Testosterone and Long Pulse Width Stimulation for Denervated Muscles after Spinal Cord Injury

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1. Background and rationale

Spinal cord injury (SCI) is a devastating medical condition that increases one's risk for type II diabetes mellitus (DM), dyslipidemia and cardiovascular disease.[1-5] The prevalence of individuals with SCI has been estimated to be 250,000-400,000 with a 14% growth since 1988.[6,7] The Department of Veteran Affairs cares for more than 46,000 Veterans with SCI-related disability. Due to advances in healthcare, individuals with SCI are now expected to have similar lifespans to able-bodied controls. The national aggregate direct costs of SCI in the United States have increased with a concomitant decline in mortality over the first year after SCI.[8] Medical care costs per case may exceed 1 million dollars for acute stabilization and rehabilitation, with annual charges thereafter ranging from $41,000 to 182,000.[6-8] The estimated lifetime costs can exceed 12 million dollars.[8] Secondary health-related consequences challenge the productivity, quality of life and well-being of those with SCI, but may be reversible with appropriate **exercise or pharmaceutical interventions** that minimize SCI-related secondary disorders and favorably influence healthcare costs after SCI.

The first few months post-injury, there is a rapid onset of skeletal muscle atrophy[9-15], and decrease in fat free mass (FFM).[9,16,17] This is accompanied by an increase in fat mass (FM)[9-13], waist circumference[13] and visceral adiposity (VAT). [15,16] The extensive muscle atrophy has been attributed to a number of factors including reduction in physical activity, unloading, disuse and reduction in anabolic hormone secretion as well as an increase release of pro-inflammatory cytokines. Cellular changes within skeletal muscle include decreased protein synthesis and increased protein degradation.[9,16-18] Individuals with SCI suffer dramatic muscle atrophy that begins within a few weeks of injury and it continues at least until the end of the first year.[16-18] Skeletal muscle cross-sectional area (CSA) could be as low as 50% compared to healthy able-bodied (AB) controls.[16] Muscle atrophy is accompanied by extensive adipose tissue infiltration including intramuscular fat (IMF).[17] IMF has been determined to account for a 70% reduction in glucose tolerance in individuals with complete SCI.[20] Ectopic adipose tissue has been demonstrated to secrete large amounts of proinflammatory cytokines, including interleukin-6 (IL-6) and tumor-necrosis factor-α (TNF-α). This stimulates hepatic production of C-reactive protein (CRP) which is predictive of vascular inflammation.[21-23]

Denervation atrophy after SCI. Excellent reviews highlight in detail the deleterious changes in skeletal muscle following lower motor neuron (LMN) denervation.[24,25] LMN denervation results in Wallerian degeneration, degeneration of neuromuscular junctions, loss of voluntary muscle contraction and dramatic changes in skeletal muscle fibers and quality.[25,26] This loss impairs the electro-chemical signals between peripheral nerves and skeletal muscles through the neurotransmitter synapses. It also diminishes the trophic support of the peripheral nerves to the muscle. This impacts muscle morphology and results in denervation atrophy. [24-26] Denervation stops muscle electrical and contractile activity and leads to a decrease in the rate of muscle protein synthesis and an increase in the rate of protein degradation.[24] Denervation atrophy is accompanied by a significant disorganization of the internal ultrastructure of fibers, and loss of striations and contractile materials.[24-26] This is also accompanied by dramatic changes in the vascular bed, infiltration of fatty materials and accumulation of interstitial connective tissue.[24-26] The changes may be further exacerbated by associated reductions in the anabolic hormones, testosterone (T), and growth hormone (GH) and the GH secondary messenger insulin like growth factor-1 (IGF-1).[18, 27-29] Growth hormone release is blunted and chronically depressed in SCI, as evidenced by reduced levels of IGF-1[29], a convenient indicator of chronic GH secretion.[18, 27] Reduction in IGF-1 has been associated with skeletal muscle atrophy and increase in the FM in rats and humans.[28,29] A fundamental difference between upper motor neuron and LMN SCI is spasticity, which has been shown to preserve muscle size up to 22% in

persons with higher compared to lower level of injury (T10 and below).[18, 31] Moreover, spastic muscle is associated with 44% greater release of IGF-1 compared to less spastic muscle.[18]

Effects of Muscle Atrophy on Metabolic Activity. The loss of metabolically active muscle mass results in reduction of the resting energy expenditure (EE) which accounts for ~84% of total daily energy expenditure. [32, 33] Reduced muscle mass and resting energy expenditure (1634 ± 290 vs. 1735 ± 295 kcal/d) was observed in SCI vs. monozygotic AB twins.[33] Another study found an association between whole body insulin-mediated glucose uptake and skeletal muscle mass in tetraplegics, suggesting loss of muscle mass as the primary reason for insulin insensitivity.[34] The extensive loss of muscle mass may explain why up to one half of persons with chronic SCI have been shown to develop glucose intolerance and insulin resistance [35,36], with up to 50% and 80% of individuals with paraplegia and tetraplegia, respectively, having type II DM.[36] Additionally, there is a decrease in high-density lipoprotein cholesterol (HDL-C) that correlated with an increase in serum triglycerides (TG).[36] Nash et al. reported that 76% of individuals with paraplegia had HDL-C less than 40 mg/dl and 34% met the Adult Treatment Panel III-defined cut-point for metabolic syndrome.[37] Another study reported that 55% of individuals with SCI were at risk of developing metabolic syndrome.[38]

Effects of Muscle Atrophy on Mitochondrial Health. Mitochondria are the main site of oxygen consumption and energy production of all tissues including skeletal muscle. Mitochondrial size and activity are impaired in metabolic disorders such as obesity, type II DM, metabolic syndrome and cardiovascular disease.[39] Individuals with SCI occupy the lowest end of the physical activity spectrum and a VO_2 peak as low as 0.9 l/min. This lower VO_2 peak was attributed to smaller muscle fibers, transformation to type II fibers, and/or a decrease in aerobic-oxidative enzyme activity.[40] Reduction in VO_2 peak is due to reduction in the size, number or the activity of mitochondria in chronic SCI.[41] Mitochondrial dysfunction leads to decreased fatty acid oxidation and may contribute to insulin resistance. This is associated with reduction in several of the key enzymes including cytochrome c oxidase, succinate dehydrogenase (SDH), pyruvate dehydrogenase and carnitine palmitoyltransferase I.[42] Mitochondrial biogenesis is driven in part through peroxisome proliferator-activated receptor (PPAR) coactivator 1 alpha (PGC-1α).[43, 44] This master regulator of mitochondrial biogenesis, and its downstream targets were decreased following nerve denervation in rodents. PGC-1α expression is known to influence fiber type phenotype and enhances the shift from fast to slow myosin heavy chain. Its expression is increased with increasing ATP cellular demands during exercise and GLUT-4 expression resulting in insulin sensitizing effects.[54] PGC-1α is decreased in obese, diabetic individuals and following denervation in animal models. Denervation results in reduction in the size and the number of mitochondria as well as decreased electron transport chain (ETC) activity.[45, 46] Martin et al. reported a 48-67% lower SDH activity per unit fiber volume, complex II of the ETC, in tibialis anterior muscle fibers of patients 2-11 years after injury compared with AB controls.[61]

Conclusion. Preserving skeletal muscle integrity is vital for several activities of cellular and whole body metabolism.[48-50] Skeletal muscles serve as a large paracrine gland that controls the interplay between the musculoskeletal system and other physiological systems.[48-50] Recent studies documented that skeletal muscle hypertrophy releases important myokines that may regulate atrophic pathways, bone and endocrine glands.[49,50] Thus, *it is crucial to maintain skeletal muscle vitality following LMN denervation* to maintain the integrity of other physiological systems.

Surface Neuromuscular Electrical Stimulation (NMES) after SCI. Recent guidelines recommend a minimum of twice weekly exercise for persons with SCI in order to ensure adherence and compliance.[51] Previous studies have used surface neuromuscular electrical

stimulation (NMES) in individuals with chronic SCI to evoke exercise-induced resistance training (RT) using ankle weights.[52-55] Eight weeks of twice weekly NMES-RT restored knee extensor muscle size to 75% of size at six weeks post-SCI.[52] Twelve weeks of training increased skeletal muscle hypertrophy by more than 40% and improved glucose tolerance many years after injury.[53] Ryan et al. noted an improvement in mitochondrial capacity by 25% following 16 weeks twice weekly NMES-RT.[54] NMES-RT may increase mitochondrial capacity to utilize fat as a source of energy during exercise and further improve insulin sensitivity.[55] Twelve weeks of twice weekly NMES-RT can elicit ~ 35% increase in skeletal muscle size, decreased IMF and VAT, increased insulin sensitivity and increased IGF-1 by 25%.[55] The mechanisms responsible for protein turnover and its impact on muscle hypertrophy following resistance exercise training in persons with SCI are unknown. An acute bout of NMES-RT has been shown to increase protein levels of phosphorylated- and total AMP-activated protein kinase (AMPK)-α and protein kinase B (Akt). This suggests that paralyzed muscle remains mechanically sensitive to triggers of cellular pathways that can increase protein synthesis via activation of IGF/AKT/mechanistic mammalian target of rapamycin complex (mTORC1)[56-58]; implying that muscle plasticity is still intact after years of SCI (see preliminary data).

Singe-muscle stimulation has an impact on systemic physiological markers. Mahoney et al. previously noted that 12 weeks of NMES-RT resulted in enhanced glucose disposal. Another study noted that following 12 weeks of NMES-RT of the knee extensor muscle group, there was improvement in glucose area under the curve, serum insulin concentration and triglyceride profile in persons with chronic SCI. The same study noted a 35% increase in plasma IGF-1 that was negatively correlated to the decrease in VAT. Our preliminary data supported the notion that single muscle stimulation for 16 weeks in conjunction with TRT resulted in significant increase in BMR (see preliminary data).

It is worth noting that training with surface NMES may not be possible for a large segment of the SCI population because of peripheral denervation. The increase in the muscle depth because of atrophy, increase of subcutaneous fat thickness and excessive infiltration of IMF may diminish the spread of the current density to activate the target muscles.[24] Standard surface NMES (pulse width of 150-600 μs) fails to stimulate denervated muscles because of an increase in the minimum time required, chronaxie, as demonstrated on the strength-duration curve.[24] This requires higher current for the direct depolarization of the muscle fibers. The limited pulse width is inversely associated with an increase in the magnitude of the current required to stimulate the muscle. Most commercially available stimulators have amplitudes that do not exceed 200 mA because of an increased risk of skin irritation and burns, especially in individuals with SCI. However, this low amplitude of the current is unlikely to cause muscle stimulation following denervation in persons with SCI. Despite the aforementioned benefits of NMES applications, 25% of the SCI population cannot benefit from the standard NMES because of LMN denervation.[24-25]

Fig 1

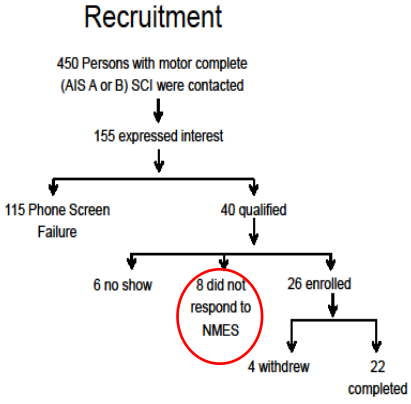


Figure1. A schematic diagram of the process of recruitment, notice 8 persons were disqualified because of no response to NMES.

The PI has just completed a clinical trial (NCT01652040) entitled “Resistance Training and Testosterone after SCI”. During the course of the trial, the study team enrolled 40 individuals with chronic (>1 year) motor complete SCI. **Eight participants (20%) failed to respond to biphasic surface NMES** and were excluded because of LMN denervation of the stimulated knee extensors (**Figure 1**). MRI revealed that the muscle fibers become significantly smaller and were replaced by fat as well as connective tissue

Rationale for using long pulse width stimulation (LPWS) following denervation in persons with SCI. The European project, Research and Innovation Staff Exchange (RISE), has introduced long pulse width (LPW) NMES to restore muscle size following denervation in people with SCI.[24-25] The effects of home based functional electrical stimulation (FES), introducing a LPW (120-150 ms) at an intensity of 250 mA for 5 days/week has been studied for two years in 25 SCI persons with complete LMN denervation.[59-62] The trial showed an increase (24%) in knee extensor cross-sectional area (CSA) following the first year and an additional 7% in the second year, respectively, with no changes in the hamstring muscles.[59] In previous trials, the length of the training was extended for 2 years on a daily basis without incorporating additional benefits to the trained muscles.[59] The technology of utilizing long pulse width stimulation (LPWS) has not been tested or approved for use in Veterans with chronic denervation after SCI. **Successful completion of this study will lead to a new rehabilitation intervention to restore muscle size after denervation in persons with SCI.** As the amplitude or pulse width increase, the nerve fibers nearest the electrodes are depolarized to activate the target muscle. All available NMES applications are based on direct excitation of neural structures and indirect activation of the target muscle. Due to the denervation, muscular contractions can only be elicited by depolarizing the cellular membrane of each single muscle fiber. Biphasic rectangular impulses with duration between 30 and 150 ms may have to be applied with amplitudes higher than that of standard NMES (450 μs). This imposes direct activation of the myofibers independent of LMN denervation. The LPWS stimulation has the capacity to penetrate deeply and activate muscle fibers.

Rationale for using testosterone replacement therapy (TRT) in persons with SCI. Testosterone (T) is an anabolic and androgenic hormone that is associated with growth. The majority of testosterone production takes place in the Leydig cells of the testes. Approximately 60-70% of testosterone is tightly bound to sex hormone binding globulin (SHBG), 30-40% is bound loosely to albumin, and 0.5-2% is free.[63-67] Sixty percent of men with SCI have low T levels in the first six months after SCI.[63] A previous study demonstrated a 37% lower level of T in men with tetraplegia compared to AB controls.[65] Testosterone signaling through the androgen receptor also results in increased brain-derived neurotrophic factor expression and restored the withdrawn synapses in acute models with peripheral denervation.[68-69] The use of an androgen receptor antagonist blocks the effects of exercise on the regenerative capacity after LMN, emphasizing the significance of using testosterone replacement therapy (TRT) in promoting axonal growth and restoring muscle size.[68-69] The effect of TRT on cardiovascular risk has been controversial.[70,71] There are data showing that hypogonadism is a risk for cardiovascular disease.[70] Some replacement studies show increased risk, but another study showed decreased mortality in veterans receiving testosterone.[71] One study in older men reported that injectable testosterone may be associated with increased cardiovascular risk but topical testosterone was not.[72] Based on the available scientific evidence, we felt that application of transdermal patches is the more feasible and safe approach for persons with SCI.

Rationale for using TRT following denervation. TRT is an FDA-approved therapy used to treat hypogonadism and often results in significant improvement of muscle strength and fat-free mass in hypogonadal men. In rats with complete SCI, TRT has been shown to attenuate muscle atrophy and the decline in oxidative and glycolytic enzymatic activities.[66] Transdermal TRT of 5-10 mg/day has been shown to increase lean mass and basal metabolic rate (BMR) in hypogonadal men with SCI.[67] In an acute denervation model, TRT acts on the nervous system using two different mechanisms, genomic and non-genomic. The genomic mechanism involves binding of the hormone to its nuclear receptor. The non-genomic mechanism is fast-driven and involves interaction of the hormone with membrane or neurotransmitter receptors. This leads to

changes in cells of the nervous system and effects on neurotrophic factors and regeneration. However, applications of TRT in chronic models of denervation have not been established. The role of TRT on muscle size independent of the changes in the peripheral nervous system has not been investigated. The anabolic steroid nandrolone reduced denervation atrophy after 56 days of sciatic nerve transection.[73] Furthermore, TRT was shown to increase muscle size, strength and increase total body potassium.[73] TRT increases skeletal muscle mass by inducing hypertrophy of both types I and II muscle fibers in men through its actions on androgen receptors. TRT promotes differentiation of a pluripotent mesenchymal cell line into myogenic lineage and inhibits differentiation into an adipogenic lineage. These effects were mediated through an androgen pathway. Androgen receptors are expressed in mesenchymal precursor cells within skeletal muscle (satellite cells). After 20 weeks of adding 100 nm of TRT to cultures of satellite cells and myocytes, the numbers of myonuclei per fiber increased from 3.2% to 4.2% and the percentage of myonuclei that tested positive for androgen receptors increased from 51% to 78%. Furthermore, the number of satellite cells as a percentage of myonuclei increased from 3.3% to 5.6% and percentage of satellite cells that were androgen positive increased from 89% to 96%.[74]

Innovation and rationale for combining both TRT and LPWS following denervation. We hypothesize that 1 year of electrically evoked TRT+LPWS will result in a significant muscle hypertrophy of 25% or more. This will be associated with a significant increase in leg lean mass (>10%) and concomitant improvement in overall metabolic profile by 20%-30%. The rationale is that elevating levels of T and free T to the physiological level will optimize the outcomes on muscle mass and will render the LPWS protocol more effective. Previous studies suggest that LPWS may require up two years of 5 days per week to restore muscle size in SCI persons with LMN denervation. Clinically, this is not feasible considering the barriers related to dressing, bowel and bladder movements, transportation, and loading and unloading of wheelchairs. These barriers would lead to decreased adherence to the protocol. Our preliminary data showed 95% compliance when an NMES protocol is administered twice weekly for 16 weeks. We are hypothesizing that the addition of TRT to LPWS may facilitate the increase in leg lean mass and allow optimization of the stimulation protocol in 1 year compared to 2 years. Therefore, this approach of combining both physical and pharmacological interventions may allow twice weekly of LPWS to be highly effective in restoring muscle size. **Administering TRT is necessary to maximize the outcomes of LPWS and provide balancing effects against several of the secondary health related complications after SCI.**

2. Significance of this research to the VA population.

Currently, 46,000 individuals are listed in the national VAMC spinal cord dysfunction registry, and more than 50% of them have insulin resistance resulting in glucose intolerance or type II DM, dyslipidemia and cardiovascular disease. The Department of Veteran Affairs has made research designed to limit secondary complications of people with disabilities its primary goal. The health consequences of losing more than 25% of muscle mass has considerable impact after SCI. Furthermore, **20-25% of those with SCI may not benefit from standard rehabilitation techniques necessary to restore muscle size because of LMN denervation.** Denervated muscles are 6 times smaller than innervated muscles **(Figure 2)**. This special population already has significant functional deficits including reduced mobility, myocardial atrophy, restrictive lung disease, and bowel and bladder dysfunction. Failure to restore muscle size may lead to other serious secondary health complications including obesity, type II DM and cardiovascular diseases. This may limit benefits from advances in therapeutic interventions such as stem cell and gene therapy or exoskeleton training. Therefore, a complementary approach of LPWS and TRT may work as an effective rehabilitation strategy to counterbalance the deleterious effects of denervation on muscle size among Veterans and civilians with SCI.

3. Preliminary Studies

a. Skeletal Muscle CSA in two persons with motor complete SCI

	Age (yrs)	*Weight (kg)*	*Height (m)*	*TSI (yrs)*	*Level of Injury*	*AIS classification*
Innervated	*45*	*87*	*1.88*	*26*	*C6*	*A*
Partially denervated	*47*	*67*	*1.78*	*25*	*T11*	*B*

Even though these individuals were matched based on age and time since injury (TSI), the two primary factors that likely to impact changes in lean mass and muscle size, note the dramatic muscle atrophy in the right thigh following SCI in a person with partial denervation (**Figure 2**). Contractile tissue is completely gone and muscle fibers were replaced with infiltrated IMF and connective tissues.

Fig 2

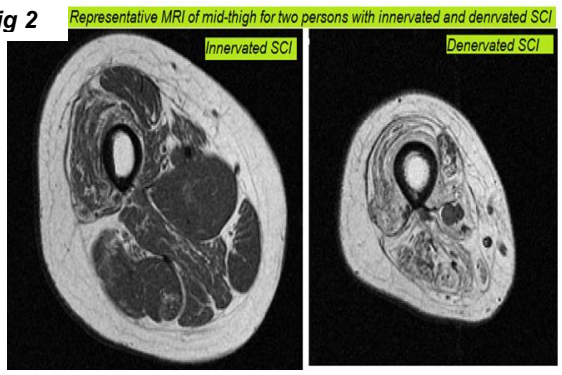


Figure 2. Representative MRI of mid-thigh for two persons with innervated and denervated muscles after SCI.

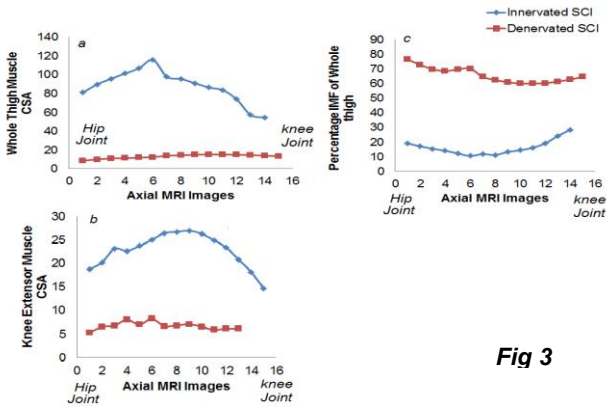


Fig 3

Figure 3. Skeletal muscle CSA of a) whole thigh, b) knee extensor and c) percentage intramuscular fat (IMF) between two persons with innervated and denervated SCI.

Figure 3 shows that whole thigh muscle CSA and knee extensor CSA were 6 times smaller, respectively in individuals with denervation (**Figure 3A and B**). Percentage IMF was 50% greater in the denervated thigh compared to the innervated one (**Figure 3C**). These results highlight the need to establish an effective rehabilitation protocol that can restore muscle size and prevent excessive accumulation of IMF in persons with SCI who suffer from LMN denervation.

b. Electrically Evoked Resistance Training.[55] Nine individuals with motor complete SCI (C5-T11) were randomly assigned into one of two groups; RT + diet (n= 5) or diet only (n=4). Weekly feedback was provided to maintain a standard diet at 45% carbohydrate, 30% fat and 25% protein. **Figure 4** shows increased knee extensor muscle size in the RT+diet group. This hypertrophy was associated with a 25% increase in plasma IGF-1. This increase in IGF-1 following training was negatively associated with ectopic adipose tissue accumulation.

Fig 4

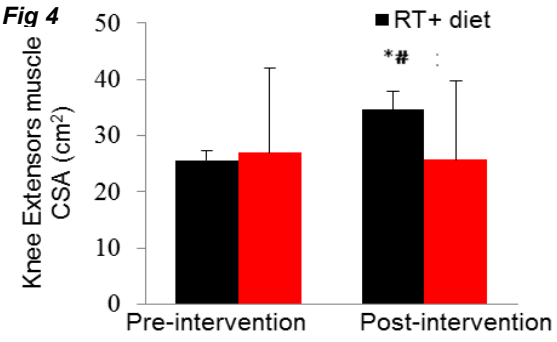


Figure 4. Skeletal muscle hypertrophy as measured by magnetic resonance imaging (MRI) of the knee extensors before and after 16 weeks of NMES-RT

c. TRT & NMES-RT. The PI's CDA2 career award findings support the view that twice weekly NMES-RT and daily TRT had adherence rates above 95% in persons with SCI. In this project 22 participants were enrolled in NMES+TRT (n=11) or TRT (n=11) groups for 16 weeks. We have safely administered 2-6 mg of T daily for 16 weeks using patches for persons with SCI to improve body composition. Blood work was taken every 4 weeks and patch compliance was monitored every month (95-100% adherence). Our data showed that 18% and 55% of subjects had baseline serum T-level below 300 and 400 ng/dl, respectively. Transdermal patches were alternated daily between the right and left shoulders. Based on the T baseline level, participants were assigned the following dose (**Table 2**). Serum T level increased from 413±147 to 525±223 ng/dl following 16 weeks of transdermal applications in NMES+TRT group.

Table 2. *TRT dose based on the serum T-l*

T- level (ng/dl)	less than 300 ng/dl	300-600 ng/dl	greater than 600 ng/dl
Patches	*6 mg/day*	*4 mg/day*	*2 mg/day*

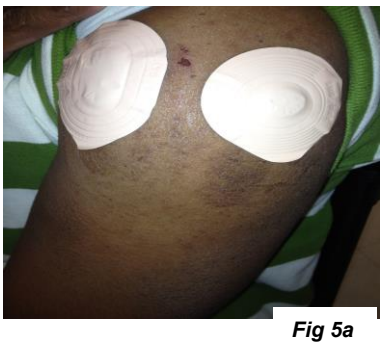


Fig 5a

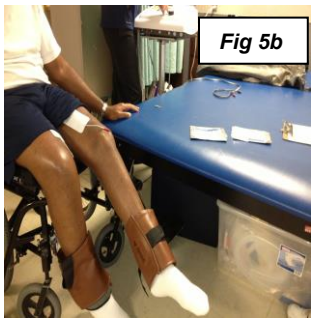


Figure 5. A) Transdermal patches applied daily and **B)** twice weekly of electrically evoked RT using ankle weights in persons with SCI.

Measurement of the T level was repeated every 4 weeks over the course of 16 weeks and the dose was adjusted accordingly. In the event of skin irritation or itching, subjects were prescribed topical hydrocortisone cream and the site of the patches was moved up or down on the shoulder away from the irritation point. TRT was prescribed for 7 days/week for 16 weeks. New patches were placed at night before going to bed. Each participant was asked to turn in a monthly log-in sheet to ensure adherence to the protocol and refill occurred every 30 days.

Progression of electrically evoked RT

RT constitutes four sets of 10 repetitions that was performed twice weekly for 16 weeks. The first week of RT was conducted with no ankle weights to ensure that the knee extensor muscles could extend the weight of the lower leg against gravity. Once full knee extension was achieved in a sitting position, two pounds were added on a weekly basis with the criteria that full knee extension was achieved before more weights were added. Surface NMES was applied to the knee extensor muscles via surface electrodes. Current from the stimulator (30 Hz, 450 µs pulses) was manually increased in 5-second intervals to evoke full knee extension with a 3-minute rest between sets. Lifted weight significantly (P< 0.0001) progressed over training period for the right (19.6±6.5 lbs.) and the left (20.0±6.1 lbs.) legs (**Figure 6**).

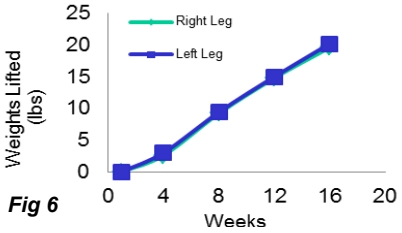
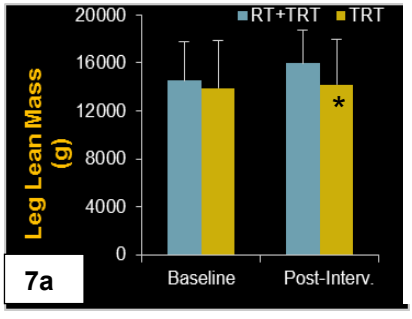


Fig 6

Figure 6. Progression of ankle weights over 16 weeks of training in the NMES-RT+TRT group (n=11).

d. Changes in body composition as measured by dual energy x-ray absorptiometry (DXA)

Sixteen weeks of NMES-RT+TRT increased leg lean mass by 1.5 kg (P=0.003) and decreased leg %fat mass (FM) by 2% (P=0.037) (**Figure 7a**).



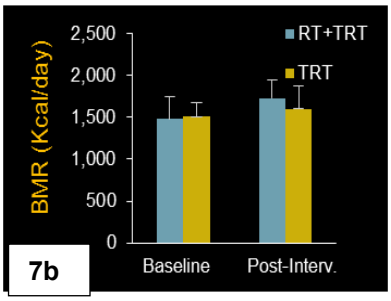


Figure 7. Changes in **a)** leg lean mass and **b)** BMR in the NMES-RT+TRT (n=11) and TRT (n=11) in persons with motor complete SCI.

Association between the changes in lean mass and BMR

The increase in whole body and leg lean mass drives the increase in BMR noted in **Figure 7b**. The increase in lean mass following NMES-RT explained 51% of the variance in BMR (n=22)
The increase in BMR of 218 kcal/day may equate to FM loss of 0.93 lb. per month or 11 lbs. per year. This highlights the significance of restoring lean mass on combating the increase in body FM, obesity and cardiovascular comorbidities after SCI. Preliminary MRI data showed 13% increase in whole muscle CSA after TRT.

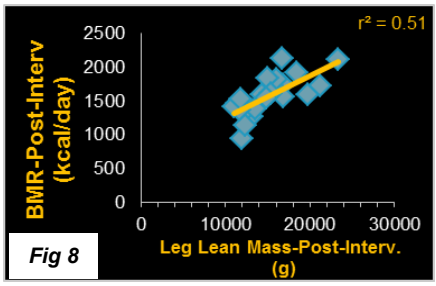


Figure 8. Relationship between leg lean mass and BMR post-intervention in 22 persons with SCI

e. Protein expression following electrical stimulation training[75]

In our previously published training studies, we have experienced wide variance in the primary outcome variables in response to electrical stimulation training. Studying molecular signaling pathways may provide insights on why skeletal muscle responds differently to FES protocols in persons with SCI. It is unknown why some individuals may respond well and experience large increases in muscle size while others do not. Understanding the variability in signaling pathways may help in the design of future clinical trials. To investigate the cellular changes, expression of three skeletal muscle proteins involved in glucose disposal, energy regulation and mitochondrial biogenesis (GLUT-4, AMPK, and PGC-1α, respectively) were measured. Participants underwent 16 weeks of 5 days/week of FES cycling. Our results show that GLUT-4, PGC-1α and AMPK increased by 3.8, 2.3 and 3.4 fold, respectively, in the vastus lateralis (VL) muscle. There was no change in protein expression of untrained muscles (Triceps m.). The study demonstrates our successful collaboration with the James J. Peters VA Hospital (Dr. Cardozo).

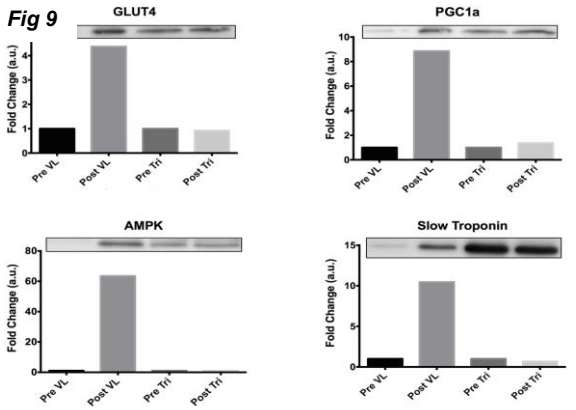


Figure 9. Immunoblots for protein levels of GLUT-4, AMPK, and PGC-1α in VL muscle following 16 weeks of FES training in persons with motor complete SCI.

f. Mitochondrial Health after SCI[76]

We also investigated the body composition factors that may influence mitochondrial density (citrate synthase) and activity (complex III) in the VL as measured by enzymatic assays. The relationship between CS or CIII activity and thigh skeletal muscle CSA is shown in **Figure 10** (n=16 for CS and n=14 for CIII). There was a positive correlation between CS activity and thigh muscle CSA (r=0.82, p=0.0001) and knee extensor (KE) CSA (r=0.74, p=0.001). Similarly, there was a positive relationship between CIII activity and thigh muscle CSA (r=0.84, p=0.0001) and knee extensor CSA (r=0.84, p=0.0002).

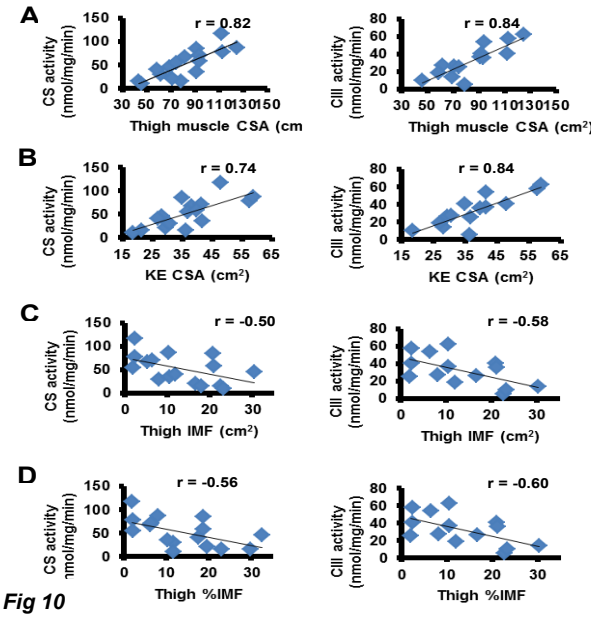


Figure 10. Citrate synthase (CS; n=16) and antimycin A-sensitive complex III (CIII; n=14) activity related to thigh muscle cross sectional area (CSA; A), knee extensor (KE) CSA (B), thigh intramuscular fat (IMF; C), and thigh %IMF (D).

4. Research Design and Methods

The goal of this randomized prospective controlled study is to investigate the effects of one year of TRT+LPWS versus TRT+standard NMES on muscle size (primary outcome variable), leg lean mass and percentage IMF, metabolic profile (BMR, carbohydrate and lipid profile) and protein synthesis and degradation pathways as well as mitochondrial health. The long-term goal is to develop a rehabilitation strategy to mitigate the deleterious changes in muscle size and lower leg lean mass in persons with denervation following SCI.

Subjects. 24 chronic (1 year or more post-injury) individuals with motor complete SCI will be recruited from the Hunter Holmes McGuire VA Spinal Cord Dysfunction registry (n=~1,717-1800) and Virginia Commonwealth University (n=750-1000) over 4 years. The Spinal Cord Injury and Disorders Outcomes (SCIDO) for FY2105 suggests that there are ~ 20% of our outpatient evaluation with LMN denervation as result of SCI. Approved flyers and advertisements will also be posted at the VAMC, VCU Hospitals and clinic sites. Training will be conducted twice weekly using a progressive RT model described in preliminary studies and previously by our group.[55].

Inclusion. All participants will be between18-70 years old, male, and greater than one-year post SCI. The age upper limit was set at 70 years to reduce the likelihood of developing any cardiovascular side effects from using TRT. Participants must have traumatic complete or incomplete T10 SCI or below (AIS A or B; i.e. motor deficit below the level of injury) with denervation of both knee extensor muscles and tolerance to the LPWS paradigm.

Exclusion. Participants with the following pre-existing medical conditions will be excluded: cardiovascular disease, uncontrolled type II DM, (HbA1c >7.5), uncontrolled hypertension (resting blood pressure >140 /90 mmHg), and those on insulin, or who have pressure sores greater than stage 3, hematocrit above 50% or severe urinary tract infection. Those with a hyper-physiological testosterone level (above 800 ng/dl) will and those who fail to tolerate the LPWS paradigm will also be excluded. Lower extremity fracture around the knee joint within the last 2 years will be excluded. After signing a consent form, all participants will undergo knee DXA scans and knee bone mineral density (BMD) less 0.6 gm/cm^2 or hip BMD T-score below -3.5 will result in exclusion from the study to prevent fracture at the distal femur or proximal tibia during training.

After informed consent, each subject will undergo a complete physical examination by a physiatrist board certified in SCI medicine (Dr. Goetz or Dr. Lavis or Dr. Castillo), including neurologic assessment and American Spinal Injury Impairment Scale Classification (AIS) examination. Participants will be evaluated every 3 months after treatment initiation and then annually to assess any adverse effects and to check compliance. Testosterone measurements will be acquired every 4 weeks during the intervention to determine the serum level and the dose will be adjusted to allow ~30% increase from baseline (Dr. Adler). A prostatic abnormality on digital rectal examination will also lead to exclusion. An increase in serum or plasma prostate specific antigen (PSA) of 1.4 ng/ml above baseline will result in immediate cessation of TRT.

Measurements. All participants will undergo body composition assessments (**specific aim 1**), metabolic studies (**specific aim 2**) and muscle biopsies (**specific aim 3**). All testing procedures will be conducted one week prior to training (**baseline**), 6 months following training (**mid-intervention**) and one week after the end of 1 year of training (**post-intervention**). At each time point, measurements will include estimation of body composition, anthropometry, and dual x-ray absorptiometry (DXA). MRI scans will be obtained for thigh skeletal muscles to determine IMF and CSA.[14-18] Participants will remain at the VA Medical Center for dinner, and overnight. After an overnight fast the subject will be gently awakened at 6 AM to measure BMR. At 6.30 AM an IV line will be placed and blood will be drawn for serum total T and IGF-1 concentrations at 6.30, 7.00 and 7.30 AM. Resting blood pressure and fasting metabolic markers will be obtained

including HbA1c, as well as lipid panels, CRP, IL-6, TNF-alpha, and free fatty acids (FFA). This will be followed by a 3-hour intravenous glucose tolerance test (IVGTT) which will begin at 8 AM and terminate at 11 AM. During the IVGTT, a dietitian will meet with each participant individually to ensure that they will follow a standard diet pattern during the 1 year-intervention (45% carbohydrate, 35% fat and 25% protein) to avoid any confounding effects on our measurements. All participants will be asked to maintain a **3 day food record** monitoring their energy intake during the course of the study. The diaries will be evaluated weekly by the dietitian to provide monthly feedback. All participants will meet with the dietitian three times during the study to ensure appropriate adherence to the diet pattern throughout the study. The vastus lateralis muscle will be biopsied to measure protein expression and to determine mitochondrial enzymatic activities.[75, 76]

Twenty-four participants will be randomly assigned to either one year of TRT+LPWS (n = 12) or a TRT+ standard NMES (n = 12). Testosterone patches (Tp; 4
-8 mg/d) will be re-placed daily on alternating skin sites at bedtime for 1 year. Randomization will be done at the end of the two-day assessment period using a random number generator computer program **(baseline).** Participants will be block randomized based on the degree of denervation (lower or higher than 50% of compound muscle action potential (CMAP) of the standard normal femoral nerve values). Both groups (TRT+LPWS & TRT+ standard NMES) will undergo 1 year of supervised unilateral progressive RT, twice weekly, using ankle weights. The two-day assessment period will be repeated using the same sequence at 6 months and after 1 year. Prior to training, we will perform multiple neurophysiological tests to confirm LMN denervation of the knee extensor muscle group. The first technique is to place the surface NMES electrodes on knee extensor muscle group and increase the current (30 Hz, 450 μs) gradually up to 200 mA. If the knee extensors show visible elicited contraction, then the participant will be disqualified from the study. If there is no response, participant will then be escorted to the EMG lab and further testing for evidence documenting muscle denervation will be performed by a trained electromyographer. Briefly, participants will undergo a femoral nerve motor conduction study with recording of the CMAP from the vastus medialis (VM) with supramaximal femoral nerve stimulation just below the inguinal ligament. The participant will undergo needle EMG of the vastus medialis to determine the presence and intensity of spontaneous muscle fiber fibrillation potentials to quantify the level of denervation. A monopolar EMG needle electrode will be inserted into the VM muscle to record spontaneous intramuscular activity in the resting muscle.

LPWS or Standard NMES. Training will be conducted twice weekly using a S88X dual output square pulse Grass Stimulator (for research use only) and the current will be set as shown in Table 3.[22,25] According to the Food and Drug Administration (FDA), all functional non-invasive electrical stimulators are considered a non-significant risk device and do not necessarily need IDE application and only require IRB approval prior to the trial. Long pulse duration (120-150 ms) is used to offset the low resting membrane potential of the denervated muscles.[22-25] When tetanic contraction is achieved, the pulse duration will be shortened gradually by 5-10 ms/ month to reduce the likelihood of skin irritation (**Table 3**). We will explore the best stimulation parameters necessary to evoke twitches/ tetanic contraction of the knee extensor as far as the frequency and amplitude of the current for each participant. Initially, we will set the pulse duration at 150 ms, frequency at 2 Hz and the amplitude of the current will be gradually increased from 0 mA by 10 mA intervals up to 200 mA. The current (mA) and the pulse duration (ms) that causes full knee extension will be recorded for each session. An example progression of the stimulation parameters is listed in table 3. Based on previous work, some participants may take up to 6 months to lift their legs against gravity.[24, 62] Therefore, a one year intervention may be a reasonable duration to load the muscle and evoke hypertrophy especially after years

of denervation. For the TRT+ standard NMES (control group) , a Theratouch 4.7 stimulator (0-200 mA) or battery operated stimulator (0-100 mA) that elicits direct current, a 5 second/5 second work/rest ratio will be used with a 3-minute rest between sets, 30 Hz, 450μs pulses.[52-55] For the control group over a 1-year period, participants will have the option to perform a home based training using our established videoconference telehealth system to monitor their training using a hand-held battery operated stimulator (see supplement). Study visits will be limited to once a month throughout the 1-year period to refill their 30 days stock of TRT patches. Training sessions will consist of 4 sets of 10 repetitions of LPWS NMES-induced knee extensions and will last for 30-40 minutes. All training procedures will be conducted with the participants sitting in their wheelchairs with enough space to clear their foot off the ground.[52-55] Two large 8 X10 cm^2 (Uni-Patch, Wabasha, MI, USA) adhesive carbon electrodes will be placed on the skin over the knee extensor muscle group.[52-55] Once the participant completes 40 repetitions of knee extension, ankle weights will be increased gradually by 2 lbs. per week (not to exceed 30 lbs.). In our prior work the maximum weight was below 30 lbs. because of concerns over the possibility of causing condylar fractures. For the control group (TRT+ standard NMES), the direct current at 450 μs will be turned up to 200 mA for 10 times/set; which is unlikely to cause either twitches or tetanic contraction of the stimulated muscle.

Table 3. Example of progression of the stimulation parameters over 1 year [24]

Months of Training	Pulse Width (ms)	Inter-Pulse interval (ms)	Frequency (Hz)	Amplitude of current (mA)	Weights (lbs.)
1-3	120-150	400	2	up to 200	0
4-6	90-120	400	15-25	up to 200	0
6-9	60-90	100-400	25-30	up to 200	2 lbs./ 40 reps/session
9-12	30-60	10-12	25-30	up to 200	2 lbs./ 40 reps/session

Testosterone replacement Therapy (TRT). Testosterone will be administered via transdermal patches (Androderm Corp., Palo Alto, CA) that will deliver between 4-8 mg/day.[39] The Endocrine Society guidelines recommend one or two patches of 5 mg/day. Because Transdermal Androderm patches are either 2 or 4 mg we will proceed with 4-8 mg/day. Two patches that deliver 4-8 mg/day initially will be worn at all times except when bathing. Participants will be instructed to change patches once a day before bedtime. Patches will be worn daily on a dry skin over the right or the left shoulder, abdomen or thighs and the application sites will be rotated to avoid skin irritation for 1 year.[67] TRT will be administered for 1 year because it has the maximum effects on lean mass after SCI. The dose will be decreased to 2 mg/day if the serum T concentration is more than 1000 ng/dL (34.7 nmol/L) and the participant will be reeducated in the patch technique if the concentration is less than 250 ng/dL (8.7nmol/L) above the pretreatment concentration. Serum prostate-specific antigen (PSA) level will be measured to ensure normal concentration according the guidelines provided by the Endocrine Society. Patch compliance will be monitored by inventory of the number of returned patches each month.[78] Free T will be calculated using total T, sex hormone binding globulin (SHBG) and albumin equation.[79]

Specific aim 1. We will compare the effects of TRT+LPWS compared to TRT+ standard NMES (control group) on the size of thigh skeletal muscle, intramuscular fat (IMF) and leg lean mass. Both groups will receive TRT for 1 year. Anthropometrics, DXA and MRI will be performed at baseline, 6 and 12 months post-interventions to measure thigh skeletal muscle size, IMF and leg lean mass.

Body mass index (BMI): Each participant will be asked to void his bladder and then will propel onto a wheelchair weighing scale. After weighing the participant and his wheelchair (1), he will be helped to transfer to an adjustable mat and his/her wheelchair will be weighted empty (2). The weight of each participant will be determined by subtracting (**2**) from **(1)** (kg). The height will be determined in the supine position. Two smooth wooden boards will be placed at the participant's head and heels and the distance between them will be taken as the height to the nearest cm. Every effort will be taken to maintain the knees in an extended position. BMI (Kg/m^2) will be calculated as weight (Kg) divided by height^2 (m^2).[15]

Dual energy x-ray absorptiometry (iDXA): iDXA will be used to measure body composition including regional and total FM, FFM and bone mineral density (BMD). Total body and regional scans will be performed using an iDXA scanner (Lunar Inc., Madison, WI) bone densitometer to determine regional bone mineral density and T-scores for hips and knees. We will perform testing after lower extremity elevation for at least 20 minutes to minimize fluid shift. All scans will be performed and analyzed by a trained, certified DXA operator. The subject will be assisted to lie on a padded table and both legs will be strapped proximal to the knees and the ankles. The arms and legs will be positioned to ensure proper alignment and the ability to lie still for 10 minutes during the scan. Total and regional (%FM and FFM) will be determined using total and regional DXA software. The coefficient of variability of two repeated scans is less than 3%.[15]

Magnetic resonance imaging (MRI): MRI will be performed using a 1.5 Tesla magnet (GE).[14-18] The skeletal muscle CSAs will be determined at baseline, 6 months (mid-intervention) and 1 year after training (post-intervention). Both lower limbs will be strapped together using a soft Thera-band to avoid any movement inside the magnet. Participants will be instructed to lie still inside the magnet and they will be provided with earplugs to protect their ears against the magnet noise. The duration of the whole scan including the preparation time should not exceed 10 minutes. Images of both thighs will be collected using the following scanning parameters (repetition time, 500; echo time, 14; field of view, 20cm; matrix, 256×256). Transaxial images, 8 mm thick and 4 mm apart, will be taken from the hip joint to the knee joint using a localized coil. Images will be downloaded and analyzed using X-vessel software.[17,19,20, 31,55]

Testosterone and PSA Concentration. The serum testosterone concentration and PSA levels will be measured at the beginning, every 4 weeks and 1 year after intervention. IV line will be placed after overnight fast, serum total T and PSA concentrations will be measured in duplicates.[78] Samples will be sent for analysis using a standard procedure assays.

Specific Aim 2. We will determine the association between the changes in skeletal muscle size, IMF, leg lean mass and the metabolic profile as determined by measuring basal metabolic rate (BMR), lipid panel and carbohydrate profile. Participants will undergo an array of metabolic measurements at baseline, 6 and 12 months post-interventions including BMR, fasting lipid panel and 3 hour IVGTT. Other metabolic and inflammatory biomarkers (plasma IGF-1, IGFBP-3, TNF-alpha, IL-6 and CRP) will also be measured at the 3 time points. We ***hypothesize*** that the increase in lean mass following TRT+ LPWS will increase BMR and improve both carbohydrate and lipid profiles as well as associated with decrease in FFA and inflammatory biomarkers.

BMR and respiratory exchange ratio. After an overnight fast, participants will be kept in a dark room for 20-30 minutes to attain a resting state during which BMR will be measured by using a canopy. The gasses (VCO_2 and VO_2) collected will used to determine the respiratory exchange ratio. This will help to determine the changes in the percentage of substrate utilization (% fat vs. % carbohydrate) after the interventions.[15]

Serum total, free testosterone and IGF-1. The plasma T and IGF-1 will be measured in the morning (2 ml/ sample).[78,79] The analysis of total T will be performed by radioimmunoassay after sample extraction and column chromatography. The interassay coefficient of variation (CV) is 12.5% or less for all quality control samples analyzed. Plasma IGF-I and IGFBP-3, concentrations will be measured by immunoluminometric assay (Quest Diagnostics, Madison,

NJ) and RIA (Diagnostics Systems Laboratories Inc., Webster, TX), respectively. Intra-assay precision of IGF-1 is 4.6% at 50 ng/ml and 3.6% at 168 ng/ml.

Blood lipids. Each subject will have fasting lipid profiles (HDL-C, LDL-C, total cholesterol, and TG) assessed, with total cholesterol: HDL-C ratios utilized as the criterion variable. Concurrent with the IVGTT and following a 12-hour fast, 10 ml of blood will be collected from the indwelling venous catheter and lipids determined by standard analyses procedures.[15]

Inflammatory biomarkers. Before starting the IVGTT and following a 12-hour fast, 10 ml of blood will be collected from the indwelling venous catheter and CRP, IL-6, TNF-α , and FFA will be determined by standard procedures using commercially available assay kits.[21]

Intravenous Glucose Tolerance Test (IVGTT) An IVGTT will be used to determine insulin sensitivity and glucose effectiveness. Each subject will undergo an IVGTT test 3 times. After a 10 to 12-hour fast, an indwelling catheter with an intravenous saline drip (0.9% NaCl) will be placed in an antecubital vein, and another intravenous line will be placed in a contralateral hand vein to facilitate infusion of glucose and blood sampling during the IVGTT. Glucose samples will be taken at –6, -4, -2, 0, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes after the rapid glucose injection (0.3 gm/kg IV over 30 seconds at time zero). Twenty minutes after the glucose injection a bolus of insulin (0.02 U/kg) will be injected to determine insulin sensitivity. Plasma glucose will be measured by the Autoanalyzer glucose oxidase method and plasma insulin concentrations will be determined by commercial radioimmunoassay using single-antibody kits. The S_I (glucose disposal rate per unit of secreted insulin per unit time) and S_G (glucose mediated glucose disposal rate) will be calculated from a least-squares fitting of the temporal pattern of glucose and insulin using the MINMOD program.[80] The coefficient of variation is approximately 15%. KG, a measure of glucose tolerance, is calculated as the least square slope of the natural log of absolute glucose concentration between 5 and 20 minutes after the glucose bolus.[80] The homeostatic model of assessment of insulin resistance (HOMA-IR) will be calculated and insulin sensitivity will be determined using Matsuda and Defronzo formula. [81,82]

Specific Aim 3. We will investigate the cellular mechanisms responsible for evoking skeletal muscle hypertrophy following TRT+LPWS compared to TRT+ standard NMES. Participants will undergo 3 muscle biopsies of vastus lateralis at baseline, 6 and 12 months post-intervention to measure gene and protein expression and perform mitochondrial enzymatic assays. Considering the limited muscle tissue in persons with denervation atrophy, four biopsy samples of the vastus lateralis muscle (total: 25-50 mg wet wt) will be obtained by a 14 gauge Tru-Cut needle using a sterile technique and local anesthesia (2% lidocaine). The biopsy samples will be frozen in liquid nitrogen and stored at -70C until further analysis. We hypothesize that the TRT+LPWS will upregulate protein synthetic and downregulate protein degradation pathways, gene expression and mitochondrial enzymatic ETC activities compared to the TRT+ standard NMES group.

Protein content. Muscle biopsy samples will be homogenized on ice using the appropriate buffers. Equal amounts of protein will be resolved by SDS-PAGE after which proteins will be electrophoretically transferred to a PVDF membrane. Western blot analysis will then be performed to determine the protein concentrations as described in preliminary data and previously by our lab.[75] After blocking, membranes will be probed with primary antibodies for activated pathways including AMPK, p-AMPK PGC-1α, IGF-1, Akt, p-Akt, mTOR and protein degradation pathways (FOXO1/3, atrogin-1, MURF). followed by incubation with the appropriate secondary antibody. Western blots will be quantified by scanning with A GS800 densitometer. Optical densities of the Western blots will be measured using image-analysis software (Molecular Analyst; Bio-Rad).

Real-time quantitative PCR (qPCR). Quantification of mRNA levels by RT qPCR (real-time PCR) in muscle biopsy samples will follow well-established procedures in our lab. Briefly, frozen muscle biopsy samples will be added to Trizol reagent and immediately homogenized then

centrifuged to separate the chloroform and aqueous phases. mRNA will be extracted using commercially available kits. Residual genomic DNA will be removed by on the column DNAse I digestion. cDNA libraries will be synthesized by reverse transcription of total RNA using commercially available kits. Expression of individual target genes (IGF1, PGC1alpha, AMPK, Akt, mTOR) will be evaluated by qPCR using 18S RNA or housekeeping genes as loading controls. The effects of interventions on mRNA expression levels will be expressed as fold-change where fold change is calculated using the $2^{-\Delta\Delta Ct}$ method.[74,83]

Mitochondrial ETC activities. The assays will be performed using fresh cholate-treated skeletal muscle homogenates. ETC complex activities will be measured spectrophotometrically as specific donor-acceptor oxidoreductase activities in 0.1M phosphate buffer (HP 8453 and Lambda 35 UV/VIS). Rotenone-sensitive NADH cytochrome c reductase will measure complexes I and III. NADH coenzyme Q reductase will be measured as the rotenone-sensitive oxidation of NADH with decylubiquinone as acceptor, and assesses complex I. NADH ferricyanide reductase measures NADH dehydrogenase in complex I. Cytochrome c oxidase will be measured as the oxidation of reduced cytochrome c and expressed as the first order rate constant.[42,43] Flurometric measurements will be conducted to measure the oxidative and glycolytic enzymes' activities including citrate synthase and SDH.[42, 43, 76]

Statistical Analyses. Means and standard deviations or frequencies and percentages will be reported for all data. Similar summaries will be provided for all outcome and biomarker data separately at baseline, 6-, and 12-months. A repeated-measures ANOVA will be used to analyze the primary study outcome, skeletal muscle size, with treatment, time, and the interaction of these variables included in the model. A first order autoregressive correlation structure will be used to capture the dependency within subjects' outcomes. A specific contrast will be used to determine if the change from baseline for the treatment group is different than that of the control group. A model selection procedure will be performed due to the relatively large number of predictor variables compared to the number of subjects for specific aims 2 and 3. A penalized random effect model will be used to suggest the important predictors for each outcome separately. These include each specific predictor mentioned in the specific aims as well as injury characteristics.[84] A parsimonious model will be chosen by the Bayesian information criterion. Since the aforementioned model is well-suited for model selection, but results in biased parameter estimates, an unpenalized random effects model will be fitted based on the parsimonious model and used for inference. Study period will be included in all analyses, and a time by group interaction will be included for the analyses associated with Specific Aim 3. All statistics will performed with R (3.3.0) with the 'glmmLasso' package. Prior to any statistical analysis, any of the biomarkers may be transformed to ensure all statistical assumptions are met. A simulation study was used to determine the power achieved from the proposed study if 5% of subjects were assumed to drop out at the 12-month observation point. One thousand datasets were created with Cohen's *d* varying between 0.5 and 2.0, with the anticipated 24 subjects being equally allocated to the treatment and control groups and the subjects' outcomes having a correlation of 0.25. A linear mixed-effects model was used to analyze the simulated data using a one-sided test at α=0.05. Results of the study show that this design will have the power to detect differences of d=1.28 with 80% power and d=1.48 with 90% power. An intent-to-treat approach will be used to deal with missing data. If the missing data is due to drop-out, the statistical methods are valid as long as the data mechanism is considered missing at random or missing completely at random.

Dissemination and Implementation Plan

The target audiences for dissemination of the results from this study include the VHA and its practitioners, the national SCI/D Services Office, the general healthcare community, and the veteran population. We will share our findings with the SCI community. Our SCI/D Services and PVA publish a quarterly newsletter that is sent to SCI practitioners across the VA system. We

will inform the VA community of the impact of these findings. We will report our findings to the scientific community via the American Congress of Rehab Medicine, American College of Sports Medicine and American Spinal Cord Injury Association (ASIA). We will target JAP, MSSE and SCI journals to publish our reports.

5. Human Subjects

I. Human Subjects Involvement and Characteristics:

Twenty-four individuals with chronic (1-year post-injury) motor complete SCI will be recruited from the Hunter Holmes McGuire VA Spinal Cord Injury & Dysfunction (SCI&D) registry (n=1,800) over 4 years to participate in the study. The following table showed the primary reason for admission to the SCI&D at the McGuire VA Medical Center based on the 2015 fiscal year.

Primary reasons for admissions	Number of Patients
Total number of SCI between 18-70 years	1717
1- ***Inpatient admission***	**778**
- Acute rehab. CARF	~ 58
- Inpatient annual evaluations	~337
- Wound Care	~350
- Respite	~ 88
- Palliative and other chronic pts	~ 47
2- Outpatient visits	**939**
- Outpatient annual evaluations	417
- SCI walk-in Clinic	400
- Total Patients in Richmond	250-275

We are expecting that 20-25% of the entire 46,000 VA SCI population (~11,500 persons) and the entire 243,000-347,000 SCI civilians (60,750-86,750 persons) according to the 2016 SCI facts and figures in US may benefit from outcomes of the current study. In our VA facility in Richmond and since the initial submission, there were additional 8 men with motor complete SCI who failed stimulation because of lower motor neuron (LMN) denervation (37.5±13 years old, 27±6 kg/m^2, T10-L1 SCI). All of them were interested in strategies that can help restoring their leg muscle. This means that we currently have access to 16 participants (8 from applicant's CDA2 study) with LMN denervation without even starting an active recruitment

process ready to be contacted to participate in the study and this will ensure the recruitment target will be met.

After informed consent, each subject will undergo a complete physical examination by a board certified physiatrist in SCI medicine, including neurological assessment and ISNCSCI examination to confirm their level of injury and determine if it is safe for them to participate. The physical exam will include the measurement of body weight using a wheelchair weighing scale, and the performance of electrocardiograph (ECG) to rule any cardiac abnormalities including abnormal heart rhythms and myocardial infarction.
All 24 participants will undergo body composition assessments (specific aim 1), metabolic studies (specific aim 2) and muscle biopsies (specific aim 3). IRB approved flyers and advertisements will also be posted at the VAMC and VCU hospitals and clinic sites.

a. Inclusion: All participants will be between 18-70 years old, male, with traumatic motor complete SCI and level of injury of T10 and below, only participants with LMN denervation as determined by EMG testing (see research design section). Participants must also have an absence of reflexes, denervation of both knee extensor muscles and tolerance to LPWS paradigm, both knee extensors will also have to be unresponsive (i.e. no observed tetanic contraction or twitches) to standard electrical stimulation procedures (stimulation frequency: 30 Hz; pulse duration:450 μs and amplitude of the current:200 mA). All participants will undergo International Standards for Neurological Classification of SCI (ISNCSCI) examination for neurological level and function and only those with American Spinal Injury Classification (AIS **A and B**; i.e. motor deficit below the level of injury) will be included. We have chosen to set 70 years as the upper limit of the study, because individuals with SCI are living longer and baby boomers with SCI are likely to represent the majority of the VA population (age 60 and above). The study should be able to generalize the findings to the general population with LMN denervation, cardiovascular complications from TRT are also less likely to occur in individuals under the age of 70 years.

b. Rationale for recruiting veterans and non-veterans for this trial
The Hunter Holmes McGuire VAMC and the surrounding 13 spokes will be notified of the clinical trial once it is approved for imitation. Despite the large Spinal Cord Dysfunction (SCD) registry (n=1,800), SCI persons with LMN denervation represent a small subgroup of the entire population and recruitment may be challenging. The primary focus will be to include Veterans with SCI and the Civilians with SCI will be only considered in case we fall short to complete our target sample size. The inclusion of Civilians with SCI will allow generalizing the findings of the current trial to the entire SCI population.

c. Rationale for conducting the trial up to 1 year
In the current trial, we will include SCI persons with different levels of denervation. It is clear that skeletal muscles with denervation atrophy may take 3-6 months to evoke a strong tetanic contraction that is capable of moving the leg against gravity. Our data showed that loading the muscle is the best approach to evoke muscle hypertrophy and therefore, adding another 6 months may be a reasonable period to load the muscles. Besides the duration of the intervention has also considered the frequency (twice weekly) and not to overly stress the muscle to cause exercise induced muscle damage. Regarding applications of TRT, it is clear that using transdermal TRT patches may also need up to 1 year to induce a modest effect on lean mass.

d. Inclusion of women and minorities: Women will not be included in the current study

because administering TRT is neither appropriate nor safe. Department of Veteran Affairs has limited access to Veteran women with motor complete SCI. They are also at risk of virilizing actions of testosterone, therefore, we were planning to limit this trial only to men with SCI. No vulnerable populations will be included and persons under 18 will be excluded.

e. Exclusion: Every effort will be made to ensure each subject's safety or to exclude participants with current medical conditions that may confound the results. A conditional exclusion is those who will fail to tolerate the LPWS paradigm will also be excluded. Pain tolerance will be examined by gradually increasing the current in 10 mA intervals starting from 0 mA. If pain arises before a visible contraction, the subject will be automatically excluded from the trial.

The list of exclusion criteria will include the followings

1. Diagnosis of neurological injury other than SCI;
2. Pre-existing medical conditions will be excluded (cardiovascular disease, uncontrolled type II DM and those on insulin requirements) or other concurrent medical conditions judged to be contraindicated by the site physician.
3. Hematocrit above 50% and severe urinary tract infection or symptoms
4. Those with hyper-physiological testosterone level above 800 ng/dl.
5. Those who will fail to tolerate the LPWS paradigm
6. Progressive condition that would be expected to result in changing neurological status;
7. Lower extremity fracture around the knee joint (distal femur or proximal tibia) within the last 2 years from enrollment in the study;
8. Knee BMD < 0.60 gm/cm^2;
9. Total hip BMD T-scores < -3.5;
10. Untreatable severe spasticity judged to be contraindicated by the site Physician;
11. Untreated or uncontrolled hypertension (systolic blood pressure >140 mmHg; diastolic blood pressure >90 mmHg);
12. Pressure ulcer of the trunk, pelvic area, or lower extremities of grade 3 or more
13. Psychopathology documentation in the medical record or history that may conflict with study objectives

f. Sources of Materials

Existing electronic medical records will be reviewed for each subject enrolled by an IRB-approved investigator (Dr. Gorgey). All research data (recruitment and collected) will be stored in the PI's locked office designated specifically for SCI Research. No VA research data will be destroyed.

II. Study Procedures

The study procedures will include assessment of vital signs, EMG to quantify level of denervation, anthropometry, body composition assessments including DXA, MRI, metabolic assessment and muscle biopsy. These procedures will be performed by Dr. Gorgey in the McGuire SCI Exercise and Body Composition Research Lab except for muscle biopsies that will be performed at the minor procedure surgical room at the McGuire VAMC. All supplies and equipment for completion of tests are available in the Research Lab and pose no cost to the hospital. The MRI will be completed at McGuire Radiology Department. A portion of the grant funds will be used to reimburse the hospital for the resources to complete the MRI. The MRI protocol has been successfully conducted in the applicant's funded CDA-2 "Resistance Training and Testosterone after Spinal Cord Injury". The total time to scan both thighs is less than 8 minutes using an advanced localized coil.

III. Recruitment and Informed Consent

a. Plans for recruitment:

1. SCI & D providers along with staff will be briefed on the protocol by the PI at a routine monthly staff meeting. Providers will be updated and their support for recruitment reinforced at future routine staff meetings. Providers will refer patients who may be potential candidates. An opportunity will be made available to discuss the study in more depth if any provider wishes to learn more about the study. Upon notification, a review of the medical chart may be performed to determine if a subject is eligible to participate in the study.
2. Recruitment Flyers will be distributed throughout the VA Medical Center.
3. SCI Research exhibits containing *IRB approved* literature for distribution to interested persons will be displayed in the VA Medical Center at monthly Paralyzed Veterans of America (PVA) meetings/events in addition to various VA Medical Center events.
4. IRB *approved* literature will be published on PVA websites in addition to other VA websites.

b. Detailed strategies for the Recruitment Plan

Every effort will be made by the applicant to implement a successful recruitment plan, and to avoid any source of coercion during the actual process.

The following timeline will be implemented to meet our recruitment goals, and determine screening failure using the following recruitment methods. Our goal is to recruit, train and test 8 participants per year. This is feasible considering our previous experience recruiting and conducting similar trials. We plan to have 4 participants recruited in the first month of the trial in the first year and we will recruit an additional 4 participants every 6 months using the following timeline to meet our sample size. Approximately 2-3 subjects will be screened every other month. They will then be randomized to either group. The goal is to recruit 8 participants per year (4 participants every 6 months) and in order to finish data collection in 3-3.5 years.

Research Activities	***Year 1***		***Year 2***		***Year 3***		***Year 4***	
Months	1-6	6-12	1-6	6-12	1-6	6-12	1-6	6-12
Equipment & Supply Purchase	→							
Recruitment (n=4 per quarter)	→	→	→	→	→	→		
Screen and enroll subjects	→	→	→	→	→	→		
Data Collection		→	→	→	→	→	→	
Progress Report		→		→		→		→

Data entry/coding/cleaning		→	→	→	→	→	→	
Data analysis and interpretation		→	→	→	→	→	→	→
Presentation/Publications				→		→		→

During the first 2 months of the trial, the investigators will purchase equipment and supplies, finalize data collection forms and procedures and begin subject recruitment. Data analyses will be performed during the final six months of the trial. It is anticipated that preliminary data will be presented at meetings of the American Spinal Injury Association, American Congress of Rehabilitation Medicine, American College of Sports Medicine, in addition to the VA RR&D Annual Conference. Three manuscripts that address the primary specific aims will be submitted to the Journals of Spinal Cord, Archives of Physical Medicine & Rehabilitation and Applied Physiology.

C. Feasibility of recruitment

There is an emphasis on feasibility of the trial recruitment to ensure we reach our target sample size, especially considering the one year commitment necessary from participants. Below we provide a step-by-step description of our recruitment strategies to demonstrate feasibility

- Prior to submission of the current work, eight participants were identified in our preliminary work.
- After submission, eight additional participants were identified which already represents 33% of our target population. We have provided the physical characteristics of these participants.
- We are planning to use both VCU and Sheltering & Arms as active recruitment sites for our trial. Currently, Sheltering and Arms is partnered with PMR-VCU under the leadership of Dr. David Cifu, Co-investigator.
- We will contact every SCI physician, nurse and rehab. providers about our ongoing protocol through our weekly clinical rounds and grand rounds.
- We will send an email blast through our secured network to our 13 Spokes sites and extended SCI Centers inviting out of state participants who may be eligible and offer them lodging opportunity during the course of the trial.
- We will send electronic flyers as well as making our research coordinator available to present at our SCI meetings, PVA monthly meeting, SCI quarterly newsletters, hub and spoke conference, and SCI national Chief teleconference meeting.
- We have been approved by our Service Chief to use one lodging room to house participants during the training period (see letter from Dr. Lavis).
- We will provide the telehealth videoconference option to the control group (n=12), they will be likely to use standard NMES unit and we have tested the safety and the feasibility of this approach. This is likely to reduce the burden on the study resources and provide more focus on the TRT+LPWS group. We cannot offer the same option for the TRT+LPWS, because all training procedures have to be completed under full supervision.
- Dropout is likely to happen with longitudinal interventions, in our CDA-2 trial, we had 26 participants enrolled and 4 withdrew as previously highlighted. During the trial, we strived to replace them and we were very successful in accomplishing this goal. According to our initial power calculation, we require a sample size of 20 participants to achieve 80% power and we completed the trial with 22 participants.

- Our SCI&D service has a designated wheelchair van that we have been using on a regular basis for transportation of our participants back and forth during the course of clinical trials, assuming they reside in the Richmond area. This accessible transportation has encouraged several participants to be enrolled in our previous VA trials.

d. Recruitment Methods

1. Flyers and brochures
2. Annual health fairs at McGuire VA Hospital and VCU Medical Center
3. Virginia local chapter of SCI (the applicant was previously invited to present in their monthly meetings).
4. The PI will notify all the physicians, physician assistants and therapist working on the SCI service about the protocol and provide them with a print out of the inclusion/exclusion criteria. The PI will plan to present quarterly at grand rounds to educate the staff and involve them in recruitment.
5. All medical providers will be provided with an inclusion/ exclusion criteria that allows them to screen participants and to notify the PI about potential applicants.
5. A letter with the inclusion/exclusion criteria will be mailed out to all potential applicants in the SCI registry, we will start first with the local participants (n= ~275) and we will subsequently include other participants in the registry.

e. Process for obtaining informed consent:

The PI will seek informed consent from subjects with an IRB approved consent form. Determination of a subject's capacity to consent will be made by the investigator. Input may be solicited from an SCI primary provider who knows the potential subjects. Potential subjects will have the full study verbally explained to them by the PI. If they voice an interest in the study they will have the written consent form provided and ample time to consider their participation. All concerns and questions will be answered. Emphasis will be placed on explaining all study procedures. Family members will be included in the discussion as desired by the subjects. They will be told that participation is voluntary and can withdraw at any time with no impact on the care they receive from the VA. They can discuss the study with their primary SCI provider. The subjects will be given a copy of the consent form once it is signed. Potential subjects will be allowed sufficient time and opportunity to read the consent alone and have all questions and concerns answered. They will be allowed to take consent home for additional time prior to making decisions. Voluntary participation and withdrawal will be reinforced. All aspects of the approved consent will be reviewed with all subjects. All signed consents will be scanned into CPRS attached to a CWAD note indicating enrollment in study. In addition to a consent note entered into CPRS for all subjects, progress notes of their active participation will also be entered. In summary, the following main points will be considered in this process

1. The PI will approach all potential applicants, only after the provision of a written medical clearance from their primary provider stating their eligibility to participate in the study.
2. The PI will discuss individually with the potential applicant and provide a detailed explanation about the study as well as offering the applicant sufficient time to consider reading the consent form, discuss with relatives and primary provider. A list of study benefits/risks will be highlighted and the applicants will sign the consent in presence of a witness.
3. Subjects will be allowed to withdraw from the study at any time without impacting their medical care or health benefits at the local VA facility.

6. Limitations and Alternatives.

The current interventions **may not be applicable** to those who have pain or intolerance to the stimulation parameters. ***1)*** Only those with LMN denervation may benefit the current protocol. ***2)***

Although LMN injury may evenly spread across different segments of the cord, we are interested in studying the large knee extensors because of its established metabolic activities. ***3)*** Every effort will be made to recruit individuals with TSI less than 5 years to ensure appropriate response to LPWS; however, the study will include others who have been injured for more than 5 years to ensure generalization to those with long-term LMN denervation. ***4)*** The Grass S88X is intended for research use only and not for clinical applications. However, successful completion of this proposal will allow clinical translation and ensure safety of introducing these stimulation parameters to the clinical field. The unit does not require FDA approval to be used for human with SCI, it does only require IRB approval prior initiation of the trial.

Other Limitations and Alternatives: The current study is limited by
1) The fact that the effects of training and increased muscle mass may be influenced by increased dietary intake, which may confound the primary outcome measures. We will evaluate the caloric intake reports from our participants on a monthly basis. This will allow us to closely monitor extra-caloric intake and we will regularly instruct our participants if the caloric intake exceeds 300-500 Kcal/week of their pre-measured BMR.
2) Some individuals may complain of skin rashes when wearing the transdermal patches; glucocorticoid cream will be prescribed for use after each patch is removed and participant will be excluded if the problem is persistent.
3) Concerns about frequent muscle biopsy samples and the size of the muscle biopsy. The muscle biopsy is performed by our Chief of Surgery, Dr. Rivers who has done more than 40 subjects with no adverse events in different SCI clinical trials. She relies on making a tiny incision and she uses a 14 gauge Tri-Cut needle. Because we are aware that SCI persons with LMN denervation may have limited size muscle, we are planning to limit our analysis to specific proteins of interest and specific gene expression that helps elucidate pathways for triggering muscle hypertrophy. For the ETC, we have proposed to study complex I and complex III as well as citrate synthase, this is likely to provide a clear indication of mitochondrial activity and size in persons with LMN denervation.
4) Another concern is the ability to recruit the suggested sample size. The PI has previously recruited and supervised 22 individuals during his CDA-2 award (see preliminary work). We have also provided a plan to maximize recruitment (*see human subject research plan for details about reasons for admissions and recruitment plan*). <u>As we highlighted earlier, we currently have access to 16 participants who meet the study inclusion criteria without starting an active recruitment process.</u>
5) Concerns about frequent blood sampling, the procedure has been previously IRB approved in two funded grants and no single adverse incident has been reported.
6) Concerns about **<u>retaining participants</u>** to finish the 1 year study; transportation is the greatest impediment to subject compliance for this population. Transportation will therefore be arranged in advance to allow for subjects personalized schedules and financial cost.

a) The health benefits of actively enrolled in the trial will be clearly highlighted.
b) A scheduled pick-up and drop-off time will be established to ensure subjects are more likely to attend all testing sessions.
c) A portion of the budget will be dedicated for transportation using the CARE van, a transportation service for individuals with disabilities.
d) <u>Compared to our initial submission, we obtained an approval from our Service Chief (Dr. Lavis) to provide a lodging unit (1 bed room) to house our training group during the study (see the support letter from Dr. Lavis).</u>
e) <u>A portion of $3000 subjects' reimbursement fee will be dedicated to reserve a room for non-veteran participants who may need lodging at Candlewood suites which is located 4.5 miles away from our center.</u>

f) For the TRT+ standard NMES (control group; n=12), we will provide the option to carry out the training at their home using our established videoconference telehealth system and monitor the training using a hand-held battery operated stimulator, which is pre-customized and ready to be used (see supplement). We have 5 Empi stimulators in our laboratory and do not need to buy any additional units. This will ensure subject retention at least in the control group and will allow the study team to focus on the retention of the other 12 participants in the TRT+LPWS group.
g) Providing financial reimbursement, social interaction during training sessions and access to the data at the conclusion of the study are additional methods to be utilized to reduce subject attrition.
h) Dr. Gorgey will work closely with each participant to resolve any scheduling barrier and will allow morning and evening schedules.
i) We will also provide a comprehensive team approach that will allow our participants free consultations with dietitian, psychologist and participation in our recreational activity program.

7. Risk to Subjects

Potential Risk	Potential effects of risk	Potential seriousness
Anthropometrics	Bruising, discomfort	Occasionally
Venous catheter insertion and blood draws	Localized swelling, soreness, bruising, and chance of infection, bleeding, pain, lightheadedness or possible fainting. A total of 12 tablespoons will be collected.	Occasionally
IV line failure	Discomfort, swelling, redness over the IV line site causing failure to use the line. Another IV will need to be placed in another part of the arm.	Occasionally
Insulin Sensitivity Tests	Hypoglycemia (low blood sugar) with occasional dizziness, sweating, and nausea, seizures, coma, or death	Occasionally Unlikely
Basic Metabolic Rate	Anxiety, apnea, and claustrophobia	Occasionally
Autonomic Dysreflexia	Slow heart rate, high blood pressure, headache flushing and sweating.	Unlikely
DXA	Fall during transfer This study will involve exposure to radiation from 3 whole body DXA scans and regional scans for hips and knees. This radiation exposure is not necessary for medical care and is for research purposes only. All radiation increases the risk of developing cancer in the future. The total amount of	Unlikely

	radiation in this study is equal to less than one day of exposure from natural background radiation. The McGuire VA Medical Center Radiation Safety Committee will review the use of radiation in this research study and will approve the use as involving acceptable risk and necessary to obtain the research information desired. Physicians will be informed about the research to help their participants to make the correct decision.	
MRI	Anxiety, dizziness, and claustrophobia	Occasionally
Muscle Biopsy	Localized swelling, soreness, bruising, chance of infection, bleeding, pain, lightheadedness or possible fainting. The numbing medication can cause allergic reaction including local skin rash and rapid heart rate.	Uncommon
Resistance training and LPWS electrical stimulation	1. Light-headedness, shortness of breath and altered heart rate& blood pressure leading to autonomic dysreflexia. Muscle soreness at your neck, upper back, shoulders, arms & hands 2. Fracture 3. Autonomic dysreflexia (slow heart rate, high blood pressure, headache flushing & sweating) which may be life threatening 4. Pressure Ulcers 5. Fainting, heart attacks or death	Unlikely
Testosterone Replacement Therapy	**Serious reactions** Severe rash at site of the patches, worsening heart failure that may cause difficulty for pumping blood, swelling of the body, enlarged prostate causing difficulty in urination, increase in red blood cells which may cause blood clots in the legs (cause swelling), chest pain, shortness of breath and rarely death and brain damage (causing a stroke), infertility, prostate cancer, difficulty in breathing during sleep, blood in urine. **Common reactions** Application site reactions, back pain, enlarged prostate, headache, irritations of the skin,	Occasionally occurs

	depression, enlarged breasts, increase cholesterol which may increase the risk of heart disease, chills, diarrhea, fatigue, frequent urination, pain during urination, reduced sex drive, inflammation of prostate, rash, acne, confusion	

a. Protection against Risk:

Every effort will be made by the PI and the study team to minimize potential risks. Any procedures will be carried out in accordance with any standard operating procedures and study staff will receive the necessary training to mitigate risks. There will be strict adherence to the inclusion & exclusion criteria of the protocol. Appropriate health screening and strict compliance with the established exclusion criteria will minimize attrition due to medically related causes. Subjects will be closely monitored by study personnel at all visits. All subjects will be provided with 24-hour contact information for the investigator and instructed to call for any concerns. Unscheduled visits may be performed if required for evaluation of safety. If a subject has an adverse event, more frequent visits may be necessary.

b. Adequacy of Protection from Risk:

Protection of skin integrity during Anthropometry:

Skin will be closely checked following each measurement to ensure no redness or scratches.

Protection from risk associated with transfers:

To minimize risk, an investigator will be available to assist in all transfers, and provide a slide board or any other assistive devices needed. Our labs are equipped with ceiling lifts that facilitate the transfer process from wheelchairs to any other station.

Protection against risk of autonomic dysreflexia (AD).

Our sample is limited to T10 and below, we have developed a protocol by carefully monitoring blood pressure and heart rate every 2-3 minutes in persons with SCI. Moreover, our training protocol provide us with an opportunity to space out time between NMES repetitions in persons who are susceptible of developing AD risk. This ensures the participants can safely continue the training and reduce the potential risk. Following 4-8 weeks of training, participants' free nerve endings become acclimatized to the electrical signals during NMES.

Protection from risk associated with Dual Energy X-ray Absorptiometry (DXA) Scan:

To minimize risk, only the required scans will be performed. An overhead lift system has been installed to ensure safe transfers on and off scanner. All study procedures have to be approved by our Radiation Safety committee prior obtaining IRB approval.

Protection from risk associated with MRI:

To minimize risks an MRI safety checklist will be used to ascertain it is safe for subjects to have an MRI. Only board certified radiologists at McGuire VAMC will be utilized.

Protection from risk associated with venous catheter insertion and blood draws:

To reduce risk, all blood drawing will be performed by certified personnel using aseptic techniques and universal precautions. All blood draws will be performed by well-trained certified nurses who are working in our SCI center. IVGTT dose calculation and test will be carried out and administered by a medical doctor and the participant will be under full medical supervision during the test.

Protection from risk associated with muscle biopsy:
The muscle biopsy will be conducted at the McGuire VA Medical Center. Three biopsy samples of the vastus lateralis muscle (25-50 mg wet wt.) will be obtained by a 14 gauge Tru-Cut needle by Dr. Rivers. Biopsies will be obtained before the trial (baseline), mid-intervention (6 months) and after 1 year of completing the trial (post-intervention) using a sterile technique and local anesthesia (2% lidocaine). The skin overlying the muscles will be prepped and draped in a sterile fashion and 3cc 2% lidocaine will be locally administered. A 5 mm skin incision will then be made with a #10 scalpel. The biopsy samples will be obtained from one site incision, after which the site will be closed with Steristrips and an overlying sterile adhesive dressing. Furthermore, a pressure dressing will be administered for at least 10 minutes. Following the muscle biopsy, Dr. Gorgey will provide the participants with extra supplies in case the dressing needs to be changed. One of our SCI physicians will examine all the participants within 48 hours after conducting the muscle biopsy. This procedure has been successfully completed in our previous CDA2 trial (n=22) without a single adverse event reported.

Protection from risk associated with resistance training and LPWS neuromuscular electrical stimulation
Dr. Adler will review all DXA scans to determine that participants can safely exercise without risk of fracture. Participants with knee BMD less than 0.6 g/cm^2 and hip T-scores below -3.5 SD or previous bony fracture around the knee joint within the last 2 years will be excluded from the trial. Dr. Gorgey will supervise all aspects related to the protocol including proper ankle weight positioning, placement of the electrodes and monitoring vital signs during electrical stimulation.

Protection from risk associated with Testosterone (T) Replacement Therapy (TRT)
Dr. Adler will clinically and medically supervise all our participants during the course of the study. Possibility of skin rashes will be treated with glucocorticoid cream after removal of T patches. The PI will supervise all the participants closely and provide a weekly call to identify any issues. Participants will be monitored for any changes in baseline health. Participants will undergo blood work to determine serum T-level as well as their Prostate-specific antigen (PSA). There is no specific normal or abnormal level of PSA; however, a value above 1.4 ng/ml or increase of the PSA above 1.4 compared to baseline values will result in immediate cessation of TRT. We will also check the HCT every 4 weeks to ensure that hematocrit (HCT) is less than 54%, value greater than 54 may result in polycythemia.
Moreover, a staff physician who is board certified in SCI medicine will evaluate all participants who enroll in the study. This physician is competent to assess for urologic irritative or obstructive voiding symptoms and for changes in erectile function. This is a routine component of SCI medicine practice for patients with urologic symptoms related to both neurogenic voiding dysfunction and prostate hypertrophy. The physician will assess each participant for new symptoms every three months.

Study removal criteria may include:
- Extensive skin irritation or rashes or itching as result of TRT applications
- Medical history of carcinoma of the breast, carcinoma of the prostate, venous thromboembolism, pulmonary embolism, stroke, cholestatic jaundice, hypertension, serious heart problems, heart failure, myocardial infarction, ventricular arrhythmia, exertional chest pain, insulin dependent diabetes, hypercholesterolemia, hypertriglyceridemia, impaired liver function, or significant renal dysfunction.
- cardiovascular events similar to acute myocardial infarction, acute coronary syndrome, congestive heart failure, any cardiovascular-related hospitalization, stroke, or cardiac arrest, and the development of new significant ischemic findings or symptoms during the course of

the study (including development of new major abnormalities on serial EKGs or symptoms of typical angina)
- Serum PSA >4.0ng/ml
- Increase in serum PSA of >1.4ng/ml compared to baseline
- HCT >54%
- Liver enzymes (AST / ALT) >1.5 times normal upper limit
- Hemoglobin >17.5 g/dL
- Serum calcium between 10.5 – 11.2mg/dL with symptoms of hypercalcemia
- Gynecomastia
- Development of severe peripheral edema, as classified as 2+ or higher
- Development of any other SAE that the Medical Director, Dr. Adler, feels necessitates study withdrawal for participant safety

Protection against possible subjects' coercion.
The $3000 dollar amount dedicated as a reimbursement fee may create subject coercion. This amount reflects the cost of gas, transportation and commitment throughout the 1-year period. To protect against any coercion, the figure will be split into 6 payments ($500 every two months or 16 visits). This means that each participant will receive ~ $31.25 per study visit. The $3000 figure will be re-calculated incase participants decide to lodge at Candlewood suites or perform the study at home using our established telehealth system. Finally, all our study procedures will be approved by the IRB and the PI will work closely with the IRB to avoid any coercive influence on any of our study participants.

c. Adverse Events
Participants will be monitored for any changes in baseline health. Their SCI provider will be notified if this occurs. An adverse event is any experience that has taken place during the course of research project, which, in the opinion of the investigators, was harmful to a subject participating in the research, increases the risks of harm in the research, or had an unfavorable impact on the risk/benefit ratio. Adverse events (AE) will be monitored throughout the study via exams, vital signs, laboratory tests, review of medical charts, and verbal concerns voiced by the participant or an associated friend or family member. Any adverse event will be documented in their files. Files will be reviewed for adverse events, protocol violations, and reasons for dropouts/withdrawals every ten days. The PI will provide the McGuire VA IRB & Office of Research Subjects Protections with an annual summary of adverse events. All serious adverse events (SAEs), such as those that are life-threating or involve hospitalization, s will be promptly reported to the McGuire IRB Research Subjects Protection Program. Participants' health conditions will be monitored during study visits.

When study procedures begin, the importance of reporting any perceived problem, adverse event or change from baseline health will be stressed to the subjects. Examples will be given to the subject of potential problems requiring immediate medical attention and potential problems requiring a phone call to the investigator. Above all, it will be stressed to the subject that direct phone access to the study staff is available 24hrs/day and if there is ever any doubt or concern on the part of the subject a call should be made. At the completion of this study all subjects will continue to receive their health care from their assigned SCI provider or other provider of subjects' own choice.

All subjects will be identified by an assigned number and their initials. Subjects' research charts will be kept inside a locked file in the PI's locked office. Only study staff listed on the Personnel List will have access to subject study records and medical information. A master sheet of

participants' full names will be kept in a locked file in the PI's office. All completed study files will be stored in the PI's SCI research office 1V-129. At the end of the 4 year period, all records will either be stored in the SCI Research Exercise Laboratory, which is a locked room or sent to Dunmar Storage Facility per direction of McGuire IRB.

8. Subjects' burdens

The current study ***was designed to reduce and minimize subject burden*** including transportation and participation time. Although participation may impose a significant burden, every effort will be taken by the study team to reduce the stressors and burdens of participation. Over the 1 year period, persons will be asked to visit the lab **4 times** (1 visit for screening and consenting and 3 testing sessions for conducting outlined procedures) for testing. All exercise training will be conducted twice weekly over 1 year. Each training session should not last more than 30 minutes. The current study design will allow each participant to serve as his own control over all the genomic, dietary, body composition, metabolic and SCI factors that are likely to impact the outcomes of any training study. Every effort will be taken to retain participants in the study for 1 year. We have significant experience retaining participants up to 9 months that adhered to the applicant's CDA-2 study protocol. In the applicant CDA-2, the study constituted of 3 phases; a delay entry approach phase for 4 weeks, 16 weeks training phase and 16 weeks detraining phase. Out of the 26 participants who completed the first 2 phases, the applicant was successful in retaining 13 participants for the 3 phases (50%). The CDA2 study was designed to examine only 50% of the participants in the detraining phase (i.e. 6 participants/ group). Moreover, each participant is allowed to miss up to 10% (12 visits) of the scheduled visits without withdrawing from the study to consider unanticipated events similar to sickness, medical appointments and vacations. We believe that we can retain participants for up to one year using similar strategies.

a) Previously we provided transportation using our facility owned Van (free of charge) to allow transportation to and from the VA facility.
b) We have allowed operation and training beyond regular business hours to accommodate education and work related schedules.
c) Total time commitment is less than 2 hours/ week, twice a week. Compared to other longitudinal clinical trials this is considered by far the least training volume, which was adopted according to the American College of Sports Medicine guidelines.
d) During data collection, we will provide sufficient time for participants to have their meals and use medications.
e) Trained research staff will help in the process of testing and data collection.
f) The SCI Exercise Physiology Laboratory is equipped with a sound system and is in the process of installing a flat screen TV that will allow participants to listen to music or watch TV during exercise providing an interactive and motivational environment.
g) A comprehensive team approach will be adopted that will allow participants to engage in recreational activity programs and to consult with a psychologist, dietitian or other clinical staff (PT, OT, etc.) as needed.

a. Benefits and Burdens

Potential benefits of research to subjects and others:

The risks associated with this study seem appropriate for the anticipated benefits. Study information will be under continuous review for any information that may impact on the risk/benefit ratio; i.e. adverse events, unanticipated problems, and complaints regarding the research. SCI persons with LMN denervation exhibit greater loss in muscle mass. The significant loss in muscle mass has been linked to several metabolic and cardiovascular health

related consequences. Individuals with SCI are at increased risk for many chronic diseases such as type II diabetes mellitus, dyslipidemia, cardiovascular disease and osteoporosis; it is important to continue developing exercise interventions that benefit these individuals and thereby decrease their risk for chronic disease. This understudied population represents 25% of the whole SCI population and cannot benefit from the standard applications of surface NMES on restoring muscle size similar to persons with SCI with intact LMN system. Results from the current investigation will facilitate a greater understanding of the methodology necessary to accurately determine the impact of long-term exercise on muscle hypertrophy, metabolic and local cellular adaptations. SCI predisposes individuals with denervated muscle to extreme muscle atrophy, decreased BMR, glucose intolerance and insulin resistance. Exercise has the potential to improve BMR, glucose effectiveness and insulin sensitivity in this population. *Moreover, the benefits of long-term rehabilitation interventions are limited in individuals with lower motor neuron (LMN) denervation following SCI. The current proposal may provide a simple rehabilitation intervention to help restore muscle size and prevent heath related consequences***.** We hope findings from this application will help reduce the costs associated with managing secondary health related complications in Veterans and Civilians with SCI. The research team will extend every effort to disseminate the knowledge gained from this trial at various scientific meetings and journals to share nationally and internationally with other academics/clinicians. While there may be no direct benefit to the subjects besides the monetary reimbursement, the information learned in this study has the potential to help others in the future.

b. Importance of knowledge to be gained: The prevalence of individuals with SCI has been estimated to be 250,000-400,000 with an estimated 14% growth in the prevalence since 1988. Of the more than 46,000 Veterans with SCI-related disability, more than 60% are overweight or obese, more than 50% are glucose intolerant and 20% are frankly diabetic. In the last two decades, the national aggregate direct costs of SCI in the United States have increased with a concomitant decline in mortality over the first year after SCI.[6] Our preliminary data suggested that 20-25% experience SCI with LMN denervation. Currently, there is no available rehabilitation intervention following lower motor neuron (LMN) denervation. Denervation to lower extremity muscles results in even more deleterious sub-lesional skeletal muscle atrophy and is accompanied with several SCI health-related consequences. The current proposal will provide a novel rehabilitation strategy, combining both TRT+LPWS to determine the effects of mitigating muscle loss following LMN denervation. Establishing the LPWS rehabilitation protocol is a rigorous process and may require several attempts to refine and establish the best stimulation protocol necessary for stimulation of LMN denervation. The addition of TRT may provide additional benefits of increasing lean mass and accelerate the actions of LPWS. Previous attempts indicate that TRT may increase lean mass and BMR. This will be the first clinical attempt to determine the role of TRT on muscle size and lean mass in persons with LMN denervation independent of neural structures. This is a promising combination of physical and pharmacologic therapy that is likely to improve body composition and other cellular functions which may improve other metabolic health biomarkers. Several of the mechanisms involved in increasing muscle size, including proteins and gene expression will be studied, which will allow us to design specific interventions that target specific abnormalities at the cellular level which have the potential to impact body composition and metabolic adaptations in future studies.

9. Clinical Trials Requirements. This is a drug trial and registration with Clinical Trials is mandated. We are aware of this requirement and are experienced in complying with all regulatory requirements. The PI has previous experience working with Clinical Trials and he successfully registered two clinical trials.

10. Data Safety and Monitoring Board (DSMB):

The Data Safety Monitoring Board (DSMB) will consist of Dr. Randall E. Merchant, PhD, Dr. Steven L. West, PhD, CRC, Dr. Carolyn W. Graham, PhD and Dr. Sean McAvoy, MD. The DSMB will meet to review the protocol prior to data collection and will meet at least annually to evaluate subject safety, data quality, and study progress and execution. The DSMB will review protocol for any major concern prior to implementation. They will also evaluate safety, study conduct, and scientific validity and integrity of the trial. They will also assess the performance of overall study operations and any other relevant issues, as necessary. Elements in the letter will include the followings:

- Evidence of efficacy according to pre-established statistical guidelines, if appropriate;
- Evidence of study-related adverse events
- Data quality, completeness, and timeliness
- Performance of the study
- Adequacy of compliance with goals for recruitment and retention, including those related to the participation of women
- Adherence to the protocol
- Factors that might affect the study outcome or compromise the confidentiality of the trial data (such as protocol violations)
- Factors external to the study such as scientific or therapeutic developments that may impact participant safety or the ethics of the study.

DSMB will include the following members

Randall E. Merchant, Ph.D.
Professor of Anatomy & Neurobiology, Neurosurgery, Physical Medicine & Rehabilitation
Executive Director of the Center for Rehabilitation Science & Engineering (CERSE)
Virginia Commonwealth University

Steven L. West, PhD
Associate Professor in the Department of Physical Medicine and Rehabilitation and Associate Director of the Center for Rehabilitation Science and Engineering at Virginia Commonwealth University) Principal Investigator (PI): He has extensive experience in conducting research on substance abuse and disability related issues.

Carolyn W. Graham, PhD
Research Methodologist, Center for Rehabilitation Sciences and Engineering
Director of Research, RRTC for Employment of Individuals with Physical Disabilities
Associate Professor, Department of Physical Medicine and Rehabilitation
Virginia Commonwealth University

William McKinley, MD
Professor in in the Department of Physical Medicine and Rehabilitation
Director, Physical Medicine and Rehabilitation Residency Program
Director of Spinal Cord Injury Services at the Rehabilitation and Research Center.

McKinley is a professor and director of the residency program in the Department of Physical Medicine and Rehabilitation with special interests and expertise in spinal cord injury, electrodiagnosis and musculoskeletal rehabilitation and has been active in clinical research in SCI and pain assessment.

Sean McAvoy, MD
Attending Physician, Spinal Cord Injury and Disorders
Department of Veterans Affairs
Hunter Holmes McGuire VA Medical Center
Phone: 804-675-6227
Email: sean.mcavoy@va.gov