each person went out of his or her way to help me with organization or methodology of the study.

Dr. Tanda has advised me well for the past four years. Most importantly, he taught me to work towards what I want to do and to worry less about things that are beyond my control.

Thank you to the people at the HTC who have helped me along in life and set examples as great people and leaders: Mrs. Huber, Mrs. White, Mr. Haworth, Ms. Clodfelter, Associate Dean Frith, and Dean Webster. Without the opportunities and encouragement from the HTC, I would not have had the chance to be involved with such interesting research!

Finally, thank you to my family (Mom, Dad, Brian, and Leo) and friends for everything they give to my life.
Table of Contents

Introduction - 5

Materials and Methods - 27

Results - 38

Discussion - 59

Future Directions - 64

References - 66

Appendix A: Institutional Review Board Approval and Informed Consent Documents
INTRODUCTION

Overview of Project:

The world’s elderly population is increasing and healthcare costs are outpacing this growth (1, 2). As people age, their muscles progressively weaken, often leading to physical disabilities and functional limitations later in life. Eventually, muscle weakness can interfere with a person’s ability to complete everyday activities, such as bathing, walking a few blocks down the road, or safely getting up from a chair (1). For elderly people, maintaining or improving muscle strength helps to maintain the ability to safely complete daily tasks. This ultimately leads to decreased healthcare costs and sustained independence.

There are many well-established methods to improve muscle function in elderly people, including endurance exercise, strength training, and flexibility training (32). All of these modalities of exercise work through a variety of mechanisms to induce specific training effects that enhance muscle function. However, there are other forms of exercise that may provide unique benefits to muscle function that have been less extensively studied; chiefly, sprint exercise.

Sprint exercise has been shown to promote an adaptation that has yet to be seen in response to any other form of exercise: improved calcium release from the sarcoplasmic reticulum of skeletal muscle (33). This adaptation is significant because calcium release within the muscle is an essential step in the process of muscle contraction. Surprisingly, however, sprint exercise is not even mentioned by the
American College of Sports Medicine in its recommendations for older individuals (32). This is likely due to concerns that it would not be well-tolerated due to risks related to high intensity exercise and the stress that it places on the joints.

Consequently, it may be of great benefit to develop a physical therapy intervention that could mimic the effects of sprint training for people that cannot safely execute sprint exercise. Such an intervention could be especially beneficial for older individuals, as traditional sprint exercise is seldom recommended.

Russ and colleagues (37) recently developed a treatment protocol that induces muscular adaptations in young people that are similar to those caused by sprint exercise. This protocol is based on an established physical therapy intervention known as neuromuscular electrical stimulation (NMES), which works by sending electric current through electrodes to stimulate nerves and activate specific muscles. NMES has been used in both laboratories and physical therapy clinics for decades and is commonly used as a treatment to improve muscle strength or endurance. The protocol developed by Russ et al., hereafter referred to as T.R.A.N.S.I.T. (Therapeutic Rapid Activation of the Neuromuscular System for Interval Training), applies NMES technology in a new way that improves peak rate of calcium release within the muscle (an essential step in muscle activation). This is of particular importance because improved calcium release has only been observed in response to sprint exercise training (33, 37). Because calcium release declines in aging muscle (27, 28), we expect that T.R.A.N.S.I.T. will improve muscle function more in elderly people than in young people.
Therefore, for my thesis project, I proposed to test the effect of T.R.A.N.S.I.T. on muscle quality (muscle force / muscle size) in older individuals. Due to specific changes in muscles that occur with age, we expected that T.R.A.N.S.I.T. would be of even greater benefit to older individuals than it was to younger individuals.

**Background:**

The number of older individuals in the United States is rapidly rising. As of 2008, there are 39 million people over the age of 65, making up 13% of the total United States population. By 2030, the number of older individuals is expected to nearly double to 72 million (1). Healthcare costs for elderly people are also expected to increase drastically. As of 2011, $555 billion was spent on Medicare; by 2020, this cost is projected to reach over $900 billion (2). A major upcoming challenge in medicine is providing effective and economical healthcare for such a large population of people with a limited pool of healthcare providers.

As people age, they often encounter physical disability and functional limitations. This impedes people from completing activities that are a part of independent living, such as bathing, safely standing up from a chair, or walking a few blocks down the road. In 2007, 42 percent of elderly Americans reported that they had at least one functional limitation that prohibited them from completing an everyday task (1). Unfortunately, dysfunction in one area of life often spills over into another, and muscle function and mobility can drop off exponentially. Although there are many factors that can lead to physical disability in people as they age, one of the
primary causes is muscle dysfunction as manifested by low muscle strength (3). In fact, after age 60, muscle strength declines by approximately 3% per year. Further, elderly people with muscle weakness have an elevated risk of falls, leading to hospitalization and significantly elevated risks for other conditions (e.g. osteoporosis and other degenerative conditions) (4). Certainly, muscle weakness in the elderly can be an extremely debilitating condition with serious consequences. Therefore, it is important to minimize losses in muscle function due to aging through the most effective and efficient interventions we can develop.

Muscular Dysfunction and Muscle Quality

The loss of muscle mass with aging, known as sarcopenia, was originally thought to completely account for the age-related loss of muscle strength. However, accumulating data indicate that loss of muscle mass can only explain a portion of the loss in muscle strength seen in aging.

For example, data from the Health Aging Body Composition Study indicate that, with age, muscle strength in the quadriceps declines at a faster rate than muscle size (5). In other words, the loss of muscle mass in the quadriceps only partially explains the loss of muscle strength observed in aging. The same study showed that, surprisingly, even if subjects maintained or increased their muscle mass, their muscles still weakened with aging (5). Consequently, there must be factors other than muscle mass that influence the rapid decline in muscle function that accompanies aging.
In light of this finding, a term known as “muscle quality” was coined as a new and important metric for evaluating muscle function (48). Muscle quality is defined as muscle strength divided by muscle size. For example, between two muscles of the same size, the stronger of the two would have a higher muscle quality. Muscle quality can be determined using either isometric (contraction without movement) or dynamic (contraction with movement) parameters. As a result, muscle quality is now a central focus of skeletal muscle physiology research and intervention development. For these reasons, we have chosen muscle quality to be the primary outcome of interest for our study because of its importance and observed decline in aging.

As detailed in a review by Russ et al (6), there are many variables that may influence the typical decline in muscular quality observed during aging. Given the complexity of the process, there are likely molecular mechanisms that have yet to be discovered. However, age-related changes in these factors can be delineated into two broad categories based on the tissue in which they occur: neural or muscular. Neural factors include age-related changes in central activation, supra-spinal properties, and structure and function of the neuromuscular junction. Muscular properties include age-related alterations in contractile filaments, muscle size and composition, muscular force transmission, and excitation-contraction coupling.

It is important to emphasize that while we can separate out these neural and muscular properties to some extent in the activation process of skeletal muscle, it takes the cooperative function of all of these processes (the neuromuscular system) for a muscle to work properly. In other words, many of these processes are inter-related,
and changes in one property are likely to affect several others. In this way, it is difficult to place relative importance on any one process without understanding how it interacts with the other processes involved. Therefore we will first provide an overview of the basic terminology and process of typical voluntary skeletal muscle activation before proceeding to a more detailed discussion of the age-related changes in the process.

Overview of Voluntary Skeletal Muscle Activation

Briefly, voluntary activation of skeletal muscle is initiated in the brain from the pre-motor cortex. From here, electrical signals known as action potentials are

![Figure 1 - E-CC - used with permission by Russ et al (6).](image-url)
modulated and travel down to the spinal cord. Next, these signals synapse with an alpha motor neuron, which sends its axon out of the spinal cord and branches to innervate the muscle fibers that compose its motor unit. The alpha motor neuron then branches to multiple endpoints, each of which innervates a single muscle fiber. The point at which the alpha motor neuron and muscle fiber meet is known as the neuromuscular junction (NMJ). At the NMJ, muscle fibers are signaled to contract by initiating excitation-contraction coupling (E-CC) (Figure 1).

When stimulated, the alpha motor neuron releases the neurotransmitter acetylcholine (Ach) from its endpoint, known as the terminal button. Released Ach then diffuses through the space of the NMJ to the muscle fiber membrane, known as the sarcolemma. A specific region of the sarcolemma, known as the motor end-plate, contains receptors sensitive to Ach (AchRs). When AchRs bind Ach, they trigger the opening of cation channels that permit influx of sodium and efflux of potassium ions through the motor end-plate region of the sarcolemma. Relatively more sodium ions flow into of the cell than potassium ions out, leading to a change in electrical potential known as a motor end-plate potential. This end-plate potential then spreads to the surrounding sarcolemma as an action potential. Meanwhile, the end-plate potential at the sarcolemma is terminated as membrane-bound acetylcholinesterase breaks down ACh bound to AchRs, therefore closing cation channels on the motor-endplate. The action potential propagates across the sarcolemma, and into the transverse-tubule system (t-tubules).
T-tubules are invaginations of the sarcolemma that act as direct passageways for the electrical signal to spread through the cylindrically-shaped muscle fiber. The action potential travels down t-tubules and activates voltage-gated dihydropyridine receptors (DHPRs), which in turn activate their coupled ryanodine receptors (RYRs). The RYRs open and permit calcium to flow from the lateral sacs of the sarcoplasmic reticulum (SR) into the cell’s cytoplasm. This is known as voltage-induced calcium release (VICR). Calcium ions released then can bind with calcium-gated RYRs, and trigger calcium-induced calcium release (CICR). While both calcium release mechanisms (VICR and CICR) occur in skeletal muscle, VICR is the principle mechanism of calcium release in skeletal muscle, whereas CICR is the primary mechanism of calcium release in cardiac muscle. Once in the sarcoplasm, calcium binds to troponin-C, inducing a conformational change in tropomyosin. This conformational change exposes a myosin binding site on the actin myofilament. As a result, myosin and actin can bind, allowing for cross-bridge cycling. Cross bridge cycling leads to sarcomere shortening and produces force by using the energy stored from the hydrolysis of ATP by actin-myosin ATPase.

Following muscle contraction and cessation of calcium release, calcium is then pumped back into the SR by SR-calcium ATPase (SERCA). Tropomyosin then resumes its conformation in wherein it blocks myosin binding sites on actin, and the muscle fiber relaxes (26).
Neural Properties that Influence Muscle Quality

The neural properties of muscle function describe any process that occurs in the brain, spinal cord, efferent (motor), or afferent (sensory) neurons that either signal or receive input from muscle tissue. Changes in specific properties such as central activation, supra-spinal, and other neural properties all may play a role in changes in muscle function that occur with aging.

Central activation is imperative to muscle function. Central activation is the ability of the central nervous system to activate skeletal muscle voluntarily. When a person voluntarily contracts a muscle, they first must fire neurons in the primary motor cortex of the brain to activate motor neurons in the spinal cord. Motor neurons then innervate skeletal muscle fibers and activate them via aforementioned signaling at the NMJ. Research is mixed as to whether central activation decreases or does not change with age. However, studies may contradict each other in part because they examine different muscles during their tests for central activation. Depending on the specific muscle tested, there appear to be variations in the extent to which central activation decreases with aging. Specifically, while the dorsiflexor muscles do not experience a significant loss in central activation with aging, the knee extensor muscles do exhibit a reduction in central activation of approximately 11% (7, 8).

Therefore, there is likely considerable variation in the extent to which age impacts central activation, depending on the muscle. It is not clear what may cause this differential impairment in central activation between muscle groups. However, a new study from Clark et al (9) shows that the weakest tertile of older individuals may
display greater deficits in central activation, suggesting that there is an association with impaired central activation and muscle weakness among older individuals, but only below a certain threshold of strength.

Other neural factors that may influence muscle quality in aging are related supra-spinal properties, or properties of the brain that are involved with the activation of skeletal muscle. One study involving cadaveric dissection demonstrated that individuals over 65 years old exhibited a 43% reduction in neural volume of the premotor cortex (the region of the brain that is responsible for initiating muscle contraction) compared to people under 45 years of age (10). Perhaps this reduced cell size affects the ability of the premotor cortex to generate electrical signals or the ability to synapse with other neurons. In addition, another study found that reduced cortical plasticity (adaptability of the cortex of the brain) can occur with age (11). Together, these studies suggest an association between these properties of the brain that initiate skeletal muscle contraction and diminished muscle function and adaptation with age.

Voluntary muscle contraction is driven by the activation of independent motor units. The manners in which motor units are coordinated and/or stimulated together determine force production and movement. A motor unit is composed of a single motor neuron and the muscle fibers that it innervates. In order for the central nervous system to communicate with the somatic nervous system and elicit muscle contraction, a single motor neuron converges with muscle fibers at NMJs. There is evidence that changes in neural properties of the neuromuscular junction may influence muscle
quality observed in aging through two primary mechanisms: a reduction in the total number of motor units (12) and more muscle fibers per motor unit (13). Further, Kido et al report that spinal excitability, or the ability to activate motor neurons, decreases with aging (14).

Motor unit discharge rate may decrease with age, thereby increasing the intensity of input necessary to reach threshold for some motor units. This, in turn, can affect force production, as higher motor unit discharge rates induce twitch summation, yielding higher forces of contraction (41). Specifically, Kamen et al (15) found that the discharge rate of muscles in the intrinsic hand is significantly lower in older adults compared with young adults.

Taken together, these changes in neural properties (central activation, supraspinal properties, and neural properties of the neuromuscular junction) may significantly contribute to the reduction in muscle quality that occurs in aging. However, neural properties are only a part of the potential cause of diminished muscle quality with age; we must also consider mechanisms within muscle.

Muscular Properties that Influence Muscle Quality

In addition to the neural factors described above, there are many changes that occur within aging skeletal muscle tissue itself that likely contribute to the age-related decline in muscle quality. These factors include contractile filaments, muscle size and composition, muscular force transmission, and E-CC.
Within sarcomeres, the functional units of skeletal muscle, muscular contractile filaments can alter with age. Actin and myosin are the two primary proteins in muscle that are essential for contraction via cross bridge cycling.

**Figure 2** – (a) Process of a single cross bridge cycle and (b) Process of cross bridge cycling in a sarcomere – figure from Sherwood (42).

In essence, cross bridge cycling is the process of myosin heads attaching to actin and producing contractile forces. The cycle begins when ATP bound to an unattached myosin head is hydrolyzed by myosin ATPase, transforming myosin into
its high energy state. Myosin then binds to actin myofilament at an exposed binding site, and changes its conformation to its low energy state, while it pulls actin toward the center of the sarcomere (thereby shortening the sarcomere), and releases bound ADP and inorganic phosphate. Then ATP binds to the myosin head in its low energy state and it dissociates from the actin filament. The cycle then repeats itself as long as the contraction is stimulated and calcium is present in the sarcoplasm (42).

In human skeletal muscle, there are three primary fiber types: type I, type IIA and type IIX, classified by the myosin heavy chain (MHC) isoform most common within its cell. Each MHC differs based on the rate of its ATPase activity: myosin ATPase activity in IIX fibers is the fastest, whereas myosin ATPase activity in Type I fibers is the slowest. As a result, different types of muscle fibers have specific functions. In general, Type IIX and type IIA muscle fibers (fast-twitch fibers) are able to produce greater forces and velocity of contraction than type I fibers (slow-twitch fibers) (19). However, there is a trade-off, as slow twitch fibers have a greater fatigue resistance than fast fibers. Klitsgaard et al have shown that, in aging muscles, there is a general shift in muscle fibers from fast to slow (20). A review article by Lexell (43) describes this shift in detail. While there may be some benefits to this shift, as slower fibers are more resistant to fatigue, there are also clear drawbacks. For example, Type I fibers have a lower specific tension than type II fibers (21). This translates to diminished maximal force production, which can be problematic as some daily activities like climbing stairs require maximal force generation for some people.
There may be functional problems within the contractile filaments with age, as old rat muscle fibers display a reduced capacity to form cross bridges (23). In another study, very old rats exhibited a reduction in the ratio of myosin to actin in the semimembranosus but not in the soleus (22). This suggests that there may be a muscle-specific decline in the balance of myosin and actin that contributes to the decrease in muscle quality.

Whole muscle size and composition change with age as well. For example, the Health, Aging, and Body Composition study found that the thigh muscles of men and women between the ages of 70 and 79 atrophy by 1% and 0.65% per year, respectively (5). Lexell et al (16) observed that most of the loss of mass can be explained by a loss of muscle fibers (all fiber types), and to a lesser extent, a reduction in size of type II fibers. Further, there may be inadequate muscle protein turnover leading to impaired anabolism of muscle tissue (17). In addition, a decline in autophagy within muscular tissue may interfere with muscular force production; autophagy helps maintain muscle size and function through its housekeeping functions by preventing an accumulation of damaged and dysfunctional cellular materials and organelles (18).

Skeletal muscle fibers must work together to yield efficient and productive movement of the skeletal system. To transmit this force, many cytoskeletal proteins are necessary to connect muscle fibers and myofilaments together. Therefore, any deficits in the dense cytoskeletal network of proteins that function to transmit the force generated by the muscle fibers would lead to diminished muscle quality. Desmin, a structural intermediate filament crucial to the structure of muscle fibers, is one such
protein in the cytoskeleton that has a potential role in the decline of muscle quality with age.

Research in rats has shown an increased quantity of desmin is produced in aging muscles (24). In another study, Boriek and colleagues observe that desmin knock-out mice produced a greater specific force than wild type mice (25). This suggests that increased desmin expression may have a negative impact on the quality of aging muscles. However, very little research on the changes in cytoskeletal proteins of aging muscle has taken place, so there may be structural proteins other than desmin that play a significant role as well (such as titin, dystrophin, or nebulin).

Changes in the process of excitation-contraction coupling (E-CC) may be responsible in part for the decrease in muscle quality with aging. The functionality and strength of muscle contraction is highly dependent on the process of E-CC. For example, impairment of the release of calcium from the sarcoplasmic reticulum would interfere with muscle fiber force and shortening velocity. In fact, research shows that there is a loss of DHPRs that occurs with aging, directly interfering with the mechanism of VICR (27). However, calcium release has also been shown to be impaired in aging muscle by another mechanism. In a study of the plantarflexor muscles in aging rats by Russ et al (28), reduced muscle quality and impaired calcium release is associated with reduced FK506-binding protein expression and binding to RYRs. However, given the complexity of E-CC and the numerous proteins involved, there may be other mechanisms that could be involved that research has yet to reveal.
Given all of this evidence, changes in the properties of skeletal muscle (contractile filaments, muscle size and composition, muscular force transmission, and E-CC) may contribute alongside neural factors to the age-related decline in muscle quality.

Treatments to Improve Muscle Function:

Given all of these factors that may contribute to the decline in muscle quality with aging, it is clear that treatment approaches must utilize a multidimensional approach to improve muscle function in older individuals.

Despite this evidence, a large portion of muscle research and, consequently, clinical practice is still rooted in the conventional wisdom that increasing muscle strength requires increasing muscle size. Consequently, there is still a large portion of research with muscle size as the primary outcome of interest.

For example, many studies look at inhibition of a myokine known as myostatin. Myostatin works through autocrine and paracrine action to influence metabolism and inhibit muscle growth (44). Consequently, people and animals that express defective forms of myostatin exhibit phenotypes with increased lean body mass and decreased fat mass. Likewise, studies on the effects of myostatin inhibition have found that decreased myostatin activity significantly increases skeletal muscle mass both in animal models and humans (29, 30). However, recent papers report decreased muscle contractile efficiency in myostatin knock-out mice (31), showing that while myostatin inhibitors increase muscle size, they also decrease muscle quality.
Given that size is only a component of muscle function, other research seeks to develop interventions to improve muscle quality as an approach to treat muscle weakness. With so many neuromuscular factors that appear to be altered with aging, it may be beneficial to elderly people to provide interventions that aim to improve muscle quality by addressing the various mechanisms that may interfere with optimal muscle function during the aging process.

Exercise is arguably the most effective way to improve muscle function; in addition to its clear systemic health benefits, it also likely works to mitigate many age-related alterations specific to skeletal muscle. The American College of Sports Medicine (ACSM) recommends that strength, endurance, and flexibility exercise be integrated into a weekly routine to maintain muscle function in elderly people (32). These forms of exercise are extremely beneficial and may each improve muscle function through their both specific and shared mechanisms.

However, the ACSM does not even mention another type of exercise, sprint training, in its recommendations. This is likely because sprinting may not be well tolerated by older individuals due to the stress it places on the joints and its required high level of exertion. However, in young people, sprint training has been shown to induce several functional benefits that have also been observed to occur in response to other forms of exercise, such as increased aerobic capacity and power output at a relatively low training volume (33). Moreover, sprint training also elicited an adaptation that no other form of exercise has been shown to offer: enhanced calcium release from the SR (34). This adaptation of increased calcium release is noteworthy
because sprinting is the only form of exercise that has been shown to be capable of enhancing this variable. We hypothesize that increased calcium release will improve the process of E-CC, due to calcium’s role in cross bridge cycling. If E-CC improves, then there may be a benefit to the maximum force of contraction in the fiber and maximal velocity, thus yielding increased muscle quality. Improved muscle quality through this mechanism might well lead to functional benefits in elderly people that cannot be achieved through other forms of exercise, given the evidence that calcium release appears to become impaired in aging muscles (27, 28). Therefore, there is a need for a clinical intervention for elderly people that can mimic the stimulus of sprint exercise on muscle, leading to enhanced muscle quality. It is first necessary, before designing a method, to consider the effects that age has on physiological responses to training.

Physiological Responses to Training in Older Individuals:

Many studies have demonstrated that older individuals respond differently from younger individuals to exercise training. For example, in an 8-week study by Wang et al (40) showed that in an eight week cycling protocol that used interval training, older and younger individuals significantly improved their VO2max, maximal cardiac output, and maximal power output. However, younger individuals improved their VO2max and maximal power output significantly more than older individuals, suggesting that the ability to adapt to exercise, while retained, is blunted in older individuals as compared to younger individuals.
Another study by Bickel et al (41) examined the effects of detraining and maintenance programs using resistance exercise varied with age. After a progressive 16-week resistance training program of the knee extensor muscles, young (mean age = 28) and older (average age = 64) adult subjects were assigned to either 3 groups: complete detraining (with no resistance activity), 1/3 volume of the original program, or 1/9 volume of the original program.

Both younger and older individuals increased their knee extensor muscle size and strength following the 16 week strength training protocol. However, while the young group maintained an increased mean myofibril CSA with either 1/3 or 1/9 volume maintenance programs, the older group regressed to baseline. This indicates that older individuals may necessitate greater maintenance program training volumes to retain any gains in muscle size achieved during a fitness program.

Interestingly, however, the regression (as observed in the starting fiber size in response to these maintenance programs) did not also occur in the gains in muscular specific strength (KE 1RM (kg)/thigh lean mass (kg)). In fact, both young and old subjects were still significantly stronger following 32 weeks of detraining than before they started the 16 week training protocol. Further, both the young and older groups assigned to the 1/3 and 1/9 volume actually increased their specific strength following 32 weeks on the maintenance program. While both groups achieved these gains, it is important to note that improvements in older individuals were attenuated compared to the young group, in both relative and absolute terms. In any case, however, these data importantly demonstrate that improvements in muscle quality from a progressive
resistance protocol can be preserved or even enhanced with a reduced (1/3 or 1/9) training volume.

In general, it takes longer for elderly people to respond to a training stimulus and often responses are blunted compared with younger people. Therefore, the training program that we design must provide both adequate frequency of training stimulus along with a long enough duration for an effect to occur.

**Novel Treatment to Improve Muscle Function:**

One possible method to achieve muscular activity similar to sprint exercise would be to use neuromuscular electrical stimulation (NMES). NMES has been shown to have distinct training effects, depending on its method of use, including increased muscular strength (35) and endurance (36). Therefore, this technology may be applicable to induce the same muscular adaptations that occur with sprint training.

NMES works by placing electrodes on the skin covering a muscle and sending a current between electrodes through the selected muscle. This depolarizes alpha motor neurons and contracts their associated muscle fibers. The activation of the motor neurons is dependent on the intensity of the stimulus (as controlled by the NMES device), the proximity of the stimulus to the neurons that fire, and the frequency of stimulation. NMES differs from volitional exercise in that it does not follow Henneman’s size principle: in which motor units are recruited in a sequential, orderly manner, with small motor units recruited before larger units. Instead, NMES activates motor units proximal to the electrodes randomly (37). In this way, we take
advantage of the non-physiological nature of NMES train motor units that may not be usually recruited in volitional exercise. Further, there is evidence that central activation can be improved with NMES (38).

New research demonstrates that NMES, while traditionally used to mimic resistance exercise, could also be applied as a treatment that provides a stimulus similar to that of sprint training. In an original protocol for the knee extensor muscles, Russ et al (37) adhere to the basic tenets of NMES, but change the process to make the treatment more comfortable for the patient while still maximally activating the muscle. This method differs from traditional NMES in which 2 large electrodes aim to activate the entire muscle belly at once. Instead, T.R.A.N.S.I.T. involves applying several pairs of smaller electrodes to the surface of the skin on small portions of the muscle, activating each of the three regions maximally with a current one at a time, which causes only muscle fibers within the electrode pair’s region on the leg to contract (Fig. 2). After maximally activating the first of three regions of the muscle, the next region would be activated, and finally the last region, each one at a time. In this way, the entirety of the muscle is activated over the duration of the treatment, and discomfort is minimized due to the relatively small size of the electrodes that activate relatively small portions of muscles.

Figure 3 - 3 pairs of electrodes (white arrows) in the T.R.A.N.S.I.T. method- used with permission by Russ et al (37).
Russ et al (37) found that 5 weeks of training with this NMES sprint protocol (3x/week) elicited significant increases in sarcoplasmic reticulum calcium release and citrate synthase activity, and decreased the proportion of the myosin heavy chain isoform IIX in young adults. Most significant of these adaptations is the increased calcium release, as that has only been observed to occur in response to sprint exercise. Further, there was a significant increase in muscle cross sectional area. Interestingly, they found a strong trend towards increased isometric strength, although this difference was not significant. Accordingly, muscle quality did not change because muscle CSA and isometric strength increased by similar proportions (~3%).

However, it remains to be seen whether this protocol would improve muscle quality in older individuals, given the apparent deficit in calcium release in older muscle. If this method of NMES does prove to be effective in this purpose, it may yield functional benefits that many elderly individuals are not normally capable of achieving through exercise.

Hypothesis:

We hypothesized that elderly individuals would display significant muscular adaptations after training with T.R.A.N.S.I.T. Volitional sprint exercise has been shown to elicit adaptations such as increased power output, citrate synthase activity, and calcium release in young adults. T.R.A.N.S.I.T. seems to mimic these effects closely in young adults. While the young group did not improve their muscle quality in response to T.R.A.N.S.I.T., we expect that this is because younger individuals do
not have impaired calcium release. However, aging muscles do exhibit impaired calcium release. Therefore, T.R.A.N.S.I.T. may help lessen or eliminate the deficiency of calcium release that occurs in aging muscles, and thus improve muscle quality.

MATERIALS AND METHODS

Subject Recruitment and Inclusion:

We set out to recruit 5-10 men and women from the Athens, Ohio community ages 60-80 to enroll in the study. To recruit subjects, we sent out an email to people who lived in the Athens community that had expressed interest in participating in research studies at Ohio University. People who responded to the email were invited to schedule a phone call to go over study components and conduct an initial screening. We described the main idea and rationale of the experiment, as well as the potential benefits and risks involved. Individuals were then given an opportunity to ask questions and voice concerns. Subsequently, we obtained consent to ask basic medical questions that related to eligibility in the study. Subjects who had no apparent contraindications to participate in the study (as determined by Dr. Russ) were then invited to come in for a final screening to determine eligibility.

Of 11 people who responded to our recruitment email and were contacted via telephone, two did not choose to participate, two were ineligible due to scheduling conflicts, and seven were invited to participate in the final screening.
Before the final screening took place, individuals were informed of the purpose and process of the study, as well as the potential risks posed. Again, we gave subjects the opportunity to ask questions and raise any concerns that they had. Once the subjects had given written informed consent (as approved by the Ohio University Human Subjects Review Board, see Appendix A), we conducted a thorough medical screening to ensure that they could safely participate in the study. The subjects were excluded for the following reasons: blood clotting disorders, uncontrolled hypertension, active cancer treatment, peripheral vascular disease, major psychiatric disease, rheumatoid arthritis, anemia, liver disease, renal disease, uncontrolled diabetes, primary neurological disorder, peripheral neuropathy of the thighs or legs, pregnancy, claustrophobia, recent alcohol or drug abuse, osteoporosis, fracture of the lower limb in the past 6 months, and metallic devices that interfere with MRI.

Further, subjects were excluded if they partook in aerobic exercise for more than 20 minutes, twice a week, or had regularly performed resistance exercise in the past six months. Subjects were also screened using the Seven-day physical activity questionnaire (SPAQ), which asks subjects to recall physical activity and sleep for the previous seven days, allowing for an estimation of activity level, sleep, and metabolic rate. Subjects who scored greater than .75 hours of very hard activity per week over the last week were excluded. Subjects who scored between .25 and .75 hours of very hard activity, but indicating that this was a low activity week relative to the previous three months, were also excluded. Further, subjects went over their medical history form with a physician who conducted a final screening to ensure that potential
participants could safely take part in the T.R.A.N.S.I.T. protocol. Following the screening procedures, six subjects enrolled and one subject was excluded due to physical activity level that was in excess of our limit (regular aerobic exercise more than 20 min, twice a week).

Isometric Muscle Force and Central Activation Testing:

Before and after the T.R.A.N.S.I.T. training protocol, subjects were tested for maximal isometric muscle force and central activation of the knee extensors of their non-dominant leg. For all testing and training, subjects were seated in a Biodex dynamometer. The knee was stabilized at 90 degrees flexion, and the hip fixed at 100 degrees flexion using a strap around the calf and a strap around the thigh. Further, subjects were secured with a seatbelt and waist belt to stabilize the hip joint and upper body from moving during contraction. This stabilization prevented subjects from using the hip extensors or other muscle groups from contributing to the force output, thereby isolating the knee extensor muscles. After stabilized, the subjects performed 3 maximal volitional isometric contractions (MVICs) with strong verbal encouragement and visual biofeedback of force output. Subjects were then given 2-3 minutes to rest and relax in between each of the three contractions. Additionally, if the force continually increased with subsequent trials or if the two greatest trials deviated by more than 5%, trials were repeatedly conducted until the subjects reached a plateau in force production. Isometric torque was measured by a force plate (in ft.*lbs.), while central activation was assessed using a doublet interpolation method, as previously
described (37). Briefly, two large electrodes were be placed on the subjects’ thigh (proximal and distally positioned) to activate the entire quadriceps muscle and stimulated with single pulses of progressively increasing current until peak twitch force was achieved. Next, the subjects executed 3 MVICs, during which a 100 Hz doublet was applied at 110% of the current that led to a plateau in twitch force (a supra-maximal intensity), and a second doublet applied to the resting muscle immediately following relaxation. Percent of central activation was calculated with the following equation:

\[
\% \text{ Central Activation} = \left[1 - \left(\frac{\text{electrically evoked force during MVIC}}{\text{resting doublet evoked force}}\right)\right] \times 100.
\]

**Peak Power Dynamic Force testing:**

Before and after the training protocol, subjects were tested for peak power of their knee extensor muscles. For this procedure, subjects extended their leg as quickly as they could with strong verbal encouragement and biofeedback. They completed three trials, with 30 seconds rest in between repetitions using a 20% resistance of their peak isometric torque (while accounting for leg weight). Peak power is defined as torque multiplied by angular velocity, to yield (N\(\times\)m\(\times\)radians/s). This can then be converted to peak power (in N\(\times\)m/s, or Watts).
Magnetic Resonance Imaging:

Before and after the T.R.A.N.S.I.T. training protocol, MRI images were taken of the non-dominant thigh (the thigh undergoing training). We used an Esaote G-Scan Brio Musculoskeletal Weight-Bearing Magnetic Resonance Imaging (MRI) scanner housed in the Academic and Research Center at Ohio University. Trans-axial images were captured over approximately the middle 1/3 of the femur (beginning from the distal 1/3 of the femur proceeding upward to approximately the upper 1/3 of the femur, depending on the leg length of the subject). 17 images were taken, each slice 10 mm apart along the length of the femur. From these 17 images, five images from each scan were selected to be used to calculate quadriceps femoris cross sectional area (CSA). These five images were selected to match between the pre-training scan and the post-training scan to represent identical cross-sections of the leg for each person. To determine which five images from each scan were to be analyzed, we matched fascial characteristics, vessel locations, as well as muscle tendon shape and location. To determine quadriceps femoris muscle and fat CSA, the muscle area and fat in the five slices were averaged for each individual, pre- and post-training using the NIH medical imaging software, MIPAV.

Isometric and dynamic muscle qualities were then calculated by dividing the peak isometric torque or peak power by the knee extensor CSA, respectively.
Contractile Properties of Muscle:

Using the data from electrically-induced muscle contractions used to calibrate the stimulator for use in determination of central activation, we were able to derive important indicators of the contractile properties of the muscle (see Figures 4a and 4b, below): time to peak twitch force, half-relaxation time, the peak rate of force production, and the peak rate of relaxation. We then measured peak resting doublet torque (which occurred after MVIC testing with electrical stimulation) and peak twitch torque (using a single pulse of electrical stimulation).
Figure 4a – Isometric force response curve to electrical stimulation. Time to peak twitch force and half-relaxation time shown.
Figure 4b – Derivative of isometric force response curve to electrical stimulation (Figure 4a). Maximal rate of force production and maximal rate of force reduction shown.
T.R.A.N.S.I.T:

T.R.A.N.S.I.T. training took place 3 times per week for 8 weeks for each subject, for a total of 24 training sessions. The training protocol targets the quadriceps femoris muscles, because knee extensor function is indicative of impending severe mobility limitation and the muscle group is used in many everyday activities, such as walking and standing up from a chair (3). To make the stimulation relatively more comfortable for the subject than traditional NMES, we used 3 sets of small electrodes (instead of 1 set of 2 large electrodes) on the muscle to activate small portions of the muscle sequentially. This permitted us to activate each region at a high rate while keeping the overall forces low at any given time. Three pairs of electrodes were used; they were placed medially, centrally, and laterally on the non-dominant thigh. The stimulation intensity was normalized between subjects by calibrating the equipment so that in each electrode pair, a 30 Hz train for 5 s produced the equivalent force of 30% of their MVIC. The subjects had their MVIC tested once every two weeks during the training to recalibrate the stimulation intensity.

T.R.A.N.S.I.T. followed the same protocol for each subject. The only differences were the intensity of stimulus as determined by the MVICs. A 10-s bout of electricity was delivered through the first electrode pair. When completed, the intensity was adjusted for the second electrode pair, and the stimulation was conducted through the second electrode pair for 10 seconds. After completing stimulation through the second electrode pair, the process was repeated for the third electrode pair. Then, we waited until 60 seconds had passed from the initiation of the first stimulus,
and restarted the process. In total, per training session, we completed 20 rounds of stimulation to each pair of electrodes, with each round taking 60 seconds. Therefore, the entire training session took 20 minutes; in all, 10 minutes of muscle activation from the electrical stimulation and 10 minutes of muscle relaxation.

Each 10 second stimulation bout consisted of 150-ms, 80 Hz trains delivered at a rate of two per second. It is important to emphasize the fact that the duration of the trains was decreased drastically (from 5 s to 150 ms), but the intensity of stimulation for the training bouts was the same (80 Hz). For this reason, we theoretically activated the same motor unit population as using the 5 second train, but the contractile force was reduced because the train was too short for twitch summation to occur during the contractions.

**Short Performance Physical Battery Test:**

The SPPB has been shown to be an accurate functional predictor of nursing home admission and short term mortality (39). It is composed of a series of functional tests such as repeated chair stands, balancing with legs together and offset, and walking speed; in essence, these are good indicators for mobility and strength. For each of these three tests, subjects receive a score from 0-4, with a maximum total score of 12 points. The initial SPPB data are useful because they help characterize the initial functional abilities of the participants and indicate if any functional deficits existed.
Scores on the SBBP for the six subjects ranged from 10-12, with an average score of 11.1. These scores show that our subjects were at a relatively low risk for both death and nursing home admission. In fact, a person with a score of 11 is at less than 1/6 the risk of death as a person with a score of 0 on the SPPB.

We expected these scores to be relatively high because of self-selection bias among subjects and because the average age of our subjects was 64.6 years old, whereas the SPPB is typically used to predict risk of mortality and nursing home admission for people who are over 70 years old.

Statistical Analysis:

This study uses a repeated-measures design and was analyzed as a case series. Therefore, statistical analysis was primarily used to observe potential trends in the data. The whole muscle variables, including peak isometric torque, muscle power, muscle CSA, muscle quality, and contractile properties were analyzed using paired student’s t-tests, with significance indicated by (p<.05). Percentage of central activation will be compared by using the nonparametric Wilcoxon signed-ranks test. However, given the low sample size of our pilot study, statistical significance was unlikely to be attained. Effect sizes (using Cohen’s d) were also calculated.

Anticipated Outcomes:

We expected that participants in our study would display enhanced isometric muscle strength, power, central activation, and muscle quality as a result of
T.R.A.N.S.I.T. Further, we expected that T.R.A.N.S.I.T. would elicit improvements greater in magnitude for individuals who exhibited weakness or functional deficits. We also expected that isometric muscle strength would improve proportionally more than muscle power.

RESULTS

Subject Adherence to Protocol and Retention:

Subjects were required to attain a minimum 87.5% attendance rate for training sessions. This allowed each subject to miss a maximum of 3 of the 24 scheduled training sessions. Of the four subjects who completed the training, all met this standard, with an average adherence of approximately 94%. Of the six subjects (four women, two men) who enrolled initially, one subject (female) withdrew after the first session due to discomfort with the training. Another subject (female) withdrew midway through the study due to an exacerbation of a pre-existing medical condition. This medical condition exacerbated during the study period to the point that attending and traveling to the training sessions presented an unnecessary risk for the subject. Therefore, four subjects (2 men, 2 women) completed the study.

Subject Tolerance to NMES Training:

To examine the subjects’ tolerance to the protocol, we asked subjects complete the Numeric Pain Rating Scale (PRS) from the NIH after their initial training session.
The PRS is a scale from 0-10 from which subjects can rate the intensity of their pain, from none (0), mild (1-3), moderate (4-6), to severe (7-10). Additionally, an investigator completed the Checklist of Nonverbal Indicators (CNVI) during the first training session. The CNVI is designed to assess non-verbal indicators of pain that the subject may or may not to admit to with the PRS on a scale. Scores on the CNVI range from 0-5, with a point given for expression of any of five non-verbal indicators of pain, such as grimaces, bracing, or vocal complaints.

During the first session, subjects rated their pain with the PRS with scores ranging from 2 to 8, with an average of 5.3, indicating moderate pain. Using the CNVI assessment, subjects displayed ratings between 2 and 5, with an average of 3.5 different types of non-verbal indicators of pain displayed per subject.

Unfortunately, despite our intentions, I neglected to repeat these measurements halfway through the study and during the final training session. We expected that subjects would experience less pain after they became acclimated to the training; however we cannot support this hypothesis with data.

However, in the study of T.R.A.N.S.I.T. on young people, Russ et al found that the subjects completed all of their training sessions, and that the mean discomfort decreased between the initial session and the mid-point for both the PRS and the CNVI (37).

During the T.R.A.N.S.I.T. protocol, we often had to toggle back the intensity of the stimulus from the pre-determined intensities of 30% of the MVIC. To do this, we used a variable resistor switch box previously constructed by Dr. Russ for the same
purpose in an earlier study. We were able to reduce the discomfort of the subjects by slightly reducing the intensity of the stimulus while still achieving adequate force production during the T.R.A.N.S.I.T. protocol. However, based on the fact that we had to regularly reduce stimulus, we will consider perhaps reducing the proportion of peak isometric torque that we use to set the intensity to below 30% of the peak isometric torque. Alternatively, we would need to devise other methods to reduce discomfort during the training sessions.

Subject 1:

<table>
<thead>
<tr>
<th>Age</th>
<th>63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>75.91</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.96</td>
</tr>
<tr>
<td>Relative Est. Daily Energy Expenditure (kcal/day/kg)</td>
<td>31.99</td>
</tr>
<tr>
<td>Resp. Rate (breaths/min)</td>
<td>12</td>
</tr>
<tr>
<td>Pulse Rate (bpm)</td>
<td>80</td>
</tr>
<tr>
<td>Blood Pressure (mmHg)</td>
<td>122/78</td>
</tr>
<tr>
<td>SPPB (0-12)</td>
<td>11</td>
</tr>
<tr>
<td>Pre-PRS (0-10)</td>
<td>5</td>
</tr>
<tr>
<td>Pre-CNVI (0-5)</td>
<td>3</td>
</tr>
<tr>
<td>Medical conditions</td>
<td>osteopenia, orthostatic hypotension, chronic fatigue syndrome</td>
</tr>
</tbody>
</table>

Table 1a: Subject 1 Health Characteristics

As shown in Table 1a, Subject 1 had unremarkable vital readings and was generally in good health. An SPPB score of 11 indicates high mobility, and a very low risk of hospitalization, nursing home admission, and death.
Of note are the diagnoses she provided: osteopenia, orthostatic hypotension, and chronic fatigue syndrome. We considered her history of osteopenia carefully, as osteopenia can progress to osteoporosis in some individuals. If she became osteoporotic at any time during the study, she would have to be withdrawn from our study based on our exclusionary criteria. However, the physician who conducted the medical screening determined that it was not limiting, and that she would be safe to participate in the study. We monitored the condition throughout the study, and asked her to report any issues that may have indicated progression of the disease. Further, we asked her to report any symptoms of orthostatic hypotension or chronic fatigue syndrome during the study. We helped minimize the risk of orthostatic hypotensive symptoms by encouraging the subject to take her time when getting in or out of the Biodex dynamometer. Subject 1 did not present symptoms of chronic fatigue syndrome during the study, and she did not report that T.R.A.N.S.I.T. treatment affected any of her conditions.
Pre-Training | Post-Training | % Change
--- | --- | ---
QF Muscle CSA (cm²) | 38.88 | 37.82 | -2.72
Peak Isometric Torque (N*m) | 137.70 | 139.25 | 1.13
Peak Angular Velocity (degrees/s) | 325.15 | 354.66 | 9.07
Peak dynamic torque (N*m) | 53.19 | 54.57 | 2.59
Peak Power (Watts) | 232.40 | 249.46 | 7.34
Isometric Muscle Quality (N/cm²) | 12.88 | 13.39 | 3.95
Dynamic Muscle Quality (Watts/cm²) | 5.98 | 6.60 | 10.34
% Central Activation | 99.93 | 95.59 | -4.34
Angle at Peak Angular Velocity (degrees) | 158.14 | 135.79 | -14.13
Angle at Peak dynamic torque (degrees) | 94.54 | 106.57 | 12.73
Angle at Peak Power (degrees) | 136.36 | 124.33 | -8.82
Time to Peak Twitch (s) | 0.10 | 0.08 | -22.24
Half-Relaxation Time (s) | 0.08 | 0.11 | 36.65
Peak dF/dt (N*m/s) | 322.15 | 293.60 | -8.86
Peak -dF/dt (N*m/s) | 119.59 | 108.24 | -9.49
Peak Resting Doublet Torque (N*m) | 56.05 | 48.53 | -13.41
Peak Twitch Torque (N*m) | 25.68 | 21.82 | -15.05

Table 1b: Subject 1 neuromuscular function characteristics and outcomes

As shown in Table 1b, following treatment with the T.R.A.N.S.I.T. protocol, Subject 1 exhibited an increased peak isometric torque (1.13%), peak angular velocity (9.07%), peak dynamic torque (2.59%), peak power (7.34%), isometric muscle quality (3.95%), and dynamic muscle quality (10.34%).

Subject 1 demonstrated a reduction in QF muscle CSA (-2.72%) and central activation (-3.79%). Further, all contractile properties were reduced: peak dF/dt (-8.86%), peak –dF/dt (-9.49%), peak resting doublet torque (-13.41%), and peak twitch torque (-15.05%).
Subject 2:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>95.45</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.705</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>32.84</td>
</tr>
<tr>
<td>Relative Est. Daily Energy Expenditure (kcal/day/kg)</td>
<td>33.23</td>
</tr>
<tr>
<td>Resp. Rate (breaths/min)</td>
<td>16</td>
</tr>
<tr>
<td>Pulse Rate (bpm)</td>
<td>76</td>
</tr>
<tr>
<td>Blood Pressure (mmHg)</td>
<td>138/82</td>
</tr>
<tr>
<td>SPPB (0-12)</td>
<td>10</td>
</tr>
<tr>
<td>Initial PRS (0-10)</td>
<td>2</td>
</tr>
<tr>
<td>Initial CNVI (0-5)</td>
<td>2</td>
</tr>
<tr>
<td>Medical conditions</td>
<td>none</td>
</tr>
</tbody>
</table>

Table 2a: Subject 2 Health Characteristics

As shown in Table 2a, Subject 2 had relatively normal vital signs, with a few exceptions. First, the blood pressure measurement of 138/82 indicated pre-hypertension. Further, a BMI of 32.84 indicated obesity. However, neither of these factors was exclusionary, and they did not preclude the subject from participating safely in the study.

Subject 2 earned a score of 10 on the SPPB, the lowest of the four subjects who completed the study. However, an SPPB score of 10 still places subject 2 at a very low risk of nursing home admission, hospitalization, or short-term mortality; in other words, it still indicated high mobility.

Interestingly, Subject 2 rated their pain during the first training session with a score of 2 with the PRS. This was the lowest rating any subject gave for the PRS. Further, Subject 2 received a score of 2 for non-verbal indicators of pain, also the lowest among all subjects. This observation points to the fact that there are wide
differences in pain perception between individuals, and that some physical therapy
treatments may be more tolerable for some people than others.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Training</th>
<th>Post-Training</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>QF Muscle CSA (cm²)</td>
<td>54.48</td>
<td>55.31</td>
<td>1.53</td>
</tr>
<tr>
<td>Peak Isometric Torque (N*m)</td>
<td>184.35</td>
<td>178.85</td>
<td>-2.98</td>
</tr>
<tr>
<td>Peak Angular Velocity (degrees/s)</td>
<td>328.30</td>
<td>371.85</td>
<td>13.26</td>
</tr>
<tr>
<td>Peak dynamic torque (N*m)</td>
<td>60.34</td>
<td>64.66</td>
<td>7.16</td>
</tr>
<tr>
<td>Peak Power (Watts)</td>
<td>249.84</td>
<td>284.80</td>
<td>13.99</td>
</tr>
<tr>
<td>Isometric Muscle Quality (N/cm²)</td>
<td>11.47</td>
<td>10.96</td>
<td>-4.44</td>
</tr>
<tr>
<td>Dynamic Muscle Quality (Watts/cm²)</td>
<td>4.59</td>
<td>5.15</td>
<td>12.28</td>
</tr>
<tr>
<td>% Central Activation</td>
<td>100.00</td>
<td>92.83</td>
<td>-7.17</td>
</tr>
<tr>
<td>Angle at Peak Angular Velocity (degrees)</td>
<td>126.05</td>
<td>148.40</td>
<td>17.73</td>
</tr>
<tr>
<td>Angle at Peak dynamic torque (degrees)</td>
<td>106.57</td>
<td>106.00</td>
<td>-0.54</td>
</tr>
<tr>
<td>Angle at Peak Power (degrees)</td>
<td>125.48</td>
<td>122.04</td>
<td>-2.74</td>
</tr>
<tr>
<td>Time to Peak Twitch (s)</td>
<td>0.10</td>
<td>0.08</td>
<td>-21.43</td>
</tr>
<tr>
<td>Half-Relaxation Time (s)</td>
<td>0.06</td>
<td>0.05</td>
<td>-28.51</td>
</tr>
<tr>
<td>Peak dF/dt (N*m/s)</td>
<td>373.92</td>
<td>456.54</td>
<td>22.09</td>
</tr>
<tr>
<td>Peak -dF/dt (N*m/s)</td>
<td>203.90</td>
<td>277.55</td>
<td>36.12</td>
</tr>
<tr>
<td>Peak Resting Doublet Torque (N*m)</td>
<td>75.61</td>
<td>62.66</td>
<td>-17.13</td>
</tr>
<tr>
<td>Peak Twitch Torque (N*m)</td>
<td>38.08</td>
<td>29.88</td>
<td>-21.54</td>
</tr>
</tbody>
</table>

Table 2b: Subject 2 neuromuscular function characteristics and outcomes

As shown in Table 2b, Subject 2 displayed increases in QF muscle CSA (1.53%), peak angular velocity (13.26%), peak dynamic torque (7.16%), peak power (13.99%), dynamic muscle quality (12.28%), peak dF/dt (22.09), and peak –dF/dt (36.12%).

Surprisingly, Subject 2 exhibited a reduction in peak isometric torque (-2.98%) and isometric muscle quality (-4.44%) after the training protocol. Further, Subject 2’s
central activation was reduced following T.R.A.N.S.I.T. (-2.57%). Peak Resting
doublet torque and peak twitch torque were reduced by (-17.13%) and (21.54%),
respectively.

Subject 3:

<table>
<thead>
<tr>
<th>Age</th>
<th>71</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (kg)</td>
<td>66.36</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.61</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.60</td>
</tr>
<tr>
<td>Relative Est. Daily Energy Expenditure (kcal/day/kg)</td>
<td>38.25</td>
</tr>
<tr>
<td>Resp. Rate (breaths/min)</td>
<td>16</td>
</tr>
<tr>
<td>Pulse Rate (bpm)</td>
<td>78</td>
</tr>
<tr>
<td>Blood Pressure (mmHg)</td>
<td>129/79</td>
</tr>
<tr>
<td>SPPB (0-12)</td>
<td>11</td>
</tr>
<tr>
<td>Pre-PRS (0-10)</td>
<td>5</td>
</tr>
<tr>
<td>Pre-CNVI (0-5)</td>
<td>3</td>
</tr>
<tr>
<td>Medical conditions</td>
<td>type II diabetes, benign essential tremor, osteoarthritis (not limiting)</td>
</tr>
</tbody>
</table>

Table 3a: Subject 3 Health Characteristics

As Shown in Table 3a, Subject 3’s vital metrics were unremarkable. Subject 3
earned a score of 11 on the SPPB, indicating high mobility.

However, Subject 3 did have a history of type II diabetes, benign essential
tremor, and osteoarthritis. The physician determined, however, that none of these
conditions would prohibit Subject 3 from safely participating in the study. We asked
Subject 3 to report any exacerbation of these conditions, and we monitored any
changes throughout the study. None of these medical conditions impacted our ability
to execute the T.R.A.N.S.I.T. protocol, and Subject 3 did not report any changes in any of the conditions during the study period.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Training</th>
<th>Post-Training</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>QF Muscle CSA (cm²)</td>
<td>39.57</td>
<td>42.24</td>
<td>6.75</td>
</tr>
<tr>
<td>Peak Isometric Torque (N*m)</td>
<td>128.32</td>
<td>140.19</td>
<td>9.18</td>
</tr>
<tr>
<td>Peak Angular Velocity (degrees/s)</td>
<td>320.86</td>
<td>320.86</td>
<td>0.00</td>
</tr>
<tr>
<td>Peak dynamic torque (N*m)</td>
<td>50.44</td>
<td>51.50</td>
<td>2.10</td>
</tr>
<tr>
<td>Peak Power (Watts)</td>
<td>212.34</td>
<td>206.04</td>
<td>-2.97</td>
</tr>
<tr>
<td>Isometric Muscle Quality (N/cm²)</td>
<td>11.49</td>
<td>11.75</td>
<td>2.28</td>
</tr>
<tr>
<td>Dynamic Muscle Quality (Watts/cm²)</td>
<td>5.37</td>
<td>4.88</td>
<td>-9.10</td>
</tr>
<tr>
<td>% Central Activation</td>
<td>100.00</td>
<td>99.67</td>
<td>-0.33</td>
</tr>
<tr>
<td>Angle at Peak Angular Velocity (degrees)</td>
<td>135.22</td>
<td>137.51</td>
<td>1.69</td>
</tr>
<tr>
<td>Angle at Peak dynamic torque (degrees)</td>
<td>103.13</td>
<td>103.13</td>
<td>0.00</td>
</tr>
<tr>
<td>Angle at Peak Power (degrees)</td>
<td>123.76</td>
<td>117.46</td>
<td>-5.09</td>
</tr>
<tr>
<td>Time to Peak Twitch (s)</td>
<td>0.11</td>
<td>0.10</td>
<td>-13.01</td>
</tr>
<tr>
<td>Half-Relaxation Time (s)</td>
<td>0.09</td>
<td>0.12</td>
<td>36.82</td>
</tr>
<tr>
<td>Peak dF/dt (N*m/s)</td>
<td>303.77</td>
<td>408.64</td>
<td>34.52</td>
</tr>
<tr>
<td>Peak -dF/dt (N*m/s)</td>
<td>126.42</td>
<td>103.03</td>
<td>-18.50</td>
</tr>
<tr>
<td>Peak Resting Doublet Torque (N*m)</td>
<td>47.54</td>
<td>58.08</td>
<td>22.19</td>
</tr>
<tr>
<td>Peak Twitch Torque (N*m)</td>
<td>28.26</td>
<td>30.87</td>
<td>9.26</td>
</tr>
</tbody>
</table>

Table 3b: Subject 3 neuromuscular function characteristics and outcomes

As seen in Table 3b, following treatment with the T.R.A.N.S.I.T. protocol, Subject 3 displayed improvements in QF muscle CSA (6.75%), peak isometric torque (9.18%), peak dynamic torque (2.10%), and isometric muscle quality (2.28%), peak dF/dt (34.52%), peak resting doublet torque (22.19%), and peak twitch torque (9.26%). There was no change in angular velocity.
Further, Subject 3 produced diminished values following training for peak power (-2.97%), dynamic muscle quality (-9.10%), central activation (-0.33%), and peak –dF/dt (-18.50%).

Subject 4:

<table>
<thead>
<tr>
<th>Age</th>
<th>66</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>104.55</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.74</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.53</td>
</tr>
<tr>
<td>Relative Est. Daily Energy Expenditure (kcal/day/kg)</td>
<td>34.37</td>
</tr>
<tr>
<td>Resp. Rate (breaths/min)</td>
<td>16</td>
</tr>
<tr>
<td>Pulse Rate (bpm)</td>
<td>62</td>
</tr>
<tr>
<td>Blood Pressure (mmHg)</td>
<td>126/83</td>
</tr>
<tr>
<td>SPPB (0-12)</td>
<td>11</td>
</tr>
<tr>
<td>Pre-PRS (0-10)</td>
<td>5</td>
</tr>
<tr>
<td>Pre-CNVI (0-5)</td>
<td>5</td>
</tr>
<tr>
<td>Medical conditions</td>
<td>type II diabetes, faintness/dizziness</td>
</tr>
</tbody>
</table>

Table 4a: Subject 4 Health Characteristics

As seen in Table 4a, Subject 4 had a few medical conditions to be considered. First, Subject 4 had a BMI of 34.93, indicating obesity. Further, Subject 4 provided a diagnosis of type II diabetes and had occurrences of faintness and dizziness when standing. On one occasion during training, Subject 4 mentioned that he had skipped breakfast and felt hypoglycemic, so we provided a bottle of juice to correct for symptoms of hypoglycemia. The subject did not indicate any symptoms of dizziness or faintness during the study, but we instructed him and all other subjects to take their
time getting into the chair and getting changed to reduce the risk of light-headedness and falling.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Training</th>
<th>Post-Training</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>QF Muscle CSA (cm$^2$)</td>
<td>52.79</td>
<td>51.36</td>
<td>-2.71</td>
</tr>
<tr>
<td>Peak Isometric Torque (N*m)</td>
<td>151.35</td>
<td>169.72</td>
<td>13.46</td>
</tr>
<tr>
<td>Peak Angular Velocity (degrees/s)</td>
<td>293.93</td>
<td>395.34</td>
<td>34.50</td>
</tr>
<tr>
<td>Peak Dynamic Torque (N*m)</td>
<td>65.88</td>
<td>71.28</td>
<td>8.20</td>
</tr>
<tr>
<td>Peak Power (Watts)</td>
<td>292.76</td>
<td>343.94</td>
<td>17.48</td>
</tr>
<tr>
<td>Isometric Muscle Quality (N/cm$^2$)</td>
<td>9.89</td>
<td>11.53</td>
<td>16.62</td>
</tr>
<tr>
<td>Dynamic Muscle Quality (Watts/cm$^2$)</td>
<td>5.55</td>
<td>6.70</td>
<td>20.75</td>
</tr>
<tr>
<td>% Central Activation</td>
<td>89.48</td>
<td>96.84</td>
<td>8.23</td>
</tr>
<tr>
<td>Angle at Peak Angular Velocity (degrees)</td>
<td>125.48</td>
<td>141.52</td>
<td>12.79</td>
</tr>
<tr>
<td>Angle at Peak dynamic torque (degrees)</td>
<td>104.28</td>
<td>101.41</td>
<td>-2.75</td>
</tr>
<tr>
<td>Angle at Peak Power (degrees)</td>
<td>125.48</td>
<td>128.92</td>
<td>2.74</td>
</tr>
<tr>
<td>Time to Peak Twitch (s)</td>
<td>0.10</td>
<td>0.08</td>
<td>-19.73</td>
</tr>
<tr>
<td>Half-Relaxation Time (s)</td>
<td>0.06</td>
<td>0.06</td>
<td>0.33</td>
</tr>
<tr>
<td>Peak dF/dt (N*m/s)</td>
<td>313.50</td>
<td>354.68</td>
<td>13.14</td>
</tr>
<tr>
<td>Peak -dF/dt (N*m/s)</td>
<td>153.50</td>
<td>144.96</td>
<td>-5.57</td>
</tr>
<tr>
<td>Peak Resting Doublet Torque (N*m)</td>
<td>51.36</td>
<td>65.55</td>
<td>27.63</td>
</tr>
<tr>
<td>Peak Twitch Torque (N*m)</td>
<td>22.94</td>
<td>23.65</td>
<td>3.07</td>
</tr>
</tbody>
</table>

Table 4b: Subject 4 neuromuscular function characteristics and outcomes

As shown in Table 4b, Subject 4 increased peak isometric torque (13.46%), peak angular velocity (34.50%), peak dynamic torque (8.20%), peak power (17.48%), isometric muscle quality (16.62%), dynamic muscle quality (20.75%), central activation (7.44%), peak dF/dt (13.14%), peak resting doublet torque (27.63%), and peak twitch torque (3.07%).

Subject 4 displayed a reduction in QF muscle CSA (-2.71%) and peak –df/dt (-5.57%) following training with T.R.A.N.S.I.T.
Combined Outcomes:

<table>
<thead>
<tr>
<th></th>
<th>Pre-Training</th>
<th>Post-Training</th>
<th>p-value</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QF Muscle CSA (cm²)</strong></td>
<td>46.43 (4.18)</td>
<td>46.68 (4.03)</td>
<td>0.81</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Peak Isometric Torque (N*m)</strong></td>
<td>150.43 (10.30)</td>
<td>156.98 (8.16)</td>
<td>0.30</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Peak Angular Velocity (radians/s)</strong></td>
<td>5.53 (.14)</td>
<td>6.30 (.27)</td>
<td>0.13</td>
<td>0.38</td>
</tr>
<tr>
<td><strong>Peak Dynamic Torque (N*m)</strong></td>
<td>57.46 (17.12)</td>
<td>60.50 (29.15)</td>
<td>0.07</td>
<td>&gt;2</td>
</tr>
<tr>
<td><strong>Peak Power (Watts)</strong></td>
<td>246.84 (3.5)</td>
<td>271.06 (4.56)</td>
<td>0.14</td>
<td>&gt;2</td>
</tr>
<tr>
<td><strong>Isometric Muscle Quality (N/cm²)</strong></td>
<td>11.32 (.61)</td>
<td>11.79 (.52)</td>
<td>0.36</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Dynamic Muscle Quality (Watts/cm²)</strong></td>
<td>5.31 (.13)</td>
<td>5.81 (.05)</td>
<td>0.27</td>
<td>1.08</td>
</tr>
<tr>
<td><strong>% Central Activation</strong></td>
<td>96.33 (2.34)</td>
<td>96.37 (1.41)</td>
<td>0.72</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Angle at Peak Angular Velocity (radians)</strong></td>
<td>2.37 (.05)</td>
<td>2.46 (.04)</td>
<td>0.68</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Angle at Peak Dynamic Torque (radians)</strong></td>
<td>1.78 (.29)</td>
<td>1.82 (.02)</td>
<td>0.57</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Angle at Peak Power (radians)</strong></td>
<td>2.23 (.13)</td>
<td>2.15 (.47)</td>
<td>0.25</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Time to Peak Twitch (s)</strong></td>
<td>0.10 (.00)</td>
<td>0.083 (.00)</td>
<td>0.001</td>
<td>&gt;2</td>
</tr>
<tr>
<td><strong>Half-Relaxation Time (s)</strong></td>
<td>0.074 (.01)</td>
<td>0.084 (.02)</td>
<td>0.44</td>
<td>1.75</td>
</tr>
<tr>
<td><strong>Peak dF/dt (N*m/s)</strong></td>
<td>445.22 (21.23)</td>
<td>513.06 (47.58)</td>
<td>0.19</td>
<td>1.32</td>
</tr>
<tr>
<td><strong>Peak -dF/dt (N*m/s)</strong></td>
<td>204.56 (25.95)</td>
<td>214.85 (55.30)</td>
<td>0.76</td>
<td>&gt;2</td>
</tr>
<tr>
<td><strong>Peak Resting Doublet Torque (N*m)</strong></td>
<td>57.64 (6.23)</td>
<td>58.71 (3.72)</td>
<td>0.88</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Peak Twitch Torque (N*m)</strong></td>
<td>28.74 (3.30)</td>
<td>26.55 (2.25)</td>
<td>0.43</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Table 5a: Average neuromuscular function characteristics and outcomes (n = 4). Cohen’s d indicates effect sizes (d > 0.2 indicates small effect size; d > 0.5 indicates a medium effect size; d > 0.8 indicates a large effect size).
Figure 5: %Change in average neuromuscular outcomes following T.R.A.N.S.I.T. *Indicates significance (p<.05)
<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>AVERAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Δ QF Muscle CSA</td>
<td>-2.72</td>
<td>1.53</td>
<td>6.75</td>
<td>-2.71</td>
<td>0.55</td>
</tr>
<tr>
<td>%Δ Peak Isometric Torque</td>
<td>1.13</td>
<td>-2.98</td>
<td>9.18</td>
<td>13.46</td>
<td>4.71</td>
</tr>
<tr>
<td>%Δ Peak Angular Velocity</td>
<td>9.07</td>
<td>13.26</td>
<td>0.00</td>
<td>34.50</td>
<td>13.76</td>
</tr>
<tr>
<td>%Δ Peak Dynamic Torque</td>
<td>2.59</td>
<td>7.16</td>
<td>2.10</td>
<td>8.20</td>
<td>5.29</td>
</tr>
<tr>
<td>%Δ Peak Power</td>
<td>7.34</td>
<td>13.99</td>
<td>-2.97</td>
<td>17.48</td>
<td>9.81</td>
</tr>
<tr>
<td>%Δ Isometric Muscle Quality</td>
<td>3.95</td>
<td>-4.44</td>
<td>2.28</td>
<td>16.62</td>
<td>4.17</td>
</tr>
<tr>
<td>%Δ Dynamic Muscle Quality</td>
<td>10.34</td>
<td>12.28</td>
<td>-9.10</td>
<td>20.75</td>
<td>8.58</td>
</tr>
<tr>
<td>%Δ Central Activation</td>
<td>-3.79</td>
<td>-2.57</td>
<td>0.00</td>
<td>7.45</td>
<td>0.27</td>
</tr>
<tr>
<td>%Δ Angle at Peak Angular Velocity</td>
<td>-14.13</td>
<td>17.73</td>
<td>1.69</td>
<td>12.79</td>
<td>3.36</td>
</tr>
<tr>
<td>%Δ Angle at Peak Dynamic Torque</td>
<td>12.73</td>
<td>-0.54</td>
<td>0.12</td>
<td>-2.75</td>
<td>2.10</td>
</tr>
<tr>
<td>%Δ Angle at Peak Power</td>
<td>-8.82</td>
<td>-2.74</td>
<td>-5.09</td>
<td>2.74</td>
<td>-3.59</td>
</tr>
<tr>
<td>%Δ Time to Peak Twitch</td>
<td>-22.24</td>
<td>-21.43</td>
<td>-13.01</td>
<td>-19.73</td>
<td>-19.10</td>
</tr>
<tr>
<td>%Δ Half-Relaxation Time</td>
<td>36.65</td>
<td>-28.51</td>
<td>36.82</td>
<td>0.33</td>
<td>11.32</td>
</tr>
<tr>
<td>%Δ Peak dF/dt</td>
<td>-8.86</td>
<td>22.09</td>
<td>34.52</td>
<td>13.14</td>
<td>15.22</td>
</tr>
<tr>
<td>%Δ Peak -dF/dt</td>
<td>-9.49</td>
<td>36.12</td>
<td>-18.50</td>
<td>-5.57</td>
<td>0.64</td>
</tr>
<tr>
<td>%Δ Peak Resting Doublet Torque</td>
<td>-13.41</td>
<td>-17.13</td>
<td>22.19</td>
<td>27.63</td>
<td>4.82</td>
</tr>
<tr>
<td>%Δ Peak Twitch Torque</td>
<td>-15.05</td>
<td>-21.54</td>
<td>9.26</td>
<td>3.07</td>
<td>-6.06</td>
</tr>
</tbody>
</table>

Table 5b: %Change in neuromuscular function for each subject and overall
**QF Muscle CSA:**

In the initial study of T.R.A.N.S.I.T. (37), young subjects significantly increased QF muscle CSA in the 5 week training protocol by approximately 3%.

However, among all subjects enrolled in our study (shown in Figure 5, Tables 5a and 5b), there was very little change in QF muscle CSA (0.55%). This finding is intriguing, as the older subjects had 8 weeks of training, as opposed to the 5 weeks of training in the previous study with younger subjects. These results could be due to the diminished hypertrophic response to training that occurs in aged muscle. While I was unable to find research into hypertrophic response in older individuals with regard to sprint training, there is evidence that sprint training can result in hypertrophy in younger individuals (47).

The wide variation between subjects in this experiment also suggests that there may be significant error with our MRI analysis method. For example, our method used the image analysis program MIPAV. MIPAV used MRI images and categorized area of muscle, fat, and bone using pixel intensities of individual pixels, and then calculating the total number of pixels within a specific range of intensities. In each MRI image, adipose tissue has a high pixel intensity (it appears relatively light), the periosteum of bone has a very low pixel intensity (it appears relatively dark), and muscle has a pixel intensity in between that of adipose tissue and bone (it appears grey).

However, because the pixels between bone, adipose, and muscle have intermediate pixel intensities that do not readily belong to one of the three distinct
categories, they could be placed into either of the categories that the pixel intensity lies between. Therefore, there is uncertainty as to where to set cutpoints, and consequently inherent error to the method. In our method, we suspected that it underestimated the amount of adipose tissue in the quadriceps femoris and overestimated the amount of muscle tissue.

Further, we decided to have subjects undergo MRI scans of ~15 minutes instead of ~30 minutes to reduce discomfort and time of the study. However, this was a trade off as MRI scans were of lesser quality than they would have been if we took images using a ~30 minute scan. This may have reduced the precision of the MRI analysis, as fewer pixels were depicted per image; therefore, they were more blurred together than a higher quality image would have been. However, if we had chosen to use ~30 minute scans, the cost of the study would have increased substantially and it would have increased the amount of time that the subject had to commit to the study.

In any case, if we conduct a follow-up experiment, we will certainly review our methods for MRI analysis and perhaps use a new imaging technique or protocol of analysis.

**Peak Isometric Torque:**

In the initial study by Russ et al (37), young subjects demonstrated a mean 2.98% increase in peak isometric torque. However, because the young subjects trained only for 5 weeks, as opposed to 8 weeks that subjects trained in this study, it is
unclear if there are any age differences. However, older subjects appear to respond at least as well as younger subjects in gains of peak isometric torque.

As we hypothesized, peak isometric torque increased by 4.71%. Interestingly, however, there may be an age-related component to this increase. The two oldest subjects, aged 66 and 71, improved peak isometric torque by 13.46% and 9.18%, respectively. In contrast, the two youngest subjects, both aged 63, changed peak isometric torque by 1.13% and -2.98%. It is possible therefore, that T.R.A.N.S.I.T. is more effective at improving isometric muscle strength with greater age.

![Figure 6: Change in angular velocity for each subject](image-url)
Peak Angular Velocity

On average, peak angular velocity increased by 13.76%. However, for Subject 4 (as shown in Figure 6), who exhibited the greatest proportional improvements of all indicators of muscle function except for QF muscle CSA, improved peak angular velocity by 34.50%. This staggering improvement likely accounted for the majority of Subject 4’s improvement in peak power (17.48%) and dynamic muscle quality (20.75%), as peak dynamic torque only improved by 8.20% for Subject 4.

Based on this finding, peak angular velocity may be the most malleable aspect of muscle function and quality. It is possible that this improvement was due to improved calcium release from the muscle, as the rate of calcium release is one of the limiting factors to contractile velocity (46).

Peak Dynamic Torque:

Peak dynamic torque increased by 5.29% on average; relatively small increases occurred in each subject. This improvement is similar to the average improvement in peak isometric torque of 4.71%. This may point to the fact that, in contrast to improvement in angular velocity, the improvement in maximal force production was relatively limited.

Peak Power

Peak power improved by an average of 9.81% following training with T.R.A.N.S.I.T. Increases for each subject were roughly proportional to improvements
in angular velocity. Accordingly, more of the improvement in peak power can be explained by increased angular velocity, as opposed to increased peak dynamic torque.

**Figure 7:** Change in isometric muscle quality for each subject

**Isometric Muscle Quality:**

In the initial study by Russ et al (37), muscle quality improved by an average of 0.79% for the young subjects.

In this study, however, isometric muscle quality improved by 4.17%, on average. Changes in isometric muscle quality (as shown in **Figure 7**) were relatively small for Subjects 1, 2, and 3 (3.95, -4.44, and 2.28%, respectively). However, Subject 4 showed a relatively large improvement of 16.62%. Given that Subject 4
displayed improvements in all aspects of muscle function measured, T.R.A.N.S.I.T. may improve isometric muscle quality to a greater extent if there is a pre-existing deficit in central activation. In fact, subject 4 was the only subject to increase central activation (7.45%).

**Figure 8**: Change in dynamic muscle quality for each subject

**Dynamic Muscle Quality:**

Dynamic muscle quality improved by 8.58% on average, but as seen in the figure below, changes differed in magnitude between subjects (shown in **Figure 8**). This variation, as with isometric muscle quality, suggests that response to T.R.A.N.S.I.T. may be dependent on the initial level of muscle function.
Central Activation:

The data for change in central activation are relatively mixed (-4.34%, -2.57%, -0.33%, 7.44%) for an average of 0.27%. These results were expected, because while NMES has actually been shown to improve central activation (38) in patients with deficits, subjects in this study displayed relatively high levels of central activation.

Subject 4 was an exception, however, as he improved his central activation by 7.44%. His low baseline levels of central activation likely contributed to his overall gains from T.R.A.N.S.I.T. training.

Angle at Peak Angular Velocity, Torque, and Power:

There was little change in the angles at which peak angular velocity, torque, or power was attained (3.36%, 2.10%, and -3.59%). Further, considering the substantial variation at which these angles were attained per subject (both pre- and post-training), our data do not indicate significant alteration of the angle at which peak angular velocity, torque, or power occur. However, these variables would certainly be important for muscle function, especially during maximal efforts. For example, the angles at which angular velocity, torque, and power occur could have implications for functional activities, such as climbing stairs or getting in and out of a car.
**Muscle Contractile Properties:**

After T.R.A.N.S.I.T., subjects exhibited significantly lower (p =.0013) time to maximal twitch force from onset of contraction (-19.10% change), despite the very small number of subjects enrolled in our study (n = 4). This finding supports our hypothesis that T.R.A.N.S.I.T. improved calcium release, because calcium release is one of the primary factors in speed of contraction (46).

All other contractile properties (1/2 relaxation time, peak dF/dt, peak –dF/dt, peak resting doublet force, and peak twitch force) exhibited large overall changes; however, they also had large variations, and were consequently not statistically significant. However, with a larger sample size, trends may show that improvements with muscle contractile properties correlate with improvements in other neuromuscular variables.

**DISCUSSION:**

With this thesis project, we set out to determine the effects of T.R.A.N.S.I.T. on muscle quality in older individuals. Our primary hypothesis was that T.R.A.N.S.I.T would improve muscle quality. Further, we hypothesized that isometric muscle quality would improve more than dynamic muscle quality. Finally, we hypothesized that muscle quality would improve more for people who had relatively low neuromuscular function.
This experiment served as a pilot study to test the feasibility of the treatment on older individuals. This gave us the opportunity to refine the protocol before initiating an investigation on a larger scale, and has shown that there are several reasons to conduct a follow-up investigation.

Perhaps the most surprising finding of this study is that, contrary to our hypothesis that isometric function would improve more than dynamic muscle function, measurements of dynamic muscle function (peak angular velocity, peak dynamic torque, and peak power) all increased to a greater extent than our measurements of isometric muscle function (peak isometric torque), despite training in an isometric mode. Specifically, peak angular velocity increased by 13.76%, peak dynamic torque increased by 5.29%, and peak power increased by 9.81%, whereas peak isometric torque increased by only 4.71% on average.

Our focal outcome of interest, muscle quality, aligned with these trends, as it is calculated as simply peak isometric torque (for isometric muscle quality) or peak power (for dynamic muscle quality) divided by muscle size. In fact, change in dynamic muscle quality was more than double that of isometric muscle quality (8.18% vs. 4.17%).

It would have stood to reason that, with T.R.A.N.S.I.T., greatest improvements would be observed in isometric indicators of force production (i.e. peak isometric torque). However, our data show the opposite.

These results contradict the principle of exercise specificity; that is, physiological adaptations are specific to the type of exercise performed, in terms of
duration, muscles used, and intensity (45). The principle of exercise specificity would have predicted that, because subjects trained isometrically, they would have improved their isometric muscle function more than dynamic contractions.

Many factors may have influenced this change. First, calcium release may have improved from the SR, as we hypothesized. Our data support this, as greatest improvements occurred in angular velocity as opposed to peak isometric or dynamic torque. Further, time to peak twitch decreased significantly (-19.10%), suggesting that calcium release from the SR may have occurred at an increased rate as a result of the training. This would be significant, as a sprint cycling protocol tested on young people has been the only form of exercise shown to improve SR calcium release (33). Likewise, T.R.A.N.S.I.T. is the only treatment that has been shown to improve SR calcium release (37), also in young people. Our data therefore suggest that older individuals may adapt similarly, or even to a greater extent. Improved calcium release would improve the rate of cross bridge cycling, thereby increasing velocity and consequently power of contraction. Power output is a critical component of muscle function, so T.R.A.N.S.I.T. may be a useful treatment to induce this adaptation, especially in cases in which there is impaired calcium release from the SR.

Interestingly, there appeared to be clear differences between subjects in their responses to the training protocol. Specifically, the starkest differences in neuromuscular outcomes occur in Subject 4. As shown in Table 5b, Subject 4 exhibits the greatest improvements in nearly every functional category. This may be due in part to the improvement in central activation that Subject 4 attained as a result
of the training (7.45%). Of note, Subject 4 was the only subject that improved central activation during the study. It is possible, therefore, that his deficit in central activation contributed to his relatively low starting values in certain neuromuscular functional characteristics measured before training. Specifically, he had the lowest values for isometric muscle quality among all subjects. After training, however, Subject 4’s central activation reached nearly 97%, a relatively high value for central activation. This suggests that T.R.A.N.S.I.T. may have rectified this deficiency in central activation, which resulted in great improvements in variables that necessitated voluntary muscle activation, such as peak isometric and dynamic torque, isometric and dynamic muscle quality, and all constituent components of these variables.

These data support the work of Clark et al (9), which show that seniors that are in the lowest tertile of strength exhibit a 20% deficit in central activation, on average. In our study, likewise, Subject 4 possessed the lowest overall values for isometric muscle quality before the T.R.A.N.S.I.T. training. However, because Clark et al measured strength isometrically, and measured the wrist flexors instead of the knee extensors, the results are not completely transitive. In fact, our data on dynamic muscle quality contradict these findings, as Subject 4 actually had the 2nd highest starting value for dynamic muscle quality. Therefore, limited central activation may not correlate as well with dynamic muscle function as it does with isometric muscle function. Further, the impact of central activation impairment may vary between muscle groups (i.e., knee extensors vs. wrist flexors).
As other subjects displayed relatively high central activation levels before the training (all greater than 99%), it is also possible that the other subjects would have displayed greater improvements in neuromuscular function measurements if they, like Subject 4, also possessed a deficit in central activation. These observations, in contrast with Subject 4, indicate that T.R.A.N.S.I.T. training may not be as useful in patients who do not have a deficit in central activation capacity.

In fact, Subjects 1, 2, and 3 all either maintained level of central activation or exhibited a small reduction. It is possible that these reductions declined as a result of age, but given that this was an 8 week study, it seems extremely unlikely. It is also possible that an undiagnosed condition somehow contributed to a decline as well (possibly neurodegenerative diseases, such as amyotrophic lateral sclerosis or multiple sclerosis); however, subjects did not inform us if any of these diagnoses came about during the study period.

More likely, reductions could have been the result of a submaximal effort during the testing following the training. If subjects did not recruit as many motor units volitionally as possible, our supra-maximal stimulation at the peak of contraction would have increased their forces by a greater magnitude than if subjects did recruit all of the motor units possible. Unfortunately, we do not have a method to precisely validate maximal contractile effort.

While T.R.A.N.S.I.T. training does appear to affect some aspects of neuromuscular function, it also seems to have no effect on other variables. For example, in our measurements of angle at peak dynamic torque, angular velocity, and
power, there does not seem to be any change following T.R.A.N.S.I.T. training.

Changes between subjects were widely variable, and based on these data, there does not appear to be an effect of T.R.A.N.S.I.T. on any of these parameters.

Further, changes between subjects in half relaxation time, dF/dt, -dF/dt, peak resting doublet torque, and peak twitch torque were extremely variable, and do not appear to correlate with any other neuromuscular function indicators that we measured. This suggests that T.R.A.N.S.I.T. training does not exert a clear influence on these physiological variables either.

Of course, as with all of the variables that we measured, we were limited by our small sample size and failed to detect trends that were present. To this end, we calculated Cohen’s d (a measure of effect size) for all of the variables we measured (Table 5a). We found that many variables exhibited a large effect size, where Cohen’s d > 0.8. Many of our primary outcome measurements, including peak torque (d > 2), peak power (d > 2), isometric muscle quality (d = 0.81), and dynamic muscle quality (d = 1.08) exhibited large effect sizes. On the other hand, because of our small sample size, it is also possible that we have detected trends that would not have been present with a larger sample size.

FUTURE DIRECTIONS:

There are many directions we could go to follow up on these results. First and foremost, we would like to conduct a follow-up study with a larger sample size,
perhaps with a control group that receives a sham treatment. One of the limitations is that we did not have a control group to account for the placebo effect and other biases.

It would also be interesting to see if there is any difference in adaptation to training in people with functional disability, as we would expect that they would show enhanced responses to training. We could aim to recruit participants that scored below a certain threshold on the SPPB that indicated risk for nursing home admission and short-term mortality, as this group would benefit the most from increased muscle quality.

Further, we could delve into the molecular mechanisms of adaptation and conduct histological studies. Specifically, we could collect muscle biopsies (pre/post) and use them to determine maximal calcium release and uptake. Further, we could analyze any array of factors, including the protein concentrations and activity involved in the E-CC pathway, as well as other proteins that may contribute to muscle quality in other ways, such as citrate synthase and lactate dehydrogenase. We could also analyze fiber types from the biopsies by staining for myosin ATPase isoforms and determine whether any fiber type shift occurs as a result of the T.R.A.N.S.I.T. training.

In any case, we believe that T.R.A.N.S.I.T. warrants further investigation as a potential treatment for impaired muscle quality with age. In fact, as with most work in science, this study leaves us with more questions to answer than with which we started.
REFERENCES:


Appendix A: Institutional Review Board Approval and Informed Consent Documents
The amendment, detailed below has been reviewed and approved by the Institutional Review Board at Ohio University.

**Amendment: Increase Compensation; Minor form Revisions; Add Research Assistants**

**Project Title:** A Pilot Study of TRANSIT to Improve Muscle Quality in Older Individuals

**Primary Investigator:** Eric Leach

**Co-Investigator(s):** Brian Clark

**Advisor:** David Russ

**Department:** Honors Tutorial College

Expiration Date: 6/2/2016
Ohio University Adult Consent Form with Signature

Title of Research: A Pilot Study of TRANSIT to Improve Muscle Quality in Older Individuals

Researchers: Dr. David Russ (Supervisor), Dr. Brian Clark (Co-Investigator), and Eric Leach (Student)

You are being asked to participate in research. For you to be able to decide whether you want to participate in this project, you should understand what the project is about, as well as the possible risks and benefits in order to make an informed decision. This process is known as informed consent. This form describes the purpose, procedures, possible benefits, and risks. It also explains how your personal information will be used and protected. Once you have read this form and your questions about the study are answered, you will be asked to sign it. This will allow your participation in this study. You should receive a copy of this document to take with you.

Explanation of Study

This study is being done because we are testing a new method to improve thigh muscle function. This method uses a specific type of electrical stimulation to activate your muscles in a unique way. We put several electrodes on parts of the front of your thigh, and then we use electricity to activate the nerves in your leg, causing the muscles in the front of your thigh to tighten up. We have tested our method previously on young people, and found that they tolerated it very well and that their thigh muscles benefited from the treatment in several ways, including increased calcium release within the muscle and increased muscle size. However, we believe that our method may be of even more benefit to older people. This study is therefore being conducted to determine if and how this treatment affects the muscles of older people.

In order to participate in this study, you must be between the ages of 60 and 80 years old. You should not participate in this study if you have a certain medical conditions, including: blood clotting disorders, symptomatic anemia, high blood pressure (greater than 140/90 mmHg), active cancer treatment, peripheral vascular disease, major psychiatric disease, rheumatoid arthritis, liver disease, renal disease, uncontrolled diabetes, primary neurological disorder (such as ALS or MS), peripheral neuropathy of the lower extremities, osteoporosis, recent fracture of the lower limb (within the last 6 months) or a compression fracture of a vertebra (bone in your back). You should also not participate if you have claustrophobia, recent alcohol or drug abuse, certain metallic implants (such as a pacemaker), or a Body Mass Index (a ratio of height to weight) greater than 30 kg/m². Finally, you cannot participate if you exercise regularly (more than 30 minutes, twice a week), or have regularly performed resistance exercise (weightlifting) in the past 6 months. We will ask you to fill out several forms to check for any of these issues. Additionally, you will see a physician to be cleared before any testing or training.
Because we will be conducting magnetic resonance imaging (MRI), you will not be permitted to participate in the experiment if any of the following apply to you:

- You have one or more medical device implants, such as heart pacemakers or inner ear implants
- You have a piece or pieces of metal close to or in an important organ.

Your participation in the study will last for 8 weeks of training, with two sessions before the training for screening and pre-training testing, and two sessions after your training for post-training testing. In total, if you decide to participate in the study, your involvement in the study will last for 9-10 weeks.

**Explanation of Your Participation in the Experiment**

**Pre-Training Testing**

For the pre-training testing, you will be asked to participate in a series of tests that measure your muscle function. The pre-training testing will take place over 2 sessions, on separate days. This testing will take place in Ohio Musculoskeletal and Neuromuscular Institute on Ohio University’s campus, located in Irvine Hall.

For your first session, you will first complete balance testing, which will take about 10 minutes. Balance testing involves standing up from a chair and balancing while standing up with your feet off-set. Next, you will complete muscle strength testing for your non-dominant leg, during which you will kick out as hard as you can against resistance. During some of these tests, we will place one set of two large electrodes on the front of your thigh. One electrode will be near your hip, and the other will be close to your knee. When activated, they will stimulate your leg to tighten with electricity. This session will take approximately 60 minutes.

For your second session, you will have an MRI on your thigh that will measure the muscle size. This will take approximately 45 minutes.

**Training Program**

After the pre-training testing, you will begin a training program to work the muscles in your non-dominant leg. The training program will involve you kicking out your non-dominant leg as hard as you can and activating portions of your muscle with three sets of two small electrodes between your hip and your knee on your non-dominant leg. These pairs of electrodes will be placed on the front of your thigh, on the inner, middle, and outer side. You will complete the training sessions 3 times a week, for 8 weeks. Each training session will take approximately 30 minutes. However, the first training session will take approximately 45 minutes so that we can fit the training equipment to you. Training can take place on 2 consecutive days, but you should not train 3 days in a row.

**Post-Training Testing**
After the training program, we will conduct the same series of tests that we used before the training program to test muscle function, and they will take roughly the same amount of time. We will not re-do the balance testing.

**Risks and Discomforts**

**Pre-Training Testing**

The balance testing carries no more risk than normal, everyday movement. Risks or discomforts that you might experience as a result from the pre-experiment muscle function testing are muscle soreness due to some of the strength testing that you will perform. If muscle soreness occurs, you will feel it usually 24-48 hours after the training session, although you may feel it anytime during the duration of the study or for a few days after its completion. You can use ice and/or over the counter pain relievers if necessary. It is also possible that you could have a strain in a muscle or a fracture as a result of the training. However, this is very unlikely, and our laboratory has not seen a single occurrence of a strain in a muscle or a fracture during our testing for hundreds of people. We will further reduce any risks by screening for bone conditions (like osteoporosis) and by using very brief electrical pulses during the testing. You will be monitored by study personnel throughout the testing and asked at regular intervals to report any abnormal discomfort. If this happens, or if you ask us to stop, the study will be stopped immediately.

There are no known risks to the MRI as long as you are cleared by the screening process.

**Training Program**

During the training program, you may also experience muscle soreness, as described above. Further, a strain or fracture is possible, but very unlikely. Because the muscle forces generated during training are lower than those during the pre-training testing, the risks are even smaller.

For this training program, you will be trained using electrical stimulation. Electrical stimulation is not known to lead to any long term risks in the body, as it has used safely for more than 50 years. However, there may be a perception of discomfort because there will be a ‘shock’ applied to your leg and your muscle will contract without your effort. There may also be discomfort associated due your own activation of your muscles. Occasionally, people are mildly allergic to the adhesives on the electrodes, leading to skin irritation, including redness and itching, and possibly a rash. If you experience any of these problems, notify the team at once and we will remove you from the study. In our experience however, such reactions to the gel on the stimulating electrodes is very rare. You will be monitored by study personnel throughout the testing and asked at regular intervals to report any abnormal discomfort. If this happens, or if you ask us to stop, the study will be stopped immediately.
Post-Training Testing

Risks or discomforts that you might experience as a result of the post-training testing are the same as those that are described for the pre-experiment testing.

Benefits

This study is important to science/society because it has the potential to help develop treatments to improve muscle function in new ways, for older individuals. The method of training we are developing may lead to a new way for medical professionals to help maintain the independence and everyday function of older individuals.

You may not benefit, personally by participating in this study. However, we expect that the strength of the leg that is trained will increase.

Confidentiality and Records

Your study information will be kept confidential by keeping your personal information in a master list that will be locked in a cabinet and backed-up on the password-protected supervisor's computer. Your data will be recorded as a code that does not contain any of your personal information. Only the investigators in the study will have access to your data, with the exception of your name and address. Because full compensation for this study may exceed $100, your name and address must be provided to the Ohio University Finance Office for accounting purposes. The Finance office may also require your social security number for tax purposes. None of the actual data recorded during any of the experiments will be shared with the Finance Office. Published or presented data will not identify you in any way. You will not be audiotaped or videotaped.

Additionally, while every effort will be made to keep your study-related information confidential, there may be circumstances where this information must be shared with: 1) Federal agencies, for example the Office of Human Research Protections, whose responsibility is to protect human subjects in research; and 2) Representatives of Ohio University (OU), including the Institutional Review Board, a committee that oversees the research at OU.

Compensation

As compensation for your time/effort, you will receive $425. If you withdraw from the study before finishing it, you will be paid on a pro-rated scale for the parts of it you completed.

Contact Information

If you have any questions regarding this study, please contact the investigator Eric Leach, (614) 556-5766 or el887611@ohio.edu, the co-investigator Dr. Brian Clark, 740-593-2354 or clarkb2@ohio.edu, or the advisor Dr. David Russ, (740) 566-0022 or russd@ohio.edu. Additionally, Dr. Law can be reached on his cell phone at 270-748-4772 to discuss health and medical issues. For urgent health and medical questions you are advised to call 911 and seek medical care.
immediately.

If you have any questions regarding your rights as a research participant, please contact Dr. Chris Hayhow, Director of Research Compliance, Ohio University, (740)593-0664 or hayhow@ohio.edu.

By signing below, you are agreeing that:
- you have read this consent form (or it has been read to you) and have been given the opportunity to ask questions and have them answered;
- you have been informed of potential risks and they have been explained to your satisfaction;
- you understand Ohio University has no funds set aside for any injuries you might receive as a result of participating in this study;
- you are 18 years of age or older;
- your participation in this research is completely voluntary;
- you may leave the study at any time; if you decide to stop participating in the study, there will be no penalty to you and you will not lose any benefits to which you are otherwise entitled.

Ohio University would like to contact you in the future about other research studies or opportunities. Please check the appropriate box below and initial:
[ ] I agree to be contacted for other research studies and opportunities
[ ] I do NOT agree to be contacted for other research studies and opportunities

Signature: __________________________________________

Printed Name: _______________________________________

Date: ________________

Administrator of Consent: ______________________________
Date: ________________

Version Date: [8/31/15]
Ohio University Adult Consent Form with Signature
(Large Print Version)

Title of Research: A Pilot Study of TRANSIT to Improve Muscle Quality in Older Individuals

Researchers: Dr. David Russ (Supervisor), Dr. Brian Clark (Co-Investigator), and Eric Leach (Student)

You are being asked to participate in research. For you to be able to decide whether you want to participate in this project, you should understand what the project is about, as well as the possible risks and benefits in order to make an informed decision. This process is known as informed consent. This form describes the purpose, procedures, possible benefits, and risks. It also explains how your personal information will be used and protected. Once you have read this form and your questions about the study are answered, you will be asked to sign it. This will allow your participation in this study. You should receive a copy of this document to take with you.

Explanation of Study
This study is being done because we are testing a new method to improve thigh muscle function. This method uses a specific type of electrical stimulation to activate your muscles in a unique way. We put several electrodes on parts of the front of your thigh, and then we use electricity to activate the nerves in your leg, causing the muscles in the front of your thigh to tighten up. We have tested our method previously on young people, and found that they tolerated it very well and that their thigh muscles benefited from the treatment in several ways, including increased calcium release within the muscle and increased muscle size. However, we believe that our method may be of even more benefit to older people. This study is therefore being conducted to determine if and how this treatment affects the muscles of older people.

In order to participate in this study, you must be between the ages of 60 and 80 years old. You should not participate in this study if you have a certain medical conditions, including: blood clotting disorders, symptomatic anemia, high blood pressure (greater than 140/90 mmHg), active cancer treatment, peripheral vascular disease, major psychiatric disease, rheumatoid arthritis, liver disease, renal disease, uncontrolled diabetes, primary neurological disorder (such as ALS or MS), peripheral neuropathy of the lower extremities, osteoporosis, recent fracture of the lower limb (within the
last 6 months) or a compression fracture of a vertebra (bone in your back). You should also not participate if you have claustrophobia, recent alcohol or drug abuse, certain metallic implants (such as a pacemaker), or a Body Mass Index (a ratio of height to weight) greater than 30 kg/m². Finally, you cannot participate if you exercise regularly (more than 30 minutes, twice a week), or have regularly performed resistance exercise (weightlifting) in the past 6 months. We will ask you to fill out several forms to check for any of these issues. Additionally, you will see a physician to be cleared before any testing or training.

Because we will be conducting magnetic resonance imaging (MRI), you will not be permitted to participate in the experiment if any of the following apply to you:

- You have one or more medical device implants, such as heart pacemakers or inner ear implants.
- You have a piece or pieces of metal close to or in an important organ.

Your participation in the study will last for 8 weeks of training, with two sessions before the training for screening and pre-training testing, and two sessions after your training for post-training testing. In total, if you decide to participate in the study, your involvement in the
study will last for 9-10 weeks.

**Explanation of Your Participation in the Experiment**

**Pre-Training Testing**

For the pre-training testing, you will be asked to participate in a series of tests that measure your muscle function. The pre-training testing will take place over 2 sessions, on separate days. This testing will take place in Ohio Musculoskeletal and Neuromuscular Institute on Ohio University’s campus, located in Irvine Hall.

For your first session, you will first complete balance testing, which will take about 10 minutes. Balance testing involves standing up from a chair and balancing while standing up with your feet off-set. Next, you will complete muscle strength testing for your non-dominant leg, during which you will kick out as hard as you can against resistance. During some of these tests, we will place one set of two large electrodes on the front of your thigh. One electrode will be near your hip, and the other will be close to your knee. When activated, they will stimulate your leg to tighten with electricity. This session will take approximately 60 minutes.

For your second session, you will have an MRI on your thigh that will measure the muscle size. This will take approximately 45 minutes.
**Training Program**

After the pre-training testing, you will begin a training program to work the muscles in your non-dominant leg. The training program will involve you kicking out your non-dominant leg as hard as you can and activating portions of your muscle with three sets of two small electrodes between your hip and your knee on your non-dominant leg. These pairs of electrodes will be placed on the front of your thigh, on the inner, middle, and outer side. You will complete the training sessions 3 times a week, for 8 weeks. Each training session will take approximately 30 minutes. However, the first training session will take approximately 45 minutes so that we can fit the training equipment to you. Training can take place on 2 consecutive days, but you should not train 3 days in a row.

**Post-Training Testing**

After the training program, we will conduct the same series of tests that we used before the training program to test muscle function, and they will take roughly the same amount of time. We will not re-do the balance testing.

**Risks and Discomforts**

**Pre-Training Testing**

The balance testing carries no more risk than normal, everyday movement. Risks or discomforts that
you might experience as a result from the pre-
experiment muscle function testing are muscle
soreness due to some of the strength testing that you
will perform. If muscle soreness occurs, you will feel it
usually 24-48 hours after the training session, although
you may feel it anytime during the duration of the study
or for a few days after its completion. You can use ice
and/or over the counter pain relievers if necessary. It is
also possible that you could have a strain in a muscle
or a fracture as a result of the training. However, this is
very unlikely, and our laboratory has not seen a single
occurrence of a strain in a muscle or a fracture during
our testing for hundreds of people. We will further
reduce any risks by screening for bone conditions (like
osteoporosis) and by using very brief electrical pulses
during the testing. You will be monitored by study
personnel throughout the testing and asked at regular
intervals to report any abnormal discomfort. If this
happens, or if you ask us to stop, the study will be
stopped immediately.

There are no known risks to the MRI as long as you
are cleared by the screening process.

**Training Program**

During the training program, you may also
experience muscle soreness, as described above.
Further, a strain or fracture is possible, but very unlikely.
Because the muscle forces generated during training are lower than those during the pre-training testing, the risks are even smaller.

For this training program, you will be trained using electrical stimulation. Electrical stimulation is not known to lead to any long term risks in the body, as it has used safely for more than 50 years. However, there may be a perception of discomfort because there will be a ‘shock’ applied to your leg and your muscle will contract without your effort. There may also be discomfort associated due your own activation of your muscles. Occasionally, people are mildly allergic to the adhesives on the electrodes, leading to skin irritation, including redness and itching, and possibly a rash. If you experience any of these problems, notify the team at once and we will remove you from the study. In our experience however, such reactions to the gel on the stimulating electrodes is very rare. You will be monitored by study personnel throughout the testing and asked at regular intervals to report any abnormal discomfort. If this happens, or if you ask us to stop, the study will be stopped immediately.

Post-Training Testing

Risks or discomforts that you might experience as a result of the post-training testing are the same as those that are described for the pre-experiment testing.
Benefits

This study is important to science/society because it has the potential to help develop treatments to improve muscle function in new ways, for older individuals. The method of training we are developing may lead to a new way for medical professionals to help maintain the independence and everyday function of older individuals.

You may not benefit, personally by participating in this study. However, we expect that the strength of the leg that is trained will increase.

Confidentiality and Records

Your study information will be kept confidential by keeping your personal information in a master list that will be locked in a cabinet and backed-up on the password-protected supervisor’s computer. Your data will be recorded as a code that does not contain any of your personal information.

Only the investigators in the study will have access to your data, with the exception of your name and address. Because full compensation for this study may exceed $100, your name and address must be provided to the Ohio University Finance Office for accounting purposes. The Finance office may also require your social security number for tax purposes. None of the actual data recorded during any of the experiments will be shared with the
Finance Office. Published or presented data will not identify you in any way. You will not be audiotaped or videotaped.

Additionally, while every effort will be made to keep your study-related information confidential, there may be circumstances where this information must be shared with: 1) Federal agencies, for example the Office of Human Research Protections, whose responsibility is to protect human subjects in research; and 2) Representatives of Ohio University (OU), including the Institutional Review Board, a committee that oversees the research at OU.

Compensation
As compensation for your time/effort, you will receive $425. If you withdraw from the study before finishing it, you will be paid on a pro-rated scale for the parts of it you completed.

Contact Information
If you have any questions regarding this study, please contact the investigator Eric Leach, (614) 556-5766 or el887611@ohio.edu, the co-investigator Dr. Brian Clark, 740-593-2354 or clarkb2@ohio.edu, or the advisor Dr. David Russ, (740) 566-0022 or russd@ohio.edu. Additionally, Dr. Law can be reached on his cell
phone at 270-748-4772 to discuss health and medical issues. For urgent health and medical questions you are advised to call 911 and seek medical care immediately.

If you have any questions regarding your rights as a research participant, please contact Dr. Chris Hayhow, Director of Research Compliance, Ohio University, (740)593-0664 or hayhow@ohio.edu.

By signing below, you are agreeing that:

• you have read this consent form (or it has been read to you) and have been given the opportunity to ask questions and have them answered;
• you have been informed of potential risks and they have been explained to your satisfaction;
• you understand Ohio University has no funds set aside for any injuries you might receive as a result of participating in this study;
• you are 18 years of age or older;
• your participation in this research is completely voluntary;
• you may leave the study at any time; if you decide to stop participating in the study, there will be no penalty to you and you will not lose any benefits to which you are otherwise entitled.
Ohio University would like to contact you in the future about other research studies or opportunities. Please check the appropriate box below and initial:

___ I agree to be contacted for other research studies and opportunities

___ I do NOT agree to be contacted for other research studies and opportunities

Signature__________________________________________

Printed
Name:__________________________________________

Date________________

Administrator of
Consent:________________________________________

Date:________________

[8/31/15] Version Date: