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Testosterone and long pulse width stimulation (TLPS) for denervated muscles after spinal cord injury: a study protocol of randomised clinical trial

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ABSTRACT

Introduction Long pulse width stimulation (LPWS; 120–150 ms) has the potential to stimulate denervated muscles and to restore muscle size in denervated people with spinal cord injury (SCI). We will determine if testosterone treatment (TT)+LPWS would increase skeletal muscle size, leg lean mass and improve overall metabolic health in persons with SCI with denervation. We hypothesise that the 1-year TT+LPWS will upregulate protein synthesis pathways, downregulate protein degradation pathways and increase overall mitochondrial health.

Methods and analysis Twenty-four male participants (aged 18–70 years with chronic SCI) with denervation of both knee extensor muscles and tolerance to the LPWS paradigm will be randomised into either TT+neuromuscular electrical stimulation via telehealth or TT+LPWS. The training sessions will be twice weekly for 1 year. Measurements will be conducted 1 week prior training (baseline; week 0), 6 months following training (postintervention 1) and 1 week after the end of 1 year of training (postintervention 2). Measurements will include body composition assessment using anthropometry, dual X-ray absorptiometry and MRI to measure size of different muscle groups. Metabolic profile will include measuring of basal metabolic rate, followed by blood drawn to measure fasting biomarkers similar to hemoglobin A1c, lipid panels, C reactive protein, interleukin-6 and free fatty acids and then intravenous glucose tolerance test to test for insulin sensitivity and glucose effectiveness. Finally, muscle biopsy will be captured to measure protein expression and intracellular signalling; and mitochondrial electron transport chain function. The participants will fill out 3 days dietary record to monitor their energy intake on a weekly basis.

Ethics and dissemination The study was approved by Institutional Review Board of the McGuire Research Institute (ID # 02189). Dissemination plans will include the Veteran Health Administration and its practitioners, the national SCI/D services office, the general healthcare community and the veteran population, as well as the entire SCI community via submitting quarterly letters or peer-review articles.

Trial registration number NCT03345576.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Long pulse width stimulation (LPWS) is safe to be administered in persons with spinal cord injury (SCI) with lower motor neuron injury.
⇒ Training twice weekly may encourage long-term compliance and adherence.
⇒ Testosterone treatment may serve as an alternative therapy for denervated muscles.
⇒ Recruitment and retaining for a duration of 12 months is rather challenging in this subpopulation with SCI.
⇒ Currently, LPWS stimulators are not clinically or commercially available in North America.

BACKGROUND

Spinal cord injury (SCI) is a devastating medical condition that increases one's risk for type II diabetes mellitus, dyslipidaemia and cardiovascular disease.[1–5] 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 Veterans Affairs cares for >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 USA have increased with a concomitant decline in mortality over the first year after SCI.[8] Medical care costs per case may exceed US$1 million for acute stabilisation and rehabilitation, with annual charges thereafter ranging from US$41 000 to US$182 000.[6–8] The estimated lifetime costs can exceed US$12 million.[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

minimise SCI-related secondary disorders and favourably influence healthcare costs after SCI.

Previous work demonstrated that surface neuromuscular electrical stimulation (NMES, ie, stimulation of intact axonal branches) evokes whole thigh and knee extensors muscle hypertrophy following exercise-induced resistance training (RT) with ankle weights in individuals with chronic SCI.[9–12] Evoking skeletal muscle hypertrophy is vital for several activities of cellular and whole body metabolism.[13–15] Skeletal muscles serve as a large paracrine gland that controls the interplay between the musculoskeletal system and other physiological systems.[13–15] Studies documented that skeletal muscle releases important myokines that may regulate atrophic pathways, bone and endocrine glands.[14 15] Twelve weeks of training increased skeletal muscle hypertrophy by >40% and improved glucose tolerance many years after injury.[10] Ryan *et al* noted an improvement in mitochondrial capacity by 25% following 16 weeks twice-weekly NMES-RT.[11] NMES-RT may increase mitochondrial capacity to use fat as a source of energy during exercise and further improve insulin sensitivity.[12] Twelve weeks of twice-weekly NMES-RT can elicit ~35% increase in skeletal muscle size, decreased intramuscular fat (IMF) and visceral adipose tissue, increased insulin sensitivity and increased insulin growth factors-1 (IGF-1) by 25%.[12] However, despite the benefits of NMES applications, approximately 25% of the SCI population cannot benefit from the standard NMES (pulse duration <1000 µs or 1 ms) because of lower motor neurons (LMN) denervation.[16 17]

Today, there are no stimulation protocols that may train the muscle following LMN denervation, because of increasing the depth of penetration following deleterious atrophy, subcutaneous fat thickness and excessive infiltration of IMF that may diminish the spread of the current density to activate the target muscles.[16] Standard surface NMES (pulse duration of 150–1000 µs which directly stimulates axonal branches or peripheral nerves) fails to activate the denervated muscles because of an increase in the minimum time required, chronaxie, as demonstrated on the strength-duration curve.[16] This requires higher current charges for the direct depolarisation of the muscle fibres. The limited pulse duration is inversely associated with an increase in the current amplitude required to activate 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. This low amplitude of the current is unlikely to cause muscle activation following denervation in persons with SCI. This, subsequently, results in failure maintaining skeletal muscle vitality following LMN denervation as well as maintaining the integrity of other physiological systems.

Long pulse width stimulation (LPWS) has the capacity to penetrate deeply and activate muscle fibres directly without reliance on stimulating the denervated peripheral nerves.[18–20] The European project, Research and Innovation Staff Exchange, has introduced long pulse width (LPW) NMES to restore muscle size following denervation in people with SCI.[21–24] The effects of home-based functional electrical stimulation, introducing an LPW (120–150 ms) at an amplitude of 250 mA for 5 days per week has been studied for 2 years in 25 persons with SCI with complete LMN denervation.[21–24] The trial showed an increase (24%) in knee extensor cross-sectional area following the first year and an additional 7% in the second year, respectively, with no changes in the hamstring muscles.[21–24] However, the safety and the feasibility of application of LPWS has not been determined within the US population with SCI or generally in North America.[25] Successful completion of this trial will provide a safe and feasible rehabilitation approach that likely enhances muscle hypertrophy in persons with SCI with LMN denervation.

We previously attempted to combine NMES-RT with testosterone treatment (TT) to attenuate cardiometabolic risk factors after SCI.[26 27] TT is an Food and Drug Administration-approved therapy used to treat hypogonadism and often results in significant improvement of muscle strength and fat-free mass (FFM) in hypogonadal men.[28 29] In rats with complete SCI, TT has been shown to attenuate muscle atrophy and the decline in oxidative and glycolytic enzymatic activities.[30] Additionally, 60% of men with SCI have low T levels in the first 6 months after SCI.[29] Administering TT may attenuate the effects of denervation on the paralysed muscle. However, applications of TT in chronic models of denervation have not been established in clinical population with SCI. The role of TT on muscle size independent of the changes in the peripheral nervous system has not been investigated. It is possible to assume that may result from upregulation of the androgen receptors that may lead to proliferation of satellite cells.[31]

We hypothesise that 1 year of TT+LPWS will result in a significant knee extensor muscle hypertrophy of 25% or more.[25] This will be associated with a significant increase in leg lean mass (>10%) and concomitant improvement in overall cardiometabolic profile by 20%–30%. Previous studies suggest that LPWS may require 5 days per week up 2 years to restore muscle size in persons with SCI with LMN denervation.[20–24] Clinically, this is not feasible considering the barriers related to dressing, bowel and bladder movements, transportation and loading and unloading of wheelchairs. We are hypothesising that the addition of TT to LPWS may facilitate the increase in leg lean mass and allow optimisation of the stimulation protocol in just 1 year. Therefore, this approach of combining both physical and pharmacological interventions may allow twice-weekly LPWS to be highly effective in restoring muscle size.

The goal of this randomised prospective controlled study is to investigate the effects of 1 year of TT+LPWS versus TT+standard NMES on muscle size (primary outcome variable), leg lean mass and percentage IMF, metabolic profile (basal metabolic rate (BMR),

carbohydrate and lipid profiles) and protein synthesis and degradation pathways as well as mitochondrial health. Carbohydrate profile will include resting plasma glucose and insulin, glucose effectiveness (Sg) and insulin sensitivity (Si), whereas the lipid profile will include the entire analysis of the lipid panel. We hypothesise that the 1-year TT+LPWS protocol will upregulate protein synthesis pathways, downregulate protein degradation pathways and increase overall mitochondrial health. Three specific aims will address these hypotheses. *Aim 1* will assess the effects of TT+LPWS compared with TT+standard NMES as a control group) on the size of thigh skeletal muscle, IMF and leg lean mass. *Aim 2* will determine the association between the changes in skeletal muscle size, leg lean mass and the metabolic profile as determined by measuring BMR, serum lipids and carbohydrate profile. *Aim 3* will investigate the cellular mechanisms (protein and messenger RNA (mRNA) expressions) responsible for evoking skeletal muscle hypertrophy following TT+LPWS. This study is novel because it provides a feasible rehabilitation intervention by combining two approaches, which are likely to improve the quality of life in persons with SCI with LMN denervation. If proven successful, the intervention will be translated into clinical practice for persons with SCI. 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.

METHODS

Study design

The entire study design and timeline is presented in figure 1. Twenty-four participants will be randomly assigned to either 1 year of TT+LPWS group (n=12) or a TT+standard NMES control group (n=12). Testosterone transdermal patches (Tp; 4–8 mg/day) will be replaced daily on alternating skin sites at bedtime for 1 year. Participants will be block randomised 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 (TT+LPWS and TT+standard NMES) will undergo 1 year of supervised bilateral (by alternating one leg at a time) progressive RT, twice weekly, using ankle weights. For the TT+standard NMES group, supervised training at home will be performed using our established telehealth programme. This programme has proven to be successful in administering electrically evoked RT in persons with motor complete SCI.

A Stimulette Den 2x stimulator (Schuhfried, Vienna, Austria and approved only for research use in our site) is used to perform the exercise training session.[25] A detailed information about the stimulator can be found in the following link (https://www.anatomicalconcepts.com/stimulette-den2x/). The progression of the stimulation parameters will be set as shown in tables 1 and 2. Each session consists of 4 sets of 10 repetitions and will last for 40–50 min. Both legs will be alternatively trained starting with the right leg and then followed with the left leg. This approach was adopted to avoid possible muscle fatigue (see table 2) that may result from electrically stimulating the denervated knee extensor muscle groups. Once the participant completes 40 repetitions of knee extension, ankle weights will be gradually increased by 2 lbs (0.907 kg) (see table 2 for details). All training procedures will be conducted with the participants sitting in their wheelchairs with enough space to clear their foot off the ground.[9–12 26 27 32] For the control group (TT+standard

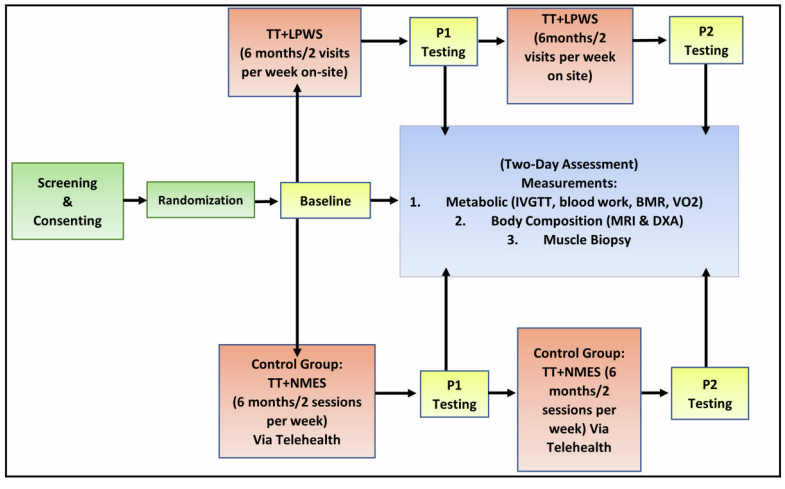

Figure 1 The study timeline (table 4) and procedure are highlighted. After screening and consent, participants will be randomised into one of two testing groups. Each participant will undergo baseline testing before beginning TT+LPWS or control TT+NMES. Each group will be tested for metabolic, body and muscle composition (P1) after a 6-month period. Each group will then complete another 6 months of electrical stimulation exercise training followed by another testing (P2). BMR, basal metabolic rate; DXA, dual-energy X-ray absorptiometry; IVGTT, intravenous glucose tolerance test; LPWS, long pulse width stimulation; NMES, neuromuscular electrical stimulation; TT, testosterone treatment.

Table 1 Example of progression of the stimulation parameters over 1-year period for TT+LPWS group

Months of training	Pulse width (ms)	Interpulse interval (ms)	Frequency (Hz)	Amplitude of current (mA)	Weight (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

LPWS, long pulse width stimulation; reps, repetitions; TT, testosterone treatment.

NMES), 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 VA video connect similar to previous work.[33] The parameters using NMES will be as follows: 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 in persons with SCI with American Spinal Injury Impairment Scale (AIS) classification A or B. However, we cannot rule the possibility of inducing muscle contraction in persons with partial denervation similar to persons with SCI with an AIS classification C. Study visits will be limited to once a month throughout the 1-year period to refill their 30 days stock of TT patches. For TT+LPWS group, participants will be trained under supervision in order to perform their LPWS training sessions.

Planned outcomes and specific aims

Muscle size as measured by MRI

The two-day period is measured at the baseline prior to training and repeated at 6 months (post-intervention (1) and after 1 year (post-intervention (2)). The 2-day assessment includes an estimation of body composition, anthropometry and dual X-ray absorptiometry (DXA), MRI scans will be obtained for thigh skeletal muscles to determine muscle and IMF CSAs.[33–42] The aim of these measurements is to compare the effects of TT+LPWS with TT+standard NMES (control group) on the size of thigh skeletal muscle, IMF and leg lean mass (*specific aim 1*).

Basal metabolic rate and metabolic profile

After obtaining the previous measurements, participants will be escorted to a nearby hotel for dinner, and overnight stay. After an overnight fast, the subject will be gently awakened at 06:00 hours to measure BMR.[26 35] At 06.30 hours, an intravenous line will be placed and blood will be drawn for serum total T and IGF-1 concentrations at 06.30, 07.00 and 07.30 hours.[26] Resting blood pressure and fasting metabolic markers will be obtained including hemoglobin A1c, as well as lipid panels, C reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor (TNF)-α and free fatty acids (FFA). This will be followed by a 3-hour intravenous glucose tolerance test (IVGTT), which will begin at 08:00 hours and terminate at 11:00 hours. The aim of these measurements is to determine the association between the changes in skeletal muscle size, IMF, leg lean mass and the metabolic profile as determined by measuring BMR, lipid panel and carbohydrate profile.[26 27 32] We hypothesise that the increase in lean mass following TT+LPWS will increase BMR and improve both carbohydrate and lipid profiles as well as associated

Table 2 Progression of the current amplitude (mA) of the stimulation parameters, and ankle weights of the right leg from week 1 to week 45 following LPWS protocol in the trial

		Repetitions	1	2	3	4	5	6	7	8	9	10
Week 1												
Pulse width: 200 ms	Set 1	Amp (mA)-set 1	160	160	160	160	160	165	165	165	165	165
Pulse interval: 400 ms	Set 2	Amp (mA)-set 2	170	170	170	170	170	175	175	175	175	175
Frequency: 1.66 Hz	Set 3	Amp (mA)-set 3	180	180	180	180	180	185	185	185	185	185
Ankle weights: 0 lbs	Set 4	Amp (mA)-set 4	190	190	190	190	190	195	195	195	195	195
Week 45												
Pulse width: 35 ms	Set 1	Amp (mA)-set 1	200	172	148	163	165	166	169	182	200	173
Pulse interval: 400 ms	Set 2	Amp (mA)-set 2	191	183	173	180	179	200	170	185	187	187
Frequency: 25 Hz	Set 3	Amp (mA)-set 3	200	177	163	194	200	199	200	200	170	191
Ankle weights: 2 lbs	Set 4	Amp (mA)-set 4	200	173	177	173	196	200	195	178	200	200

The highlighted region represents the amplitude in week 45.
Note the amplitude of the current increased approximately by 19% in week 1 from set 1 to set 4, this reflects the increase in muscle fatigue of the denervated knee extensor muscle group during one training session. This fatigue pattern decreased to only 9% in week 45 (ie, last training bout) following 12 months of training in one of the participants.
LPWS, long pulse width stimulation .

with decrease in FFA and inflammatory biomarkers (*specific aim 2*).

During the IVGTT, a dietitian will meet with each participant individually to instruct on how to 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.[12 26 27 32] 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 3 times during the study to determine their adherence to the diet pattern throughout the study.

Mitochondrial electron transport chain and signalling pathways

The third aim is to investigate the cellular mechanisms responsible for evoking skeletal muscle hypertrophy following TT+LPWS compared with TT+standard NMES.[27] Participants will undergo three muscle biopsies of the right vastus lateralis muscle at baseline, 6 and 12 months postintervention to measure gene and protein expression and perform mitochondrial enzymatic assays.[43 44] 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 anaesthesia (2% lidocaine). The biopsy samples will be frozen in liquid nitrogen and stored at −70°C until further analysis. We hypothesise that the TT+LPWS will upregulate protein synthesis and downregulate protein degradation pathways, gene expression and mitochondrial enzymatic electron transport chain (ETC) activities compared with the TT+standard NMES group.

Screening and consenting

Prior to the 2-day assessment and 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 electromyography (EMG) laboratory 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 VM to determine the presence and intensity of spontaneous muscle fibre 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.

Each participant will provide a written informed consent that was approved by the local institutional review board (IRB) (see online supplemental file 1). 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. If they voice an interest in the study, they will have the written consent form provided. Potential subjects will be allowed sufficient time and opportunity to read the consent alone and have all questions and concerns 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 that they can withdraw at any time with no impact on the care they receive from the VA. They will be allowed to take consent home for additional time prior to making decisions. They can also discuss the study with their primary provider. All signed consents will be scanned into the VA computerised patient record system and attached to a note indicating enrolment in study. In addition to the entered consent form, progress notes of their active participation will also be entered on a weekly basis.

After informed consent, each subject will undergo a complete physical examination by a physiatrist board certified in SCI medicine, including neurological assessment and 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. 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 TT.

Randomisation and allocation

Randomisation will be done at the end of the 2-day assessment period using a random number generator computer program (baseline). Participants will be block randomised based on the degree of denervation (lower or higher than 50% of CMAP of the standard normal femoral nerve values. Standard NMES (30 Hz, 450 µs and amplitude of current (mA) as tolerated) was added for the control group (TT+NMES) with the attempt to blind our participants to study design. Also, it is unclear whether standard NMES as such short pulse duration would have any effects on the denervated muscles (table 3).

Measurements

Body composition assessment

Body mass index

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

Table 3 The use of random number generators to plan randomisation of 24 participants with SCI with LMN injury into either TT+LPWS (n=12) or TT+NMES (n=12; control group) for the entire study

Subject ID	Randomisation	Assignment	Order in the group
1	1	TT+LPWS	1
2	1	TT+LPWS	2
3	0	TT+NMES	1C
4	0	TT+LPWS	2C
5	0	TT+NMES	3C
6	0	TT+NMES	4C
7	1	TT+LPWS	3
8	1	TT+NMES	4
9	1	TT+LPWS	5
10	0	TT+NMES	5C
11	0	TT+NMES	6C
12	1	TT+LPWS	6
13	1	TT+LPWS	7
14	0	TT+NMES	7C
15	0	TT+NMES	8C
16	1	TT+LPWS	8
17	0	TT+NMES	9C
18	1	TT+LPWS	9
19	0	TT+NMES	10C
20	1	TT+LPWS	10
21	1	TT+LPWS	11
22	0	TT+NMES	11C
23	0	TT+NMES	12C
24	1	TT+LPWS	12

In the randomisation column, 1 refers to TT+LPWS and 0 refers to TT+NMES. C refers to control group.
LMN, lower motor neurons; LPWS, long pulse width stimulation; NMES, neuromuscular electrical stimulation; SCI, spinal cord injury; TT, testosterone treatment.

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. Body mass index (kg/m^2) will be calculated as weight (kg) divided by height^2 (m^2).[26 27]

Dual energy X-ray absorptiometry

iDXA will be used to measure body composition including regional and total fat mass (FM), FFM and bone mineral density (BMD). Total body and regional scans (arms, trunk and legs) will be performed using an iDXA scanner (Lunar, Madison, Wisconsin, USA) bone densitometer to determine regional BMD and T-scores for hips and knees.[36 41 42] We will perform testing after lower extremity elevation for at least 20 min to minimise fluid shift. All scans will be performed and analysed 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 min 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 <3%.[42]

Magnetic resonance imaging

MRI will be performed using a 1.5 T magnet (GE).[34–41] The skeletal muscle CSAs will be determined at baseline, 6 months (midintervention) and 1 year after starting the intervention (postintervention). 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 min. Images of both thighs will be collected using the following scanning parameters (repetition time, 500; echo time, 14; field of view, 20 cm; 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 localised coil. Images will be downloaded and analysed using X-vessel software.[12 26 27 32 34]

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. Intravenous line will be placed after overnight fast, serum total T and PSA concentrations will be measured in duplicates.[41 45 46] Samples will be sent for analysis using a standard procedure assays.

Metabolic studies

BMR and respiratory exchange ratio

After an overnight fast for 10–12 hours, participants will be kept in a dark room for 20–30 min to attain a resting state during which BMR will be measured by using a canopy. The gasses (VCO_2 and VO_2) collected will be used to determine the respiratory exchange ratio. This will help to determine the changes in the percentage of substrate utilisation (% fat vs % carbohydrate) after the interventions.[12 26]

Serum total, free testosterone and IGF-1

The plasma T and IGF-1 will be measured in the morning (2 mL/sample).[26 38 41] The analysis of total T will be performed by radioimmunoassay (RIA) after sample extraction and column chromatography. The interassay coefficient of variation (CV) is 12.5% or less for all quality control samples analysed. Plasma IGF-I and insulin growth factor binding protein-3 (IGFBP-3), concentrations will be measured by immunoluminometric assay (Quest Diagnostics, Madison, New Jersey, USA) and RIA (Diagnostics Systems Laboratories, Webster, Texas, USA), 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 (high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, total cholesterol and triglyceride) assessed, with total cholesterol:HDL-C ratios used 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.[26 35 41]

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.[26 47]

Intravenous glucose tolerance test

An IVGTT will be used to determine insulin sensitivity and glucose effectiveness.[26 48] Each subject will undergo an IVGTT test 3 times. After a 10-hour 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 min after the rapid glucose injection (0.3 g/kg intravenously over 30 s 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 RIA 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.[34] The CV 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 min after the glucose bolus.[34] The homeostatic model of assessment of insulin resistance will be calculated and insulin sensitivity will be determined using Matsuda and DeFronzo formula.[49 50]

Muscle biopsies

Protein content

Muscle biopsy samples will be homogenised on ice using the appropriate buffers. Equal amounts of protein will be resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis after which proteins will be electrophoretically transferred to a polyvinylidene fluoride membrane. Western blot analysis will then be performed to determine the protein concentrations as described in preliminary data and previously by our lab.[27 43] After blocking, membranes will be probed with primary antibodies for activated pathways including Adenosine monophosphate kinase (AMPK), p-AMPK PGC-1α (phosphorylated-AMPK peroxisome-proliferator-activated receptor-γ coactivator-1 alpha), IGF-1, Akt, p-Akt, mammalian target of rapamycin (mTOR) and protein degradation pathways (Forkhead box O (FOXO)1/3, atrogin-1, Muscle RING-finger protein-1 (MURF)), followed by incubation with the appropriate secondary antibody. Western blot analysis will be quantified by scanning with a GS-800 densitometer. Optical densities of the western blot analysis will be measured using image-analysis software (Molecular Analyst; Bio-Rad).

Real-time quantitative PCR

Quantification of mRNA levels by RT quantitative PCR (real-time qPCR) 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 homogenised then centrifuged to separate the chloroform and aqueous phases. mRNA will be extracted using commercially available kits. Residual genomic DNA will be removed by the column DNAse I digestion. complementary DNA libraries will be synthesised by reverse transcription of total RNA using commercially available kits. Expression of individual target genes (IGF-1, PGC-1α, 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.[30 43]

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 0.1 M 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.[50 51] Flurometric measurements will be conducted to measure the oxidative and glycolytic enzymes' activities including citrate synthase and succinate dehydrogenase.[27 32 44 50–52]

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Statistical analyses

Means and SD 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 analysis of variance will be used to analyse 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 with the number of subjects for specific aims 2 and 3. A penalised random effects 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.[53] 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 unpenalised 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 be performed with R (V.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 analyse 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 are 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.

Recruitment strategy

The following timeline and research activities (table 4) will be implemented to meet our recruitment goals. Our goal is to recruit, train and test eight participants per year. We plan to have four participants recruited quarterly and we will recruit an additional four participants every 6 months using the following timeline to meet our sample size. Approximately two to three subjects will be screened every other month. They will then be randomised to either group. The goal is to recruit eight participants per year (four participants every 6 months) to finish data collection in 4 years. Table 5 highlights the number of participants currently enrolled, completed or withdrew from the current trial.

DISCUSSION

The current trial will provide a novel rehabilitation strategy, combining TT+LPWS to determine the effects of mitigating muscle loss following LMN denervation. Previously, our group was successful in attempting to combine electrically evoked RT with TT to attenuate cardiometabolic risk factors in persons with SCI.[26] 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.[21 25] The addition of TT may provide

Table 4 Timeline of research activities across the 4-year study duration

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 and supply purchase	→							
Recruitment (n=4 per quarter)	→	→	→	→	→	→		
Screen and enrol subjects	→	→	→	→	→	→		
Data collection		→	→	→	→	→	→	
Progress report		→		→		→		→
Data entry/coding/cleaning		→	→	→	→	→	→	
Data analysis and interpretation		→	→	→	→	→	→	→
Presentation/Publications				→		→		→

Table 5 The number of persons who were recruited, completed or withdrew from the study

Subject ID	TT+LPWS/NMES	Baseline 1	Postintervention	Postintervention 2	Sex	LOI	TSI (years)	AIS	Classification
001	TT+LPWS	C	C	C	M	T9	2	A	Paraplegia
002	TT+LPWS	C	C	X	M	T7	5	C	Paraplegia
003	TT+NMES	C	C	C	M	T11	2	A	Paraplegia
004	TT+LPWS	C	C	X	M	T11	19	A	Paraplegia
005	Withdrew	X	X	X	M	T11	12	A	Paraplegia
006	TT+NMES	C	C	C	M	T11	20	A	Paraplegia
007	Withdrew	X	X	X	M	T10	2.5	B	Paraplegia
008	TT+NMES	C	C	C	M	T10	12	A	Paraplegia
009	TT+LPWS	C	C	C	M	T12	14	A	Paraplegia
010	TT+LPWS	C	C	O	M	T12	6	A	Paraplegia
011	TT+NMES	C	C	O	M	T6	8	A	Paraplegia
012	TT+NMES	C	O	O	M	T11	28	A	Paraplegia

AIS, American Spinal Injury Impairment Scale; C, completed the study; LOI, level of injury; LPWS, long pulse width stimulation; M, male; NMES, neuromuscular electrical stimulation; O, still ongoing; TSI, Time since injury; TT, testosterone treatment; X, withdrew from the study.

additional benefits of increasing lean mass and accelerate the actions of LPWS. Previous research attempts indicate that TT may increase lean mass and BMR in persons with SCI.[28] This is the first clinical attempt to determine the role of TT on muscle size and lean mass in persons with LMN denervation independent of neural structures.

This is a promising combination of physical and pharmacological therapies that are likely to improve body composition and other cellular functions which may improve other cardiometabolic health biomarkers. Several of the mechanisms involved in increasing muscle size, including proteins and gene expression will be studied,[27 30 31 43] 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.

The consequences of LMN denervation on skin, muscle mass and bone mass have been previously highlighted.[17–20 25] Applications of LPWS required the use of two large carbon electrodes soaked with gel and placed inside wet conductive spongy pads and strapped to participant's thigh with Velcro straps. The large carbon electrodes ensure adequate dissipation of the energy density to the target muscles.[25] Following 3 years, we did not report a single incidence following applications of LPWS. We have administered LPWS to persons with T7 SCI with LMN that occurred as result of vascular infraction. LPWS (120–150 ms) has the potential to safely stimulate denervated muscles and to restore muscle size in people with SCI. The previous paradigm has focused on daily activation (5 days per week) of the denervated muscles without applying progressive loading of applying ankle weights. Daily training is not a clinically feasible approach in persons with SCI. However, the downside of conducting LPWS twice weekly is that the current dose may not be sufficient to induce strong potent stimuli to upregulate protein synthesis and evoke muscle hypertrophy similar to what reported in the innervated muscles.[27] Future studies that incorporate telehealth supervision may facilitate increasing the frequency of training to 4× per week for home-based training.[33] Moreover, previous trials did not focus on enhancing the neuromuscular homeostasis by promoting the increase in lean mass independent of LMN denervation. Administering TT may likely increase lean mass and BMR[28] and reduce the catabolic pathways that hinder anabolic profile and muscle hypertrophy in persons with SCI.[26 30]

Considering the small size of this SCI subpopulation and limited access to them, we sought to collect three levels of measurements that address body composition, metabolic profile and cellular adaptations in response to exercise and TT. Historically, changes in body composition are associated with changes in metabolic profile.[34 35] For example, loss in lean mass has been associated with decreases in BMR.[34] Waist circumference, a proxy-index of visceral adiposity, has been linked to altered cardiometabolic profile.[54] Electrically evoked RT with TT resulted in robust muscle hypertrophy and 14% increases in BMR.[26] The use of TT in the current study may result in changes in whole, regional body composition as well as non-stimulated muscles and lead to increase in whole-body lean mass, which subsequently resulted in increasing BMR. Therefore, using DXA to measure body composition assessment is rather an important measurement that may help controlling for whole and regional changes as well as explaining the findings of the current study.[41 42] The cellular mechanisms responsible for these adaptations are not well-studied.[27 31 32 44] Therefore, the cellular mechanisms may provide additional insights on whether denervated muscles responds differently from the paralysed innervated muscles as far as signalling pathways and mitochondrial activities.

Recruitment is by far the largest challenge to complete the current trial. Another concern is the ability to recruit

the suggested sample size and to retain participants to finish the 1-year study; transportation is the greatest impediment to subject compliance for this population. We have been attempting to provide transportation and allow for subjects' personalised schedules. Providing financial reimbursement, social interaction during training sessions and access to the data at the conclusion of the study are additional methods to be used to reduce subject attrition.

COVID-19 is currently another unavoidable challenge[55 56]; it negatively impacts the study's designated time frame for recruitment process. COVID-19 amplified the limitations in terms of finding, recruiting, working with and transporting participants. Most of them are concerned about the 1 year commitment and of coming to the centre on weekly basis, since it increases the risk of being exposed to the virus. However, for safety, the COVID-19 guidelines and precautions are followed like washing hands frequently, wearing a face mask and a face shield, maintaining physical distance and limiting contact with people who may be infected. These are effective ways in preventing the spread of COVID-19, which are adhered and followed at the Hunter Holmes McGuire VA Medical Center.

Few participants might not be qualified for either TT or LPWS protocol. Those with hyperphysiological testosterone level >800 ng/dL are excluded from the study. For example, we had a non-randomised participant who was considered as a screen failure due to his testosterone level of 1724.6 ng/dL. The participant relied on commercially available non-prescribed testosterone boosters to maintain his muscle mass. Another factor is the extensive skin irritation, rashes or itching as result of TT patches applications. Therefore, participants may not be complying with patches applications on a regular basis due to skin discomfort and irritation. However, patch compliance is monitored by counting the number of returned patches per month throughout the course of the trial. Neuropathic pain is one of the factors that kept participants from being compliant with the study protocol of applying LPWS.[57] Participant noted difficulty in tolerating amplitude of the current >100 mA. Despite the neuropathic pain, the LPWS did not aggravate it and participants were able to finish the 12 months of the study. The initial inclusion criteria primarily targeted those with LMN below the level of T10 AIS classification A or B; however, during the course of the trial we have screened several participants either with T6 level of injury (as result of vascular infarction) or with AIS classification C. These participants should be considered based on the limited size of the population with LMN denervation compared with the entire SCI population. However, it may be unlikely that participants with AIS classification C may not tolerate the intensity of the LPWS. Women will not be included in the current study because administering TT is neither appropriate nor safe. 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 years will be excluded.

Dissemination and implementation plan

The target audiences for dissemination of the results from this study include the Veteran Health Adminstration 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 Paralyzed Veteran Affairs (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. We will target peer-review journals to publish our reports.

Data Safety and Monitoring Board

The Data Safety Monitoring Board (DSMB) will meet annually to review the protocol prior to data collection to evaluate subjects' 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. The report will include the following:

- Evidence of efficacy according to pre-established statistical guidelines.
- 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.

Trial status

It is currently an active trial that has been open for enrolment since 1 July 2018 with anticipated completion date of 30 August 2023. The study was approved by McGuire VA IRB and R&D since 8 May 2018. Recruitment started on 1 July 2018 and will end by 30 June 2023.

Access to data

Data will be available on email communication with the principal investigator following completion of the study and receiving prior approval for data sharing from the appropriate regulatory bodies.

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Contributors All authors contributed to the current work and they were involved in several aspects of the protocol. ASG: design of the study, secured funding, IRB approval, data management; RK: IRB coordination, recruitment, scheduling, training participants, data management; MA: training participants and data management; RG: administering IVGTT and responsible for all analysis of the blood work; JR: responsible for conducting muscle biopsy; LG, TC, TLL: responsible for medically screening patients and determine their eligibility to participate in all aspects of the study; DCi: design of the study and oversee protocol completion; DCa: administering electrodiagnostic testing to confirm denervation; EL: design of the study and responsible for all mitochondria work; CC: design of the study and responsible for all intracellular signalling and mRNA studies; RA: design of the study, prescribe testosterone treatment and monitor testosterone dose throughout the trial.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data will be available on email communication with the principal investigator following completion of the study and receiving prior approval for data sharing from the appropriate regulatory.

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Department of Veterans Affairs

VA RESEARCH CONSENT FORM

Subject Name: _____ **Date:** _____

ICF Template version: 3/8/2016

Title of Research Study: Testosterone and Long Pulse Width Stimulation for Denervated Muscles after Spinal Cord injury
Sponsor: VA Merit Review
Protocol No: N/A
Investigator Name and Address: Ashraf S. Gorgey, MPT, PhD
1201 Broad Rock Blvd
Richmond, VA 23249

1. Whom should I contact for questions? (Contacts)
If you have any questions, concerns or complaints regarding this study, unexpected reactions, or you are injured and become ill as a result of participation in this study please call AM or PM:

	Office	Off Hours
Dr. Ashraf S. Gorgey	**(804) 675-5000 ext. 3386**	**(804) 750-4814**
Dr. Robert A Adler	**(804) 675-5424**	**(804) 659-0281**
Dr. Lance Goetz	**(804) 675-5455**	**(804) 351-3423**
Dr. Teodoro Castillo	**(804) 675-5000 ext. 4582**	**(804) 659-0186**
Dr. Timothy Lavis	**(804) 675-5455**	**(804) 351-0753**
Dr. Jeannie Rivers	**(804) 675-5112**	**(804) 338-1791**
Dr. Ranjodh Gill	**(804) 675-5424**	**(804) 539-7420**

If you are unable to reach any of the study staff listed and need immediate medical assistance, please call the VAMC hospital operator at 800-784-8381 and ask for the Emergency Room physician to obtain advice, or call the **Emergency Room directly at (804)-675-5527.** If you have any questions, concerns or complaints about your rights as a research subject you may contact the **McGuire Institutional Review Board (IRB) at (804) 675-5676.** The IRB is responsible for reviewing research in humans and making sure that their safety and rights are protected.

MIRB APPROVED
2/28/19

2. What is this research study about? (Introduction)

You are being asked to volunteer for this research study because you are a person with a spinal cord injury (SCI). This study involves research to determine the effectiveness of testosterone replacement therapy (TRT) and long pulse width stimulation (LPWS) neuromuscular electrical stimulation (NMES) vs. testosterone replacement therapy (TRT) and standard NMES on your muscles and metabolism. LPWS and NMES use electrical shocks to exercise your paralyzed muscles. The difference is that the LPWS is electrical pulses penetrate deeper into the muscles.

If you participate, you will be randomly assigned (like a flip of a coin) to receive TRT+LPWS or TRT+NMES for a one year period. There will be 12 subjects in each group. During study visits, both groups will exercise the knee muscles in the sitting position. A series of tests/procedures described below will be done at the beginning of your participation, after 6 months and the end of 1 year.

The expected duration of your participation is ONE YEAR (3 weeks of testing + 49 weeks of training, twice weekly).

3. What is expected of me? (Procedures)

If you agree to participate and sign this consent form the following study procedures will be done.

A. Measurements

Day 1

- You will be asked to undergo a complete physical examination including rectal exam, (One time- 30 to 45 minutes). Your blood pressure, heart rate, an electrocardiogram (EKG, heart tracing) and electromyogram (EMG) will be done. The EMG measures muscle response to nerve stimulation of the muscle. A needle will be inserted into the knee muscle group to record muscle activity. Depending on the results of these tests the study doctor will discuss with you if you are eligible to continue in this study.

- If you are eligible to continue, your weight, height, waist and abdominal measurements will be taken. Your body fat, muscle and bone mass will be measured using x-rays (DXA) while lying on a table. The measurements will be performed 3 times. Each scan takes 20 minutes.

-Magnetic Resonance Imaging (MRI) scans will be obtained to measure abdominal fat and lower leg muscles size. MRI uses strong magnets to make detailed pictures of your body. This procedure involves lying still on a table during the scanning period. The measurements will be performed 3 times. Each MRI scan takes 45 minutes.

- You will lodge in room 1V-130 at the Spinal Cord Injury and Disorders Department (SCI &D) for dinner, and will remain overnight.

Day 2

- At 6 am, you will be awakened to measure your basal metabolic rate (BMR). The BMR test is performed to determine how much oxygen your body uses at rest. This measurement requires that a large clear plastic dome be placed over your head and the air you breathe will be measured. The dome placed over your head will provide you with plenty of air. You will be instructed to remain awake, but quiet and still, during this testing procedure which will take approximately 45 minutes. Resting blood pressure will be obtained.

- Two IV lines (small plastic tubes) will then be placed in your arms, and blood samples will be drawn at 6:30, 7:00 and 7:30 am.

- This will be followed by a 3-hour intravenous glucose tolerance test (IVGTT). An IVGTT examines your sugar tolerance and how your body uses insulin. Glucose and insulin will be injected into one IV-line Blood samples will be drawn from the other IV line. Three blood samples will be obtained. Glucose will be injected in to your vein over 20 seconds at the start of the test. Blood samples will be taken at multiple times between minutes 3 and 180 of the test. Twenty minutes into the test, a small dose of insulin will be injected into your arm vein. The total amount of blood drawn during the entire study is about 12 tablespoons.

- During the 3 hour-IVGTT, a dietician will meet with you to ensure that you will follow a standard diet during the study. You will be asked to maintain a 3 day food record during the course of the study. The forms will be evaluated weekly by the dietitian to provide monthly feedback. You will be asked to meet with the dietitian two times during the course of the study (baseline and week 12) to make sure you follow the diet throughout the study.

- Immediately after the IVGTT three small muscle biopsy samples will be taken from the knee muscle group to determine the effects of exercise. A numbing medication will be injected at the biopsy sites and a 1/4 inch skin incision will be made with a small surgical scalpel. A special biopsy needle will be inserted through the skin incision and into the muscle and a small amount of muscle (3-4 pieces) will be collected, after which the site will be closed and a pressure dressing applied for at least 10 minutes.

-All of these tests, including muscle biopsy, will be done three times, before the study, 6 months and after 1 year of completing the study.

-You will be asked to complete a questionnaire about the level of your physical activity at the beginning, middle, and the end of the study. This will take approximately 5-10 minutes.

Randomization:

You will be assigned by chance (like the flip of a coin) to one of two groups **Group 1 TRT + LPWS** or **Group 2 the TRT + NMES.**

B. <u>Interventions for one year (TRT+LPWS vs TRT+NMES)</u>

- If you have been assigned to the TRT+LPWS group or the TRT+NMES (control group), you will receive one year of electrical leg shock with ankle weights that will be done while sitting in your wheelchair. These sessions will be done twice a week at the study site for a full year. Two adhesive patches will be placed on the skin over the knee muscle group. For the TRT+LPWS, we will explore the best stimulation parameters necessary to evoke twitches of the knee muscle.

- For the TRT+NMES group*, electrical current from the stimulator will be slowly increased in 5-second intervals to cause full leg extension. Once full knee extension is achieved in a sitting position, an extra 2 lbs of weight will be added on a weekly basis. Each session will be consisted of 4 sets of 10 knee extensions and it will last for 30-40 minutes. Training will be alternated between right and left legs.

  * If you are randomized in the TRT+NMES group (control group), you have the option to exercise at home using Neuromuscular Electrical Stimulation (NMES) accompanied with ankle weights and Telehealth communication (a service offered by the Department of Veterans Health Administration).

  *Telehealth communication will be provided by video conference. If you are not enrolled to participate in VA Telehealth activities then a form will be completed to register and provide permission before you may participate. Registration with SCI Home Telehealth is typically completed with the McGuire SCI Home Telehealth Coordinator, Melodie Anderson, MSN RN.

  * You will be provided a portable neuromuscular unit as well as ankle weights. You will be trained on how to use the neuromuscular unit and apply the ankle weights by the principal investigator. At the end of the one year intervention, you will return the neuromuscular unit and the ankle weights.

  * You will need to provide your own working computer with webcam, microphone, and speakers (headphones or earphones).

- Both groups (TRT+LPWS or TRT+NMES) will be asked to place a testosterone (4mg/day-8 mg/day) patch on clean dry skin of your shoulder to wear at all times. The patch will be changed once a day before bedtime on the right or the left shoulder over the course of the one year. The blood testosterone level will be measured every 4 weeks and the dose will be adjusted if necessary. You will also be asked to report to the SCI Exercise Physiology laboratory at the end of every 4

weeks and return the empty testosterone patch packages so we can determine if you are using the patches as instructed.

4. Will the research benefit me? (Benefits)
It is possible that you may receive no benefit from participating in this study. Information from this study may help others in the future.

5. What are my alternatives to being a research subject? (Alternative Therapy)
You do not have to participate in this study to receive treatment for your condition. Your alternative is to decline participation in the study.

6. What are my risks? (Risks, Inconveniences, Discomforts)
Participation in this study may involve risks that are unknown at this time. Your condition may stay the same, may improve or may worsen from study participation.

All drugs have the potential to cause allergic reactions including the drugs used in this study. Allergic reactions may be mild to severe, and include the following symptoms: chills, fever, skin rash, hives, itching, watery eyes, swelling, headache, difficulty breathing, difficulty swallowing, severely low blood pressure, organ failure, and death. Serious allergic reactions require immediate medical attention.

- **Anthropometrics**
  - Bruising, discomfort. This occasionally occurs.
- **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. This occasionally occurs.
- **IV line failure**
  - Discomfort, swelling, redness over the IV line site causing failure of the IV. Another IV will need to be placed in another part of the arm. This occasionally occurs.
- **Insulin Sensitivity Tests**
  - Hypoglycemia (low blood sugar) with occasional dizziness, sweating, and nausea. This occasionally occurs. Seizures, coma, or death, is unlikely to occur.
- **Basic Metabolic Rate**
  - Anxiety, apnea (difficulty breathing), and claustrophobia. This occasionally occurs.

- **Autonomic Dysreflexia**
  - Slow heart rate, high blood pressure, headache flushing and sweating. This is unlikely to occur, but can be a life threatening condition.

- **DXA**

  Fall during transfer. This is unlikely to occur. This research study requires you to have 3 DEXA scans which involves exposure to radiation in the form of X-rays. This radiation exposure is not necessary for your medical care and is for research purposes only. All radiation increases the risk of developing cancer in the future. The total amount of radiation that you will receive in this study is equal to about 9 extra days of exposure from natural background radiation. The McGuire VA Medical Center Radiation Safety Committee has reviewed the use of radiation in this research study and has approved this use as involving acceptable risk and necessary to obtain the research information desired. Please tell your doctor if you have taken part in other research studies or received any other medical care recently involving radiation.

- **MRI**
  - Anxiety, dizziness, and claustrophobia. This occasionally occurs.

- **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. This is uncommon.

- **Resistance training and LPWS electrical stimulation**
  - 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.
  - Fracture.
  - Autonomic dysreflexia (slow heart rate, high blood pressure, headache flushing & sweating) which may be life threatening.
  - Pressure ulcers.
  - Fainting, heart attacks or death.
  - Chemical burns to the skin.
  - These are unlikely to occur.

- **Testosterone Replacement Therapy**
  - Serious reactions:
    - Severe rash at site of the patches, worsening heart failure that may cause shortness of breath and, 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:
  - Skin irritation, back pain, enlarged prostate, headache, irritations of the skin, 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.
- This occasionally occurs.
- In case of skin irritation, steroid cream may be used and will be provided. Side effects of steroid cream include thinning of skin, increase in number and size of small blood vessels under the skin, increase risk of skin infection, and change in skin coloration.

➢ **EMG**
- Localized swelling, soreness, bruising, chance of infection, bleeding, pain.

7. Will I get paid? (Compensation)
You will receive $3,000 for your participation in this study ($500 every two months) until the end of the one year duration of the study. The compensation offsets for transportation costs and participation in the study. If you are in the TRT+NMES group and you have chosen to use Telehealth option, you will receive $1200 for your participation in the study ($200 every two months).

If you receive payments from the Department of Veterans Affairs they will be reported to the IRS along with your social security number.

8. Will I have to pay? (Cost of Participation)
You will not have to pay for care received as a subject in a VA research project regardless of whether you are a Veteran or a non-Veteran. If you get a bill for research services contact your study doctor or research nurse. Some Veterans are required to pay co-payments for medical care and services provided by the VA. These co-payment requirements will continue to apply to medical care and services provided by the VA that are not part of the study.

There is no guarantee that the medicines you will receive during this study will be continued after the study is completed. If you are a Veteran and are eligible for care you may continue to receive the same medicine after the study only if the medicine is routinely available at McGuire VAMC and your physician decides that it is the most appropriate treatment.

9. Does pregnancy prevent me from participating? (Pregnancy)
Women will not be eligible to participate because of the unknown risks that involve using Testosterone patches.

10. What if I get injured? (Research Related Injury)
In the event of injury resulting from your participation in this research study, McGuire Veterans Affairs Medical Center may or may not provide compensation, depending on applicable federal regulations. A research injury is any injury or illness caused by your participation in the study. In the event of a research injury, necessary medical treatment will be provided to assist your recovery from the injury. For research related injury, the VA must provide necessary medical treatment regardless of whether you are a Veteran or a non-Veteran.

This agreement to provide medical treatment does not include treatment for injury/illness that is not a result of the study. To help avoid injury, it is very important to follow all study directions. You are not giving up any of your legal rights by signing this form.

11. Who Will See My Information? (Confidentiality)
The confidentiality of your research records will be maintained according to professional standards of confidentiality and VA regulations. Records identifying you may be reviewed by the members of the research team, the Research and Development Committee and its sub-committees, accrediting agencies, officials from the Veterans Health Administration, the Office of Research Oversight, the VA Office of the Inspector General, Richmond VAMC, and other federal oversight agencies such as the Food and Drug Administration, Office for Human Research Protections, or as required by law. All subjects will be identified by an assigned number and their initials. Subjects' research charts will be kept inside a locked file cabinet in a locked office. Only study staff will have access to your study records and medical information.

The ways your study doctor will use your study-related health information and the people who may receive it are identified in a separate form entitled, Authorization for Use & Release of Individual Identifiable Health Information for Veterans Health Administration Research. You will be asked to sign that form to show that you give permission for these uses and sharing of your information. You do not have to sign the authorization form. However, if you do not sign, you will not be able to participate in the study.

A description of this clinical trial will be available on http://www.clinicaltrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Information published or presented about the results of this study will not identify you.

If you are a non-Veteran receiving care as part of this study, you will have an electronic VAMC medical record created for you. You will also be given a VA Notice of Privacy Practices.

12. Do I have to participate in this study or can I withdraw from the study? (Voluntary Participation and Withdrawal)

Participation in this study is voluntary and you may refuse to participate without penalty or loss of benefits to which you are otherwise entitled. The study staff will answer any questions you may have about the study. You are free to withdraw your consent and stop participation at any time. If you decide to withdraw from this study, you should contact *Dr. Ashraf S. Gorgey, MPT, PhD* to discuss termination of your participation. It is important that you do this so that *Dr. Gorgey* can withdraw you safely. Stopping will in no way change the quality of care you receive now or in the future at this institution or your right to participate in other studies.

Any significant new findings that develop during the research study that may affect your decision to continue participating will be provided to you as soon as possible.

Your participation in this research study may be ended without your consent for the following reasons:

- If the study doctor believes, for any reason, that it is within your best interest.
- If you develop side effects that are considered dangerous.
- If you refuse to take **Testosterone Patches** or fail to return for follow-up as recommended by your study doctor or fail to follow the study doctor's instructions.
- If you refuse to have tests that are needed to determine whether **Testosterone Patches** are safe and effective.
- If you require treatment with drugs that are not allowed in this study.
- If other causes prevent continuation of the clinical research study.
- **VA Merit Review,** FDA, McGuire IRB may also end the study at any time.

13. Date of Consent Form Revision, 4/20/2018, 2/22/2019

Subject Name:_____ Date:_____

Research Study Title: **Testosterone and Long Pulse Width Stimulation for Denervated Muscles after Spinal Cord injury**

Principal Investigator**: Ashraf S. Gorgey, MPT, PhD** VAMC: **Richmond**

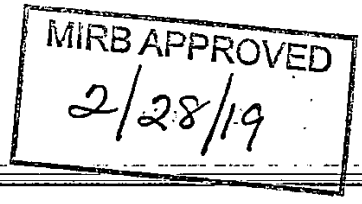
RESEARCH SUBJECTS' RIGHTS: I have read or have had read to me all of the above.

Dr. **Gorgey** (or an associate) has explained the study to me and answered all of my questions. I have been told of the risks or discomforts and possible benefits of the study. I have been told of other choices of treatment available to me.

I understand that I do not have to take part in this study, and my refusal to participate will involve no penalty or loss of rights to which I am entitled. I may withdraw from this study at any time without penalty or loss of VA or other benefits to which I am entitled. The results of this study may be published, but my records will not be revealed unless required by law. By signing below, I am agreeing to participate in this research study. I will receive a signed copy of this consent form.

_____ _____
Subject's Signature Date

_____ _____
Signature of Person Obtaining Informed Consent Date



Department of Veterans Affairs

INVESTIGATIONAL DRUG INFORMATION RECORD

1. TITLE OF STUDY
Testosterone and Long Pulse Width Stimulation for Denervated Muscles after Spinal Cord Injury

2. RESPONSIBLE INVESTIGATOR *(Individual who signed Form FD-1572)*
Ashraf S. Gorgey, MPT, PhD

3. PRINCIPAL INVESTIGATOR *(If different than responsible investigator)*
Ashraf S. Gorgey, MPT, PhD

4. ALL DESIGNATIONS FOR DRUG *(Generic and chemical, code, trade-names, other designations)*
Andoderm Testosterone

5. MANUFACTURER OR OTHER SPONSOR
Watson Phram Inc.

6. SOURCE OF DRUG *(If other than manufacturer or sponsor)*
Will be purchased from McGuire VAMC Pharmacy by Brent Gregory and reimbursed by Dr. Gorgey's research fund

7. THERAPEUTIC CLASSIFICATION AND EXPECTED THERAPEUTIC EFFECT(S)
Topical Anabolic Steriod

8. DOSAGE FORMS AND STRENGTHS
4 mg/day-8 mg/day transdermal patches will be used in this study

9A. IS THIS DRUG A CONTROLLED SUBSTANCE?
☒ YES ☐ NO *(If "Yes", complete item 9B)*

9B. CLASSIFICATION
Schedule III

10. STABILITY AND STORAGE REQUIREMENTS

A. PRIOR TO MIXING, STORAGE SHOULD BE *(Check applicable box(es))*
☒ AT ROOM TEMPERATURE ☐ IN REFRIGERATOR ☐ IN FREEZER ☐ PROTECTED FROM LIGHT ☐ OTHER(Specify)

B. AFTER MIXING, DRUG REMAINS STABLE IN REFRIGERATOR FOR *(Check appropriate box and enter quantity)*
☐ ____ MINUTES ☐ ____ HOURS ☐ ____ DAYS

11. DRUG ADMINISTRATION PROCEDURES

A. ROUTES OF ADMINISTRATION *(Check appropriate box(es))*
☐ ORAL ☐ I.V. INFUSION
☐ I.V. PUSH ☒ OTHER Topical

B. ADMINISTRATION DIRECTIONS
Rotate Application sites

C. RECONSTITUTION DIRECTIONS
N/A

12A. DRUG ADMINISTERED BY *(Also complete item 12B)*
☒ A. PHYSICIAN ONLY ☐ B. PROFESSIONAL NURSE

12B. ROUTE
Topical

13. USUAL DOSAGE RANGE
4 mg-8 mg per day

14. KNOWN SIDE EFFECTS AND TOXICITIES
See package insert

15A. DOUBLE BLIND?
☐ YES ☒ NO *(If "Yes", complete items 15B and 15C)*

15B. NAME OF INDIVIDUAL WHO HAS CODE DESIGNATION
N/A

15C. TELEPHONE NUMBERS
DAYTIME: N/A
EVENING: N/A

16. SPECIAL PRECAUTIONS *(Include drug interactions (synergisms, antagonisms), contraindications, etc.)*
See package insert

17. ANTIDOTE
See package insert-

18. STATUS *(Check one)*
☐ INVESTIGATIONAL ☐ PHASE II ☒ COMMERCIALLY AVAILABLE
☐ PHASE I ☐ PHASE III ☐ OTHER *(Specify)*

19. NAMES OF AUTHORIZED PRESCRIBERS

A. Robert Adler, MD

B. Lance Geotz, MD; Ranjodah Gill, MD

C. Timohty Lavis, MD

D. Teodoro Castillo, MD

20. SIGNATURE OF RESPONSIBLE OR PRINCIPAL INVESTIGATOR — DATE: 2/22/2019

22. PATIENT IDENTIFICATION *(I.D. plate or give name – last, first, middle)*

21. APPROVED BY

A. SUBCOMMITTEE ON HUMAN STUDIES

21A. SIGNATURE OF CHAIRPERSON — DATE: 2/28/19

B. RESEARCH AND DEVELOPMENT COMMITTEE

21B. SIGNATURE OF CHAIRPERSON — DATE: 2-27-19

VA FORM 10-9012 NOV 1989 — COMPUTERIZED VERSION Revised 9/98