BMJ Open

Effects of Testosterone and Evoked Resistance Exercise after Spinal Cord Injury [TEREX-SCI]: A Randomized Clinical Trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-014125
Article Type:	Protocol
Date Submitted by the Author:	06-Sep-2016
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Primary Subject Heading:	Rehabilitation medicine
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	SPINAL CORD INJURY, TESTOSTERONE, RESISTANCE TRAINING, BODY COMPOSITION and METABOLISM, MITOCHONDRIA, INFLAMMATORY BIOMARKERS

Effects of Testosterone and Evoked Resistance Exercise after Spinal Cord Injury [TEREX-SCI]: study protocol for a randomized controlled trial

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Abstract

Introduction: Individuals with spinal cord injury (SCI) are at a lifelong risk of obesity and chronic metabolic disorders including insulin resistance and dyslipidemia. Within few weeks of injury, there is a significant decline in whole body fat-free mass, particularly lower extremity skeletal muscle mass, and subsequent increase in fat mass (FM). This is accompanied by a decrease in anabolic hormones including testosterone. Testosterone replacement therapy (TRT) has been shown to increase skeletal muscle mass and improve metabolic profiles. Additionally, resistance training (RT) has been shown to increase lean mass and reduce metabolic disturbances in SCI and other clinical populations.

Methods and analysis: Twenty-six individuals with chronic, motor complete SCI between 18-50 years old were randomly assigned to a RT+TRT group (n = 13) or a TRT group (n = 13). Twenty-two participants completed the initial 16-week training phase of the study and 4 participants withdrew. Thirteen participants out of the 22 completed the second 16 week of the detraining phase. The TRT was provided via transdermal testosterone patches (4-6 mg/day). The RT+TRT group had 16 weeks of supervised unilateral progressive RT using surface neuromuscular electrical stimulation with ankle weights. This study will investigate the effects of evoked RT+TRT or TRT alone on body composition (muscle cross sectional area, visceral adipose tissue, %FM) and metabolic profile (glucose and lipid metabolisms) in individuals with motor complete SCI. Findings from this study may help in designing exercise therapies to alleviate the deterioration in body composition after SCI and decrease the incidence of metabolic disorders in this clinical population.

Ethics and Dissemination: The study is currently approved by the McGuire VA Medical Center and Virginia Commonwealth University. All participants read and signed approved consent

forms. Results will be submitted to peer-reviewed journals and presented at national and international conferences.

Trial Registration: NCT01652040

Keywords: RESISTANCE TRAINING, SPINAL CORD INJURY, BODY COMPOSITION, METABOLISM, TESTOSTERONE, MITOCHONDRIA, INFLAMMATORY BIOMARKERS

Strengths and limitations

- The trial will investigate the use of surface neuromuscular electrical stimulation induced resistance training (NMES-RT) to restore muscle size after spinal cord injury (SCI)
- The trial will provide evidence on the effectiveness of testosterone replacement therapy (TRT) to restore muscle size and lean mass and serve as an alternative approach for those who cannot benefit from NMES.
- The trial will determine how restoring muscle size and lean mass by RT+TRT or TRT can benefit the metabolic profile after SCI.
- The study is only limited to men with complete SCI
- Surface NMES may not benefit those with full sensation or peripheral lower motor neuron denervation

Introduction

There are approximately 11,000-12,000 new cases of spinal cord injury (SCI) in the United States annually with an overall prevalence of 250,000-400,000 [1, 2]. Persons with motor complete injury have loss of both sensation and motor function below the level of injury, while incomplete injury is characterized by preserved motor or sensory function. Chronic SCI is associated with dramatic skeletal muscle atrophy [3-5], increase of fat mass (FM) [6-8] and decrease of fat free mass (FFM) [6, 7]. Collectively, these factors put individuals with SCI at risk for metabolic disorders such as type II diabetes and cardiovascular disease.

Previous studies reveal that 60% of individuals with SCI are either overweight or obese [2, 9-11]. Individuals with SCI also have a redistribution of adipose tissue, with greater trunk FM and visceral adipose tissue (VAT) compared to age and waist circumference matched able-bodied controls [12-14]. Adipose tissue, particularly VAT, secretes proinflammatory cytokines including interleukin-6 (IL-6) and tumor-necrosis factor-α (TNF-α). Therefore, the increase in VAT after SCI may contribute to metabolic syndrome by stimulating the hepatic production of C-reactive protein (CRP) which is tied to vascular inflammation [15-18]. Another type of ectopic adipose tissue, intramuscular fat (IMF), is increased after SCI and has been correlated with reduced insulin sensitivity [19, 20].

Metabolic changes also accompany SCI, with previous studies finding that more than 50% of individuals with SCI are glucose intolerant, while one out of five is diabetic [2, 9-11, 21]. Other studies report that 55% of individuals with SCI are at risk of developing metabolic syndrome [22, 23]. Individuals with complete tetraplegia are more likely to experience decreased glucose and carbohydrate tolerance and have a higher prevalence of heart disease than

those with incomplete injuries [24, 25]. Furthermore, those with motor complete injury have lower HDL-C [24].

While previous studies have shown a link between body composition and metabolic profile after SCI, the cellular mechanisms remain unknown. Mitochondria are the site of oxygen consumption and energy production from glucose and lipid metabolism. Unfortunately, mitochondrial function is impaired in a number of diseases including neurodegenerative disease, atherosclerosis, hypertension and cancer [26-29]. Fewer and smaller mitochondria are found in skeletal muscle of insulin resistant, obese and type II diabetic individuals [30]. Previous studies found decreased muscle oxidative capacity and succinate dehydrogenase activity, complex II of the electron transport chain, after SCI [31-33].

One hypothesis is that skeletal muscle mitochondrial function is decreased in metabolic disorders, leading to decreased fatty acid oxidation which contributes to insulin resistance [34, 35]; however, this is controversial [35] . Mitochondria are dynamic organelles and undergo biogenesis, remodeling, and degradation. Mitochondrial biogenesis is driven in part through the action of peroxisome proliferator-activated receptor (PPAR) coactivator 1 alpha (PGC-1α) [36, 37]. In addition, PGC-1a integrates insulin signaling and lipogenesis in skeletal muscle [38, 39]. PGC-1α is decreased in animal models following denervation [40, 41]. Decreased mitochondrial biogenesis and reduced mitochondrial mass may result in decreased energy production and therefore play a significant role in the altered metabolic profile following SCI [42].

Body composition and metabolic changes after SCI may be further exacerbated by reduced anabolic hormones including T, growth hormone and the growth hormone second messenger insulin like growth factor-1 (IGF-1) [43, 44]. Previous studies have shown that 60%

of men with SCI have low T and that TRT increases IGF-1 in men [45-48]. In a rodent model of SCI, TRT attenuates the loss of muscle [49, 50]. TRT decreases total body fat, increases lean mass [51, 52] and increases the number of proliferating skeletal muscle satellite cells in in men [53]. These findings suggest that TRT may provide metabolic benefits to individuals with SCI.

Resistance training (RT) improves insulin sensitivity and increased fatty acid and carbohydrate metabolism as well as attenuates sarcopenia in the elderly and after SCI [54-61]. Moreover, RT has been shown to influence body composition by increasing lean mass, decreasing FM and reducing VAT, suggesting that the benefits of RT could overcome the risk of developing insulin resistance [54-57]. Neuromuscular electrical stimulation (NMES) has been used to evoke RT using ankle weights in individuals with chronic SCI [57, 60]. One study showed a 40% increase in skeletal muscle size and improved glucose tolerance after 12 weeks of training [57].

While there is evidence that loading the paralyzed skeletal muscles results in significant muscle hypertrophy, improvement of body composition and metabolic profile, depression of T after SCI may limit these effects of RT. Thus, in hypogonadal men with SCI, TRT may be an effective therapy to counterbalance the growing rate of obesity, type II diabetes and cardiovascular disease among individuals with SCI. Moreover, TRT may provide an effective intervention for those who cannot effectively benefit from NMES because of denervation. With these considerations in mind, here we report the design of an ongoing study for which the major research goal is to investigate the effects of 16 weeks of evoked RT+TRT vs. TRT on body composition (primary outcome variables; muscle cross sectional area (CSA), VAT, %FM) and metabolic profiles (secondary outcome variables; glucose and lipid metabolism) in individuals with motor complete SCI.

Methods and analysis

Study design

A randomized controlled study will be undertaken in which individuals with SCI will be randomized to receive RT+TRT or TRT alone for 16 weeks.

The study was approved by the McGuire Veteran Affairs Investigation Research Board and the Virginia Commonwealth University (VCU) Office of Research and Innovation. The trial has been registered at clinicaltrials.gov (NCT01652040). A member of the research team will explain the study and obtain written informed consent. After informed consent each subject underwent a detailed physical examination at the Hunter Holmes McGuire VA Medical Center (VAMC) by a physiatrist board certified in SCI medicine. This exam included a neurological assessment, electrocardiogram and American Spinal Cord Injury Association (ASIA) examination.

The study design and procedures are presented in Figure 1. The study visits included estimation of body composition, anthropometry, and dual x-ray absorptiometry (DXA; baselines 1 and 2 and post-interventions 1 and 2). Additionally, MRI scans were obtained for trunk adipose tissue and lower extremity skeletal muscles and IMF CSA (baseline 2, post-interventions 1 and 2). Participants were then escorted to the VCU-CRS unit (VCU Clinical Research Unit) for dinner, and remained in the VCU-CRS unit overnight for the 4 study visits.

Recruitment and Randomization

The recruitment process started in July 2012 and ended in June 2015 and data analysis is currently being performed. Recruitment details and randomization are presented in Table 1 and Figure 2. Prior to the start of the study, numbers 1-26 were randomized using the n-Query software program by the principal investigator. At the end of the two-day assessment period

(Baseline 1), the allocation of subjects into either group was performed in a blinded fashion by drawing a folded paper with a number (1-26) by the research coordinator. This number was matched with the assignment from the randomization procedure.

Twenty-six participants were recruited to participate in the study. A 4 week delayed entry period was included to obtain baseline measurements, stabilize body weight and educate participants on how to monitor their dietary intake. Four participants withdrew from the trial. At baseline 2, two participants failed to comply with study protocol and he withdrew from the study because lack of interest. Nurses failed to locate the veins on the third participant to conduct IVGTT and he was asked to withdraw from the trial. The fourth participant was diagnosed with unhealed grade IV pressure ulcer after being admitted into the trial. Following the delayed entry period, participants were randomly assigned to a RT+TRT group (n = 13) or TRT group (n = 13). TRT patches (4-6 mg/day) were replaced daily on alternating shoulders at bedtime for 16 weeks. The RT+TRT group received 16 weeks of supervised unilateral progressive RT using surface NMES and ankle weights. Following the intervention the two-day assessment period was repeated (Figure 1).

Participants and eligibility criteria

Participants were men between 18-50 years old with a body mass index (BMI) of $\leq$ 30 kg/m^2. The upper limit of age was set to 50 years to avoid unanticipated side effects that may result from TRT applications. Participants had motor complete SCI C5-L2, ASIA A or B. Participants with pre-existing medical conditions were excluded. These included cardiovascular disease, uncontrolled type II diabetes and those on insulin, pressures sores stage 2 or greater, supra-physiological T level, hematocrit above 50% and urinary tract infection or symptoms.

I. Interventions

Resistance training

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 weight was added. Surface NMES was applied to the knee extensor muscles via surface electrodes. One electrode was placed 2–3 cm above the superior aspect of the patella over the vastus medialis muscle, and the other lateral to and 30 cm above the patella over vastus lateralis muscle. Current from the stimulator was manually increased in 5-second intervals to evoke full knee extension with a 3-minute rest between sets, 30 Hz, 450μs pulses and a current sufficient to evoke full knee extension as previously described [62-64]. Four sets of 10 repetitions was performed twice weekly for 16 weeks.

Testosterone replacement therapy (TRT)

Following baseline measurements, T was administered by a patch (Androderm, Watson Pharma. Inc, Parsippany, NJ) that delivered between 4-6 mg/day [51]. Serum T concentration was measured and reviewed in a blinded fashion weekly for the first month and then every 4 weeks. The dose was decreased to 2 mg/day if the serum T concentration was more than 1000 ng/dL (34.7 nmol/L) and the participant was reeducated about use of the patch if the concentration was less than 250 ng/dL (8.7nmol/L) above the pretreatment concentration. Patches were returned after use to ensure adherence to the intervention protocol.

Detraining after 16-week intervention

Six participants from the RT+TRT group continued training once weekly using the same training approach for 16 weeks. Their TRT dose was set at 2 mg/ day. Six participants from the TRT group were followed for additional 16 weeks without intervention. Following the detraining phase, the two-day assessment period was repeated without performing skeletal muscle biopsy (Figure 1). The rationale of the detraining phase is to determine whether once weekly training can maintain skeletal muscle hypertrophy, body composition and metabolic improvements incurred by the 16 week intervention.

II. Primary Outcomes

Data will main confidential at all times and any patient identifiers will be removed prior to data analysis. Analysis for all study procedures will be performed in a blinded fashion ensuring full concealment until complete data analysis.

Anthropometrics and Body Composition Assessments

Height of each participant was determined while lying on their left side in the supine position. Two smooth wooden boards were placed at the participant's head and heels and the distance between them was measured to the nearest cm. Measurement of waist circumference was determined in duplicate by identifying the narrowest region of the trunk from sitting and supine positions. Three-site skin fold assessment was conducted in triplicates for suprailiac, abdominal and thigh.

A Lunar Prodigy Advance (Lunar Inc., Madison, WI) bone densitometer was used to measure total body and regional (lumbar spine, proximal femur, and forearm) FM) and FFM. Testing was performed after lower extremity elevation for at least 30 minutes to minimize fluid

shift. The subject was assisted to lie on a padded table and both legs were strapped proximal to the knees and ankles. The arms and legs were positioned to ensure proper alignment.

Magnetic resonance imaging (MRI)

MRI was performed at the VAMC Hospital using a 1.5 Tesla magnet (GE) as previously described [4, 19, 57, 65, 66]. Transaxial images, 10 mm thick and 10 mm apart, were taken from the hip joint to the knee joint and from knee to the ankle using the whole body coil. The location of the scan was identified by placing a mark 6 inches proximal to and distal to the patella and matched on follow up scans. To analyze VAT and subcutaneous adipose tissue (SAT) transverse slices (0.8 cm thickness) were acquired every 0.4 cm gap from the xyphoid process to the femoral heads. Images were acquired in series of two stacks with L4-L5 used as a separating point.

Analyses will be performed using commercial available software (X-vessel) as previously described [4, 19, 65, 66]. Briefly, the thigh and leg images will be segmented into fat (high intensity), skeletal muscle (mid intensity) and background/bone (low intensity). Manual selection of a pixel of skeletal muscle will highlight all skeletal muscle pixels and provide the total number of skeletal muscle pixels while excluding fat. VAT and SAT will be measured by manually tracing around the anatomical borders. The number of pixels in the highlighted region will be multiplied by the matrix size to measure VAT and SAT CSA (cm^2).

Skeletal muscle torque and specific tension

Torque and muscle CSA of the knee extensor muscle group was evaluated using a Biodex isokinetic dynamometer (Shirely, NY). Measurements were done 72 hours after the muscle biopsy to prevent acute effects. Participants were seated with both the trunk-thigh angle and the knee-thigh angle at 90º. Each participant was securely strapped to the test chair by a crossover

shoulder harnesses and a belt across the hip joint. The axis of the dynamometer was aligned to the anatomical knee axis and the lever arm was attached 2-3 cm above the lateral malleolus. Before measuring isometric torque, passive tension of the right knee extensor muscle group was measured at 5, 30, 60, 90,180, 270 degrees/sec as an index of spasticity. Isometric torque was measured using current amplitude of 50 and 100 mA at a frequency of 30 Hz and pulse duration 450 μs. Isokinetic torque was measured at 60, 90 and 180 degrees/sec using the same stimulation protocol.

Serum and plasma analysis

All metabolic profile analysis is presented in Table 2. Blood samples were collected after an overnight fast. Total T was measured by liquid chromatography with isotope dilution mass spectrometry detection after supported liquid extraction. Free T concentration was calculated using sex hormone binding globulin and albumin concentrations (www.issam.ch/freetesto.htm) [67]. Serum IGF-I concentration will be measured by an immunoluminometric assay (Quest Diagnostics, Madison, NJ). Fasting lipid profiles (HDL-C, LDL-C, total cholesterol, and triglycerides) will be determined as previously described [8, 20]. Inflammatory biomarkers CRP, IL-6, TNF-α , and free fatty acids will be determined by commercially available enzyme-linked immunosorbent assay kits (ALPCO; Salem, NH).

Basal metabolic rate

After an overnight fast for 10-12 hours, participants were kept in a dark room for 20-30 minutes to attain a resting state during which basal metabolic rate was measured as previously described [8]. Briefly, while in a supine position a canopy was placed over the subject's head. Each subject was allowed 2-3 minutes before starting the test no ensure no signs of apnea or claustrophobic episodes. All subjects were instructed to stay awake during the entire test and to

breathe normally. The canopy was then attached to a vacuum to draw the expired gases to the flowmeter of the metabolic unit (COSMED KB42). Prior to the test, the metabolic unit was calibrated using the standard procedures identified by the manufacturer. Carbon dioxide and oxygen output was used to calculate the respiratory exchange ratio and basal metabolic rate (kcal/day) was calculated using the average of the last 15 minutes of the test. This was used to measure the percentage of substrate utilization (% fat vs. % carbohydrate) [8, 68].

To determine whether NMES-RT improves exercise performance, testing was performed using a functional electrical stimulation bike (Restorative Therapies, RTI-300) against progressive resistance protocol until fatigue. The protocol started with 3 minutes resting with the participant attached to the bike, 3 minute warm-up (35-37 RPM) using the servomotor and then two minute incremental progressive resistance protocol (1 Nm, 3 Nm, 5 Nm) until fatigue. After fatigue, a one minute cool down period was allowed followed by 5 minutes of rest. Energy expenditure and cardiovascular performance [VO_2 (l/min), blood pressure and hear rate] was collected at baseline 2 and post-interventions 1 and 2.

Each participant met with a dietician at the start of the study and was asked to maintain a 5 day food dietary log monitoring their caloric intake for the duration of the study. Participants were instructed to record all liquid and food consumption and no nutritional advice was given on the size or the portion of the food. Dietary logs were analyzed on a weekly basis using a nutritional software package (Nutrition Data System for Research version 2014) under the supervision of a registered dietitian. After analysis was completed, the average caloric intake (kcal) and percentage macronutrients (carbohydrates, fat and protein) were calculated. Each participant received monthly feedback via phone call with the registered dietician on how to maintain appropriate dietary habits based on his basal metabolic rate and percentage

macronutrients (45% carbohydrates, 30% fat and 25%). Every effort was made to balance the dietary habits between the RT+TRT and the TRT group.

Intravenous Glucose Tolerance Test (IVGTT)

An IVGTT was used to determine insulin sensitivity and glucose effectiveness before training and 48 hours after the last exercise bout. After an overnight fast, an intravenous line was placed to facilitate infusion of glucose and blood sampling. Blood samples were taken every 2-3 minutes before and 30 minutes after glucose injection (0.3 gm/kg IV over 30 seconds), followed by 5-10 minutes sampling ending at 180 minutes. Twenty minutes after the glucose injection a bolus of insulin (0.02 U/kg) was injected to determine insulin sensitivity. Plasma glucose was measured by the Autoanalyzer glucose oxidase method and plasma insulin concentrations were determined by commercial radioimmunoassay (Table 2). The glucose disposal rate per unit of secreted insulin per unit time and glucose mediated glucose disposal rate were calculated from a least-squares fitting of the temporal pattern of glucose and insulin throughout the IVGTT using the MINMOD program [69]. A representative analysis of IVGTT is presented in Figure 3. The acute insulin response to IV glucose was calculated as the mean rise in plasma insulin above baseline at 3, 4 and 5 minutes after IV glucose administration. KG, a measure of glucose tolerance, was calculated as the least square slope of the natural log of absolute glucose concentration between 5 and 20 minutes after the glucose bolus [70]. The homeostatic model of assessment of insulin resistance (HOMA-IR) was calculated and insulin sensitivity was determined using Matsuda and Defronzo formula [70, 71].

III. Secondary Outcomes

Skeletal muscle biopsy

Biopsy samples of vastus lateralis muscle (~100 mg wet weight total) were obtained by a 14 gauge tru-cut biopsy needle, before and 72 hours after the interventions. The biopsy samples were quickly frozen in liquid nitrogen and stored at -80°C until further analysis. One sample was split into two halves and used for measuring activities of mitochondrial enzymes. The second sample was used for Western blot analysis. The third sample was used for immunohistochemistry.

Mitochondrial Electron Transport Chain activities

Electron transport chain enzyme activities were measured spectrophotometrically in skeletal muscle homogenates as previously described [72]. Rotenone-sensitive NADH cytochrome c reductase measured complexes I and III. Decylubiquinol-cytochrome c oxidoreductase was measured as the antimycin-sensitive reductase to assess complex III. Citrate synthase was measured as an estimate of mitochondrial mass as previously described [72].

Protein content

Proteins were resolved by SDS-PAGE then transferred to a PVDF membrane (Trans-blot; Bio-Rad). Equal protein loading was confirmed with a Ponceau S stain after the transfer. After blocking for one hour, membranes were incubated overnight at 4 ˚C with primary antibody diluted 1:1000. Primary antibodies included glucose transpoter-4, focal adhesion kinase, PGC-1α (Santa Cruz Biotechnology), total protein kinase B (AKT), phosphorylated AKT, total mammalian target of rapamycin (mTOR), phosphorylated mTOR, (Cell Signaling). Membranes were rinsed and placed in a 1:2000 solution of a horseradish peroxidase-conjugated secondary antibody (Cell Signaling) for 1 hour at room temperature. Membranes were then rinsed and

incubated with a horseradish peroxidase chemiluminescence detection reagent (GE Healthcare) for 5 minutes. Proteins were visualized using an Amersham Imager 600 (GE Healthcare). Optical densities were measured using iQuant software and all samples were normalized to the baseline values for that participant.

Histological Analysis

Immediately after muscle biopsy, samples were mounted on tongue blades by using a medium of OCT compound and tragacanth gum and stored at -70°C until analysis. Serial cross sections (8-10 μM) will be collected on glass slides and frozen at -20°C until analysis. Fiber type and CSA will be determined by histochemical staining for myosin ATPase (preincubation at pH 4.3 or 9.4) as previously described [73]. Type I fibers will be identified by dark staining after acid preincubation, type II fibers light staining, and type IIB intermediate. At pH 9.4 the staining pattern was the opposite. Haematoxylin & Eosin (H&E) staining will be performed according to conventional histological procedures. Mitochondrial complex II and IV activity will be estimated by the activity of succinate dehydrogenase and cytochrome c oxidase activity, respectively, as previously described [74, 75]. Stained muscle sections will be observed using an Olympus BX-51 fluorescent microscope (Olympus, Tokyo, Japan) and analyzed using ImageJ software.

Statistical Analyses

Paired t-tests will be used to determine differences in body composition and metabolic profile between baseline 1 and baseline 2. To determine the effect of interventions and detraining, a repeated measures ANOVA will be used. Simple linear and multivariate regression analyses were used to examine the relationship between body composition and metabolic profile variables. For protein expression, we will use paired t-tests to examine the effects of each intervention. The effect size is calculated based on the effects of RT on body composition and

metabolic profiles that were previously published [62]. The number of subjects necessary to find statistical differences in the major variables (muscle size, VAT and insulin concentration) of this study was found to be 10 participants per group. Thirteen subjects per group would provide greater than 80% power to detect a change of this magnitude using a two-sample t-test with an alpha level of 0.05. Even if three subjects were to drop out, ten subjects per group would still have 80% power to detect a change in muscle size. One participant from the RT+TRT group withdrew after developing side effects to TRT patches and the medical monitor personnel recommended to withdraw from the trial in week 8. However, we will perform intent to treat analysis on his data. We anticipate that we will collect sufficient data to determine the effects of rehabilitation interventions on protein expression, mitochondrial enzymatic and ETC activities in individuals with SCI. Statistical analysis will be performed using SPSS version 23.0 (Chicago, IL) with a level of significance set at $p<0.05$.

Ethics and Dissemination

Ethical approval has been obtained from the institutional review boards at the McGuire VA Medical Center and Virginia Commonwealth University. All participants read and signed approved consent forms prior to baseline assessment. Results of the study will be published in peer-reviewed journal and presented at national and international conferences.

Data Monitoring

The research staff will oversee and monitor the study to ensure data quality and participant compliance. Adverse events, regardless of severity, will be reported to the McGuire institutional review board. Only members of the research team will have access to data.

Discussion

Individuals with SCI experience profound skeletal muscle atrophy, deterioration in body composition and abnormal metabolic profile. Within few weeks of injury, there is a significant decrease in whole body FFM, particularly lower extremity skeletal muscle mass, and subsequent increase in FM. These changes predispose this population to the risk of glucose intolerance, insulin resistance, dyslipidemia and the development of type II diabetes and cardiovascular disease. The main purpose of this study is to investigate the effects of 16 weeks of evoked RT+TRT or TRT alone on body composition (muscle CSA, IMF, VAT, %FM, FFM) and metabolic profile (glucose, lipid and basal metabolic rate) in individuals with motor complete SCI.

Ectopic adipose tissue accumulation, IMF and VAT, has been strongly associated with altered metabolic profile after SCI [19, 20]. IMF has been determined to account for a 70% reduction in glucose tolerance in individuals with complete SCI [19]. VAT is independently associated with impairment in glucose tolerance, insulin resistance and dyslipidemia after SCI [20]. Edwards et al noted significant positive association between VAT and insulin resistance and a negative correlation between VAT:SAT ratio and HDL-C [13]. Increase in VAT is also related to leptin and plasminogen activator inhibitor-1 concentrations [14]. It is possible that increases in FM are associated with inflammatory biomarkers that trigger metabolic syndrome after SCI [22]. Ectopic adipose tissue has been demonstrated to secrete proinflammatory cytokines, including IL-6 and TNF-α. This stimulates hepatic production of CRP which is tied to vascular inflammation [15-19].

RT is an important type of exercise that has been shown to induce positive physiological adaptations such as increasing lean mass and reducing metabolic disorders in other clinical

populations. Previous work suggests that twice weekly NMES-RT can induce favorable body composition and metabolic adaptations. Twelve weeks of NMES-RT has shown to increase thigh muscle CSA by 35-40% as measured by MRI [57]. Moreover, there was a reduction in %leg FM and a trend towards decrease in VAT CSA after 12 weeks of NMES-RT [62]. The favorable adaptations in body composition were associated with decrease in plasma insulin area under the curve and plasma triglycerides [57]. These findings were attributed to an increase in plasma IGF-1. However, the effect of training appears to be limited to the trained muscle and only modestly impacted whole body composition. It is unclear whether a longer RT program greater than 12 weeks would provide additional benefits to individuals with SCI.

TRT has been shown to increase skeletal muscle mass in hypogonadal men, men with chronic illness, and older men [51-53]. Androgen deficiency in men is associated with a loss of FFM and an increase in FM [51]. In epidemiologic studies, men with decreased free T-index had lower appendicular skeletal muscle mass than those with normal T levels [76]. Previous work documented that TRT increases muscle mass with a reciprocal decrease in total body FM [51, 52]. This reciprocal action has been suggested due to a switch from adipogenic toward myogenic differentiation of mesenchymal stem cells [76]. In a randomized controlled double blinded clinical trial, TRT was shown to improve insulin sensitivity, CRP and reduce VAT [52]. Sixty percent of men with SCI have low T level and levels are associated with time since injury [47, 48]. TRT has also been reported to increase IGF-1 and several molecular mechanisms related to the protective pathways have been recently elucidated [45, 46, 49, 77]. Therefore, enhancing the decline in anabolic homeostasis by providing TRT may provide additional benefits as was previously demonstrated by increasing lean mass and resting metabolic trial in individuals with SCI.

In summary, we anticipate that this trial will provide important insights into the body composition and metabolic benefits following 16 weeks of evoked RT+TRT or TRT. If beneficial, this may be a feasible strategy for the rehabilitation of individuals with chronic SCI and increase the health of this and other clinical populations. Additionally, TRT alone may provide an alternative intervention for those who cannot benefit from training using surface NMES, because of lower motor neuron denervation or intolerance to applications of electrical stimulation. The study will also shed light on several molecular pathways that have been suggested to influence both body composition and metabolic profile.

Trial Status: Enrollment into the study started in July 2012 and as of April 2016 all participants have completed the study. Data collection and data analysis are expected to be completed in December 2016. The study is expected to be closed in June 2017.

Contributions: ASG supervised all aspects of the trial including all interventions and measurements and secured funding for the trial procedures. ASG, REK, LCO drafted the manuscript. REK, JS, RK, and LCO contributed to data collection and analysis. RG, TL, TC, DXC, CC, RA, EJL and DRG are research physicians that contributed to patient monitoring and study design. DXC and RA will provide guidance during data analysis and manuscript preparation. All authors affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Competing interests: The authors have no competing interests to declare

Funding: This work was supported by VA-RRD CDA2 to Ashraf S. Gorgey, clinical trial registration number NCT01652040.

Acknowledgements: We would like to thank Rodney Wade, Sakita Sistrun, Liron Segal for helping in the processes of data capturing and analysis as well as the study participants for their time and effort. We would also like to thank Hunter Holmes McGuire Research Institute and Spinal Cord Injury Services and Disorders for providing the environment to conduct clinical human research trials. VCU Clinical Research Service for providing space and staff to perform the blood work procedures. The work is supported by the Department of Veteran Affairs, Veteran Health Administration, Rehabilitation Research and Development Service (B7867-W).

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Table 1. Randomization of individuals with motor complete SCI into either RT+TRT (n=13) or TRT (n =13) using n Query software with a treatment ratio 1:1.

Subject ID	Assignment	Baseline 1	Baseline 2	Post-Intervention 1	Post-Intervention- 2
10001	RT+TRT	C	C	C	C
10002	TRT	C	C	C	C
10003	TRT	C	C	C	C
10004	RT+TRT	C	C	C	C
10005	RT+TRT	C	C	C	C
10006	TRT	C	C	C	X
10007	RT+TRT	C	C	C	C
10008	TRT	C	C	C	C
10009	RT+TRT	C	withdraw	X	X
10010	TRT	NA	NA	NA	NA
10011	RT+TRT	C	withdraw	x	x
10012	TRT	C	C	C	C
10013	TRT	C	C	C	x
10014	RT+TRT	C	C	C	withdraw
10015	TRT	C	C	C	x
10016	TRT	C	C	C	C
10017	RT+TRT	C	C	C	x
10018	TRT	C	C	C	x
10019	RT+TRT	C	C	C	C
10020	RT+TRT	withdraw	x	x	x
10021	RT+TRT	C	C	C	x
10022	TRT	C	C	C	C
10023	RT+TRT	C	C	C	C
10024	TRT	C	withdraw	x	x
10025	RT+TRT	C	C	C	C
10026	RT+TRT	C	C	Withdraw-week 8	x
10027	TRT	C	C	C	x

C: completed; **NA**: not assigned for #10. Baseline 1 was followed by 4 weeks of no intervention for all the participants. Prior to baseline 2, randomization was performed into RT+TRT or TRT groups. Post-intervention 1 (n=22) was conducted following 16 weeks of both interventions. Post-intervention 2 (n= 13) was conducted following 16 weeks of RT+TRT (n=7) or no intervention (n=6).

Table 2. Metabolic health variables measured at baseline 1, baseline 2, post-intervention 1 and post-intervention 2

	Quantity	Special handling	Techniques of Analysis
Insulin and Glucose	2 ml	Potassium oxalate/sodium fluoride tube (Grey Top)	ELISA and biochemistry analyzer
HBA1C		SST	Standard Procedure
Testosterone every 4 weeks	4 ml	SST	Liquid chromatography with isotope dilution mass spectrometry detection
Albumin*		SST	Standard Procedure
SHBG*		SST	Standard Procedure
IGF-1, IGFBP-1 and 3	4 ml	SST	ELISA
Inflammatory biomarkers (CRP, IL-6, TNFα)	4 ml	SST	ELISA
Free fatty acids	2 ml	Potassium oxalate/sodium fluoride tube (Grey Top)	Enzymatic colorimetric quantification
Triglycerides, total cholesterol, HDL, LDL	4 ml	SST	Enzymatic colorimetric quantification

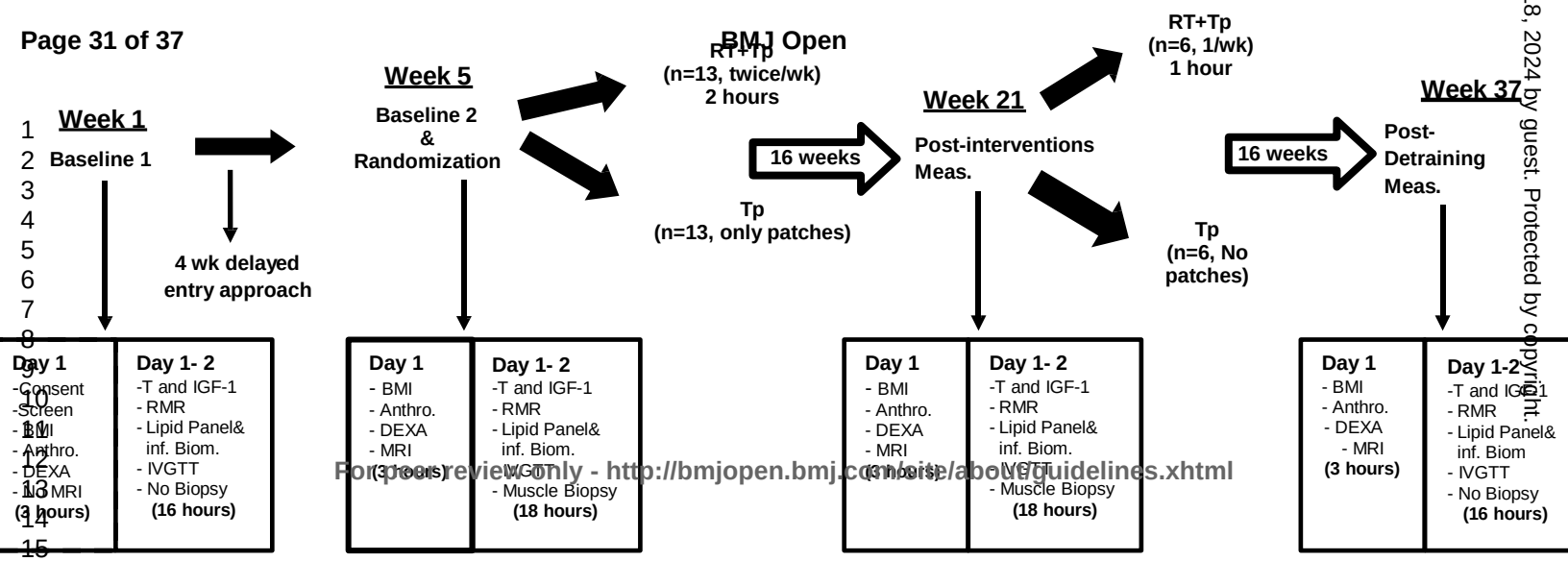
*, Only at baseline 2 and post-intervention 1 to calculate free testosterone. ELISA, enzyme-linked immunosorbent assay; HBA1C, hemoglobin A1c; SHBG, sex hormone binding globulin; SST, serum separator tube; IGF-1, insulin-like growth factor 1; IGF-BP, insulin-like growth factor binding protein; CRP, C-reactive protein; IL-6, interleukin 6; TNFα, tumor necrosis factor alpha

Figure Legends

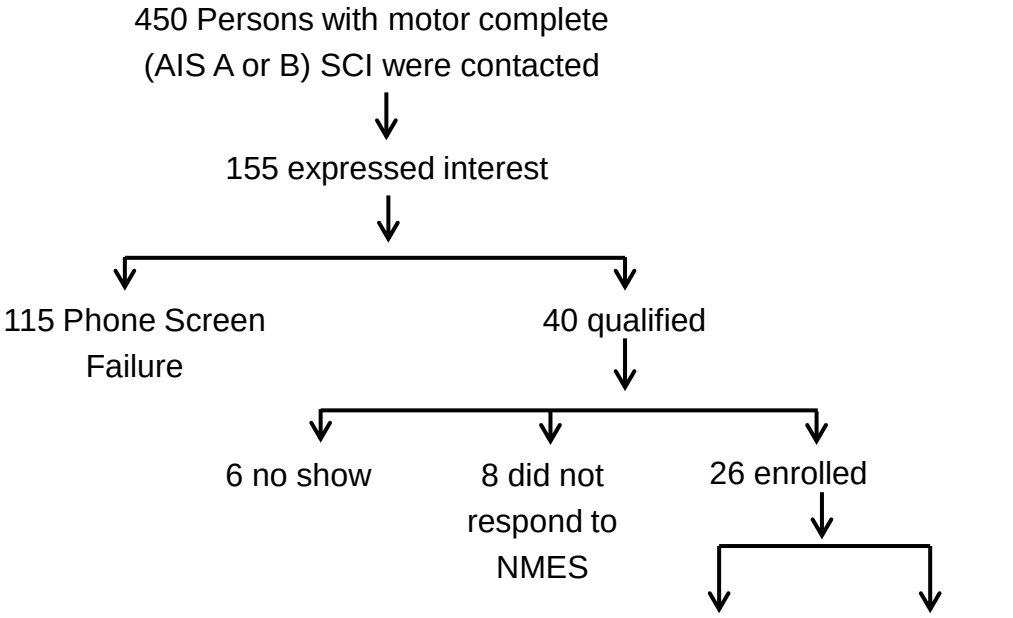
Figure 1. Timeline and main procedures of the TEREX trial for both the RT+TRT and TRT groups during baseline 1, baseline 2, post-intervention 1 and post-intervention 26 for persons with motor complete SCI.

Figure 2. Schematic diagram showing the process of recruitment over the 3 year period of the TEREX trial.

Figure 3. A representative figure of analysis for IVGTT in a person with SCI after infusion of dextrose followed by insulin 20 minutes later. The dextrose concentration is calculated by multiplying 0.3g x body weight (kg) in 50% solution. Insulin concentration is determined by multiplying 0.02 units x body weight (kg). Blue line represents fasting glucose concentration, resting, following infusion, and over 120 minutes. Red line represents the line of best fit of glucose concentration. Green line represents fasting insulin level, spike following 20 minutes of dextrose infusion and over 120 minutes. Black line represents the line of best fit for insulin concentration.



Recruitment



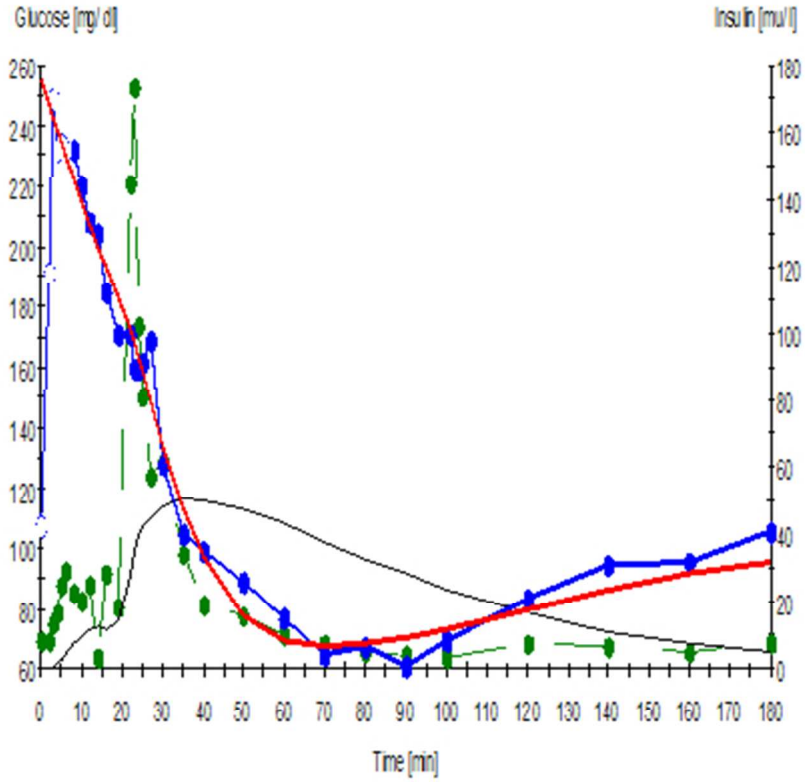


Figure 3. A representative figure of analysis for IVGTT in a person with SCI after infusion of dextrose followed by insulin 20 minutes later. The dextrose concentration is calculated by multiplying 0.3g x body weight (kg) in 50% solution. Insulin concentration is determined by multiplying 0.02 units x body weight (kg). Blue line represents fasting glucose concentration, resting, following infusion, and over 120 minutes. Red line represents the line of best fit of glucose concentration. Green line represents fasting insulin level, spike following 20 minutes of dextrose infusion and over 120 minutes. Black line represents the line of best fit for insulin concentration.

132x126mm (96 x 96 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3, 7
	2b	All items from the World Health Organization Trial Registration Data Set	3, 7
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	20
	5b	Name and contact information for the trial sponsor	21
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
	6b	Explanation for choice of comparators	5-6
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-16
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7, Fig 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	____16-17_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____7-8_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	_____7-8_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_____7-8_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____8_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____9-10_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_____10-16_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	_____17_____

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	16-17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	17
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	3, 7, 17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	3, 17
	31b	Authorship eligibility guidelines and any intended use of professional writers	20
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	attached
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Effects of Testosterone and Evoked Resistance Exercise after Spinal Cord Injury [TEREX-SCI]: A Randomized Clinical Trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-014125.R1
Article Type:	Protocol
Date Submitted by the Author:	23-Dec-2016
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Primary Subject Heading:	Rehabilitation medicine
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	SPINAL CORD INJURY, TESTOSTERONE, RESISTANCE TRAINING, BODY COMPOSITION and METABOLISM, MITOCHONDRIA, INFLAMMATORY BIOMARKERS

Effects of Testosterone and Evoked Resistance Exercise after Spinal Cord Injury [TEREX-SCI]: study protocol for a randomized controlled trial

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Abstract

Introduction: Individuals with spinal cord injury (SCI) are at a lifelong risk of obesity and chronic metabolic disorders including insulin resistance and dyslipidemia. Within few weeks of injury, there is a significant decline in whole body fat-free mass, particularly lower extremity skeletal muscle mass, and subsequent increase in fat mass (FM). This is accompanied by a decrease in anabolic hormones including testosterone. Testosterone replacement therapy (TRT) has been shown to increase skeletal muscle mass and improve metabolic profiles. Additionally, resistance training (RT) has been shown to increase lean mass and reduce metabolic disturbances in SCI and other clinical populations.

Methods and analysis: Twenty-six individuals with chronic, motor complete SCI between 18-50 years old were randomly assigned to a RT+TRT group (n = 13) or a TRT group (n = 13). Twenty-two participants completed the initial 16-week training phase of the study and 4 participants withdrew. Twelve participants out of the 22 completed 16 weeks of detraining. The TRT was provided via transdermal testosterone patches (4-6 mg/day). The RT+TRT group had 16 weeks of supervised unilateral progressive RT using surface neuromuscular electrical stimulation with ankle weights. This study will investigate the effects of evoked RT+TRT or TRT alone on body composition (muscle cross sectional area, visceral adipose tissue, %FM) and metabolic profile (glucose and lipid metabolisms) in individuals with motor complete SCI. Findings from this study may help in designing exercise therapies to alleviate the deterioration in body composition after SCI and decrease the incidence of metabolic disorders in this clinical population.

Ethics and Dissemination: The study is currently approved by the McGuire VA Medical Center and Virginia Commonwealth University. All participants read and signed approved consent

forms. Results will be submitted to peer-reviewed journals and presented at national and international conferences.

Trial Registration: NCT01652040

Keywords: RESISTANCE TRAINING, SPINAL CORD INJURY, BODY COMPOSITION, METABOLISM, TESTOSTERONE, MITOCHONDRIA, INFLAMMATORY BIOMARKERS

Strengths and limitations

- The trial will investigate the use of surface neuromuscular electrical stimulation induced resistance training (NMES-RT) to restore muscle size after spinal cord injury (SCI)
- The trial will provide evidence on the effectiveness of testosterone replacement therapy (TRT) to restore muscle size and lean mass and serve as an alternative approach for those who cannot benefit from NMES.
- The trial will determine how restoring muscle size and lean mass by RT+TRT or TRT can benefit the metabolic profile after SCI.
- The study is only limited to men with complete SCI
- Surface NMES may not benefit those with full sensation or lower motor neuron denervation

Introduction

There are approximately 11,000-12,000 new cases of spinal cord injury (SCI) in the United States annually with an overall prevalence of 250,000-400,000 [1, 2]. Persons with motor complete injury have loss of both sensation and motor function below the level of injury, while incomplete injury is characterized by preserved motor or sensory function. Chronic SCI, defined as one year post injury, is associated with dramatic skeletal muscle atrophy [3-5], increase of fat mass (FM) [6-8] and decrease of fat free mass (FFM) [6, 7]. Collectively, these factors put individuals with SCI at risk for metabolic disorders such as type II diabetes and cardiovascular disease.

Previous studies reveal that 60% of individuals with SCI in the United States are either overweight or obese [2, 9-11]. Despite a low body mass index (BMI) in 50% of the SCI population, individuals are likely to have more than 30% of their body mass as FM. Furthermore, person with SCI are 13% fatter per unit BMI than able-bodied individuals. Individuals with SCI also have a redistribution of adipose tissue, with greater trunk FM and visceral adipose tissue (VAT) compared to age and waist circumference matched able-bodied controls [12-14]. Adipose tissue, particularly VAT, secretes proinflammatory cytokines including interleukin-6 (IL-6) and tumor-necrosis factor-α (TNF-α). Therefore, the increase in VAT after SCI may contribute to metabolic syndrome by stimulating the hepatic production of C-reactive protein (CRP), which is tied to vascular inflammation [15-18]. Another type of ectopic adipose tissue, intramuscular fat (IMF), is increased after SCI and has been correlated with reduced insulin sensitivity [19, 20].

Metabolic changes also accompany SCI, with previous studies finding that more than 50% of individuals with SCI are glucose intolerant, while one out of five is diabetic [2, 9-11, 21].

Other studies report that 55% of individuals with SCI are at risk of developing metabolic syndrome [21-23]. Individuals with complete tetraplegia are more likely to experience decreased glucose and carbohydrate tolerance and have a higher prevalence of heart disease than those with incomplete injuries [24, 25]. Likewise, depressed HDL-C (<35 $mg \cdot dL^{-1}$) and a higher total cholesterol/HDL-C ratio, predictors of coronary heart disease, were noted in those with chronic SCI compared with able bodied controls [24, 26]. These are not universal findings, however, as a systematic review of carbohydrate and lipid disorders in persons with SCI did not find strong evidence of increased risk beyond that of the general population [27].

While previous studies have shown a link between body composition and metabolic profile after SCI, the cellular mechanisms remain unknown. Mitochondria are the site of oxygen consumption and energy production from glucose and lipid metabolism. Unfortunately, mitochondrial function is impaired in a number of diseases including neurodegenerative disease, atherosclerosis, hypertension and cancer [28-31]. Fewer and smaller mitochondria are found in skeletal muscle of insulin resistant, obese and type II diabetic individuals [32]. Previous studies found decreased muscle oxidative capacity and succinate dehydrogenase activity, complex II of the electron transport chain, after SCI [33-35].

One hypothesis is that skeletal muscle mitochondrial function is decreased in metabolic disorders, leading to decreased fatty acid oxidation which contributes to insulin resistance [36, 37]; however, this is controversial [37] . Mitochondria are dynamic organelles and undergo biogenesis, remodeling, and degradation. Mitochondrial biogenesis is driven in part through the action of peroxisome proliferator-activated receptor (PPAR) coactivator 1 alpha (PGC-1α) [38, 39]. In addition, PGC-1α integrates insulin signaling and lipogenesis in skeletal muscle [40, 41]. PGC-1α is decreased in animal models following denervation [42, 43]. Decreased

mitochondrial biogenesis and reduced mitochondrial mass may result in decreased energy production and therefore play a significant role in the altered metabolic profile following SCI [44].

Body composition and metabolic changes after SCI may be further exacerbated by reduced anabolic hormones including testosterone (T), growth hormone and the growth hormone second messenger insulin like growth factor-1 (IGF-1) [45, 46]. Previous studies have shown that 60% of men with SCI have low T and that testosterone replacement therapy (TRT) increases IGF-1 in men [47-50]. In rodent models of SCI, TRT attenuates the loss of muscle [51, 52]. TRT decreases total body fat, increases lean mass [53, 54] and increases the number of proliferating skeletal muscle satellite cells in in men [55]. These findings suggest that TRT may provide metabolic benefits to individuals with SCI.

Resistance training (RT) improves insulin sensitivity and increases fatty acid and carbohydrate metabolism as well as attenuates sarcopenia in the elderly and after SCI [56-63]. Moreover, RT has been shown to influence body composition by increasing lean mass, decreasing FM and reducing VAT, suggesting that the benefits of RT could overcome the risk of developing insulin resistance [56-59]. Functional electrical stimulation (FES) has been shown to improve fatty acid kinetics, carbohydrate metabolism and vascular health after SCI [60-63]. Electrically evoked RT using neuromuscular electrical stimulation (NMES-RT) and ankle weights is another form that has been shown to be effective in inducing muscle hypertrophy in individuals with chronic SCI [59, 64]. One study showed a 40% increase in skeletal muscle size and improved glucose tolerance after 12 weeks of training [59]. Another study showed that following 12 weeks of NMES-RT, whole thigh, knee extensor and flexor cross sectional areas (CSAs) increased by 28%, 35% and 16%, respectively. Moreover, the ratio of leg FFM to whole

body FFM increased by 20% following intervention. There was 32% decrease in glucose area under the curve adjusted to muscle CSA following 12 weeks of NMES-RT. However, there were only modest effects on whole body composition as well as a non-significant decrease in VAT [64]. It is possible that the limited effects of NMES-RT on parameters of body composition and VAT can be possibly explained by depressed T-level in persons with SCI. Supplementing exogenous T may optimize the outcomes of NMES-RT on parameters of body composition and metabolic profile such as increase basal metabolic rate (BMR).

TRT may be an effective therapy to counterbalance the growing rate of obesity, type II diabetes and cardiovascular disease among individuals with SCI. Moreover, results from the current trial may provide evidence that TRT is an effective intervention for those who cannot effectively benefit from NMES because of lower motor neuron denervation or intolerance to electrical stimulation. Therefore, the primary hypothesis is that the addition of TRT will maximize the benefits of electrically evoked RT on parameters of body composition and metabolic profile in men with chronic complete SCI. We, hereby, report the design of an study for which the major research goal is to investigate the effects of 16 weeks of evoked RT+TRT vs. TRT on body composition (primary outcome variables; muscle CSA, VAT, %FM) and metabolic profiles (secondary outcome variables; glucose and lipid metabolism) in individuals with motor complete SCI.

Methods and analysis

Study design

A randomized controlled study was undertaken in which individuals with SCI were randomized to receive RT+TRT or TRT alone for 16 weeks. The study was approved by the McGuire Veteran Affairs Investigation Research Board and the Virginia Commonwealth

University (VCU) Office of Research and Innovation. The trial has been registered at clinicaltrials.gov (NCT01652040). A member of the research team explained the study and obtained written informed consent. After informed consent each subject underwent a detailed physical examination at the Hunter Holmes McGuire VA Medical Center (VAMC) by a physiatrist board certified in SCI medicine. This exam included a neurological assessment according to the International Standards for Neurological Classification of SCI (ISNCSCI), including the American Spinal Injury Association (ASIA) Impairment Scale (AIS) [65].

The study design and procedures are presented in Figures 1 and 2. The study visits included estimation of body composition, anthropometry, and dual x-ray absorptiometry (DEXA; baselines 1 and 2 and post-interventions 1 and 2). Additionally, MRI scans were obtained for trunk adipose tissue and lower extremity skeletal muscles and IMF CSA (baseline 2, post-interventions 1 and 2). Participants were then escorted to the VCU-CRS unit (VCU Clinical Research Unit) for dinner, and remained in the VCU-CRS unit overnight for the four study visits. Muscle biopsies were obtained at baseline 2 and post intervention 1.

Recruitment and Randomization

The recruitment process started in July 2012 and ended in June 2015. Data analysis is currently being performed. Recruitment details and randomization are presented in Table 1 and Figure 3. Prior to the start of the study, numbers 1-26 were randomized using the n-Query software program by the principal investigator. At the end of the two-day assessment period (Baseline 1), the allocation of subjects into either group was performed in a blinded fashion by drawing a folded paper with a number (1-26) by the research coordinator. This number was matched with the assignment from the randomization procedure.

Twenty-six participants were recruited to participate in the study. A four week delayed entry period was included to obtain baseline measurements, stabilize body weight and educate participants on how to monitor their dietary intake. This allowed participants to serve as their own controls. Four participants withdrew from the trial. At baseline 2, two participants failed to comply with study protocol and withdrew from the study because lack of interest. Nurses failed to locate the veins on the third participant to conduct IVGTT and he was asked to withdraw from the trial. The fourth participant was diagnosed with a grade IV pressure ulcer after being admitted into the trial. Following the delayed entry period, participants were randomly assigned to a RT+TRT group (n = 13) or TRT group (n = 13). TRT patches (2-6 mg/day) were replaced daily on alternating shoulders at bedtime for 16 weeks. The RT+TRT group received 16 weeks of supervised unilateral progressive RT using surface NMES and ankle weights. Following the intervention the two-day assessment period was repeated (Figure 1).

Participants and eligibility criteria

Participants were men between 18-50 years old with a BMI of $\leq$ 30 kg/m^2. The upper limit of age was set to 50 years to avoid unanticipated side effects that may result from TRT. Participants had motor complete SCI C5-L2, ASIA A or B. Participants with pre-existing medical conditions were excluded. These included cardiovascular disease, uncontrolled type II diabetes and those on insulin, pressures sores stage 2 or greater, supra-physiological T level, hematocrit above 50% and urinary tract infection or symptoms.

I. Interventions

Resistance training

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 weight was added. Surface NMES was applied to the knee extensor muscles via surface electrodes (Figure 2). One electrode was placed 2–3 cm above the superior aspect of the patella over the vastus medialis muscle, and the other lateral to and 30 cm above the patella over vastus lateralis muscle. Current from the stimulator was manually increased in 5-second intervals to evoke full knee extension with a 3-minute rest between sets, 30 Hz, 450 μs pulses and a current sufficient to evoke full knee extension as previously described [64, 66, 67]. Four sets of 10 repetitions was performed twice weekly for 16 weeks.

Testosterone replacement therapy (TRT)

Following baseline measurements, T was administered by patches (Androderm, Watson Pharma. Inc, Parsippany, NJ) that delivered between 2-6 mg/day [53] (Figure 2). Serum T concentration was measured and reviewed in a blinded fashion weekly for the first month and then every 4 weeks by an endocrinologist. Baseline dose was prescribed according to the initial T level. A dose of 6, 4 or 2 mg/day was prescribed if the serum T-level was less than 300, 300-600 or above 600, respectively. The dose was decreased to 2 mg/day if the serum T concentration was more than 1000 ng/dL (34.7 nmol/L) and the participant was reeducated about use of the patch if the concentration was less than 250 ng/dL (8.7nmol/L) above the pretreatment concentration. Patches were returned after use to ensure adherence to the intervention protocol. Participants were instructed to place patches at bedtime and only remove them during showering. If skin irritation became an issue, participants were initially advised to move patches up or down on the shoulder muscles from the irritation site and if the situation was not resolved, a hydrocortisone cream was prescribed.

Detraining after 16-week intervention

Six participants from each group were followed for 16 weeks after the initial intervention. The RT+TRT group continued training once weekly using the same training approach for an additional16 weeks. For the first week, the maximum ankle weights attained during the training phase were used. The weights were then gradually decreased by two pounds per week until the lowest weight was attained (2 pounds). TRT dose was set at 2 mg/day for the entire detraining phase. Participants from the TRT group were followed for additional 16 weeks without intervention. Following the detraining phase, the two-day assessment period was repeated without performing skeletal muscle biopsy (Figure 1). The rationale of the detraining phase is to determine whether once weekly training can maintain skeletal muscle hypertrophy, body composition and metabolic improvements incurred by the 16 week intervention.

II. Primary Outcomes

Data will remain confidential at all times and any patient identifiers will be removed prior to data analysis. Analysis for all study procedures will be performed in a blinded fashion ensuring full concealment until complete data analysis.

Anthropometrics and Body Composition Assessments

Height of each participant was determined while lying in the supine position. Two smooth wooden boards were placed at the participant's head and heels and the distance between them was measured to the nearest cm. Measurement of waist circumference was determined in triplicate by identifying the narrowest region of the trunk from sitting and supine positions. Three-site skin fold assessment was conducted in triplicate for suprailiac, abdominal and thigh.

A Lunar Prodigy Advance (Lunar Inc., Madison, WI) bone densitometer was used to measure total body and regional (lumbar spine, proximal femur, and forearm) FM and FFM.

Testing was performed after lower extremity elevation for at least 20 minutes to minimize fluid shift. The subject was assisted to lie on a padded table and both legs were strapped proximal to the knees and ankles. The arms and legs were positioned to ensure proper alignment.

Magnetic resonance imaging (MRI)

MRI was performed at the VAMC Hospital using a 1.5 Tesla magnet (GE) as previously described [4, 19, 59, 68, 69]. Transaxial images, 10 mm thick and 10 mm apart, were taken from the hip joint to the knee joint and from knee to the ankle using the whole body coil. The location of the scan was identified by placing a mark 6 inches proximal to and distal to the patella and matched on follow up scans. To analyze VAT and subcutaneous adipose tissue (SAT) transverse slices (0.8 cm thickness) were acquired every 0.4 cm gap from the xyphoid process to the femoral heads. Images were acquired in series of two stacks with L4-L5 used as a separating point. TRT patches were removed 48-72 hours prior to MRI scans to avoid skin burn.

Analyses will be performed using commercial available software (X-vessel) as previously described [4, 19, 68, 69]. Briefly, the thigh and leg images will be segmented into fat (high intensity), skeletal muscle (mid intensity) and background/bone (low intensity). Manual selection of a pixel of skeletal muscle will highlight all skeletal muscle pixels and provide the total number of skeletal muscle pixels while excluding fat. VAT and SAT will be measured by manually tracing around the anatomical borders. The number of pixels in the highlighted region will be multiplied by the matrix size to measure VAT and SAT CSA (cm^2).

Skeletal muscle torque and specific tension

Torque of the knee extensor muscle group was evaluated using a Biodex isokinetic dynamometer (Shirely, NY). Measurements were done 72 hours after the muscle biopsy to prevent acute effects on protein expression. Participants were seated with both the trunk-thigh

angle and the knee-thigh angle at 90º. Each participant was securely strapped to the test chair by a crossover shoulder harnesses and a belt across the hip joint. The axis of the dynamometer was aligned to the anatomical knee axis and the lever arm was attached 2-3 cm above the lateral malleolus. Before measuring isometric torque, passive tension of the right knee extensor muscle group was measured at 5, 30, 60, 90,180, 270 degrees/sec as an index of spasticity. Isometric torque was measured using current amplitude of 50 and 100 mA at a frequency of 30 Hz and pulse duration 450 μs. Isokinetic torque was measured at 60, 90 and 180 degrees/sec using the same stimulation protocol.

Serum and plasma analysis

All metabolic profile analysis is presented in Table 2. Blood samples were collected after an overnight fast. Total T was measured by liquid chromatography with isotope dilution mass spectrometry detection after supported liquid extraction. Free T concentration was calculated using sex hormone binding globulin and albumin concentrations (www.issam.ch/freetesto.htm) [70]. Serum IGF-I concentration was measured by an immunoluminometric assay (Quest Diagnostics, Madison, NJ). Fasting lipid profiles (HDL-C, LDL-C, total cholesterol, and triglycerides) were determined as previously described [8, 20]. Inflammatory biomarkers CRP, IL-6, TNF-α , and free fatty acids were determined by commercially available enzyme-linked immunosorbent assay kits (ALPCO; Salem, NH).

Energy Expenditure

After an overnight fast for 10-12 hours, participants were kept in a dark room for 20-30 minutes to attain a resting state during which BMR was measured as previously described [8]. Briefly, while in a supine position a canopy was placed over the subject's head. Each subject was allowed 2-3 minutes before starting the test to ensure no signs of apnea or claustrophobic

episodes. All subjects were instructed to stay awake during the entire test and to breathe normally. The canopy was then attached to a vacuum to draw the expired gases to the flowmeter of the metabolic unit (COSMED KB42). Prior to the test, the metabolic unit was calibrated using the standard procedures identified by the manufacturer. Carbon dioxide and oxygen output was used to calculate the respiratory exchange ratio and BMR (kcal/day) was calculated using the average of the last 15 minutes of the test. This was used to measure the percentage of substrate utilization (% fat vs. % carbohydrate) [8, 71].

To determine whether NMES-RT improves exercise performance, testing was performed using a functional electrical stimulation bike (Restorative Therapies, RTI-300) against progressive resistance protocol until fatigue. The protocol started with 3 minutes resting, 3 minute warm-up (35-37 RPM) using the servomotor and then a two minute incremental progressive resistance protocol (1 Nm, 3 Nm, 5 Nm, etc.) until fatigue. After fatigue, a one minute cool down period was allowed followed by 5 minutes of rest. Energy expenditure and cardiovascular performance [VO_2 (l/min), blood pressure and heart rate] was collected at baseline 2 and post-interventions 1 and 2.

Each participant met with a dietician at the start of the study and was asked to maintain a 5 day food dietary log monitoring their caloric intake for the duration of the study. Participants were instructed to record all liquid and food consumption and no nutritional advice was given on the size or the portion of the food. Dietary logs were analyzed on a weekly basis using a nutritional software package (Nutrition Data System for Research version 2014) under the supervision of a registered dietitian. After analysis was completed, the average caloric intake (kcal) and percentage macronutrients (carbohydrates, fat and protein) were calculated. Each participant received monthly feedback via phone call with the registered dietician on how to

maintain appropriate dietary habits based on his BMR and percentage macronutrients (45% carbohydrates, 30% fat and 25%). Every effort was made to balance the dietary habits between the RT+TRT and the TRT group.

Intravenous Glucose Tolerance Test (IVGTT)

An IVGTT was used to determine insulin sensitivity and glucose effectiveness before training and 48 hours after the last exercise bout. After an overnight fast, an intravenous line was placed to facilitate infusion of glucose and blood sampling. Blood samples were taken every 2-3 minutes before and 30 minutes after glucose injection (0.3 gm/kg IV over 30 seconds), followed by 5-10 minutes sampling ending at 180 minutes. Twenty minutes after the glucose injection a bolus of insulin (0.02 U/kg) was injected to determine insulin sensitivity. Plasma glucose was measured by the Autoanalyzer glucose oxidase method and plasma insulin concentrations were determined by commercial radioimmunoassay (Table 2). The glucose disposal rate per unit of secreted insulin per unit time and glucose mediated glucose disposal rate were calculated from a least-squares fitting of the temporal pattern of glucose and insulin throughout the IVGTT using the MINMOD program [72]. A representative analysis of IVGTT is presented in Figure 4. The acute insulin response to IV glucose was calculated as the mean rise in plasma insulin above baseline at 3, 4 and 5 minutes after IV glucose administration. KG, a measure of glucose tolerance, was calculated as the least square slope of the natural log of absolute glucose concentration between 5 and 20 minutes after the glucose bolus [73]. The homeostatic model of assessment of insulin resistance (HOMA-IR) was calculated and insulin sensitivity was determined using Matsuda and Defronzo formula [73, 74].

III. Secondary Outcomes

Skeletal muscle biopsy

Biopsy samples of vastus lateralis muscle (~50-100 mg wet weight total) were obtained by a 14 gauge tru-cut biopsy needle, immediately prior-to and 72 hours after the 16 weeks of RT+TRT or TRT interventions. The 72 hours post-intervention was to avoid any acute effects from the last training bout on muscle protein expression to ensure that changes are due training effect. There was no muscle biopsy during the detraining phase. The biopsy samples were quickly frozen in liquid nitrogen and stored at -80°C until further analysis. One sample was split into two halves and used for measuring activities of mitochondrial enzymes. The second sample was used for Western blot analysis. The third sample was used for immunohistochemistry.

Mitochondrial Electron Transport Chain activities

Electron transport chain enzyme activities were measured spectrophotometrically in skeletal muscle homogenates as previously described [75]. Rotenone-sensitive NADH cytochrome c reductase measured complexes I and III. Decylubiquinol-cytochrome c oxidoreductase was measured as the antimycin-sensitive reductase to assess complex III. Citrate synthase was measured as an estimate of mitochondrial mass as previously described [75].

Protein content

Proteins were resolved by SDS-PAGE then transferred to a PVDF membrane (Trans-blot; Bio-Rad). Equal protein loading was confirmed with a Ponceau S stain after the transfer. After blocking for one hour, membranes were incubated overnight at 4 ˚C with primary antibody diluted 1:1000. Primary antibodies included glucose transpoter-4, focal adhesion kinase, PGC-1α (Santa Cruz Biotechnology), total protein kinase B (AKT), phosphorylated AKT, total mammalian target of rapamycin (mTOR), phosphorylated mTOR, (Cell Signaling). Membranes

were rinsed and placed in a 1:2000 solution of a horseradish peroxidase-conjugated secondary antibody (Cell Signaling) for 1 hour at room temperature. Membranes were then rinsed and incubated with a horseradish peroxidase chemiluminescence detection reagent (GE Healthcare) for 5 minutes. Proteins were visualized using an Amersham Imager 600 (GE Healthcare). Optical densities were measured using iQuant software and all samples were normalized to the baseline values for that participant.

Histological Analysis

Immediately after muscle biopsy, samples were mounted on tongue blades by using a medium of OCT compound and tragacanth gum and stored at -70°C until analysis. Serial cross sections (8-10 μM) were collected on glass slides and frozen at -20°C until analysis. Fiber type and CSA will be determined by histochemical staining for myosin ATPase (preincubation at pH 4.3 or 9.4) as previously described [76]. Type I fibers will be identified by dark staining after acid preincubation, type II fibers light staining, and type IIB intermediate. At pH 9.4 the staining pattern was the opposite. Haematoxylin & Eosin (H&E) staining was performed according to conventional histological procedures. Mitochondrial complex II and IV activity was estimated by the activity of succinate dehydrogenase and cytochrome c oxidase activity, respectively, as previously described [77, 78]. Stained muscle sections will be observed using an Olympus BX-51 fluorescent microscope (Olympus, Tokyo, Japan) and analyzed using ImageJ software.

Statistical Analyses

Paired t-tests will be used to determine differences in body composition and metabolic profile between baseline 1 and baseline 2. To determine the effect of interventions and detraining, a repeated measures ANOVA will be used. Simple linear and multivariate regression analyses were used to examine the relationship between body composition and metabolic profile

variables. For protein expression, we will use paired t-tests to examine the effects of each intervention.

The effect size is calculated based on the effects of RT on body composition and metabolic profiles that were previously published [62]. The number of subjects necessary to find statistical differences in the major variables (muscle size, VAT and insulin concentration) of this study was found to be 10 participants per group. One participant from the RT+TRT group withdrew after developing side effects to TRT patches and the medical monitor personnel recommended him to withdraw from the trial in week 8. However, we will perform intent to treat analysis on his data, which means that despite his early withdrawal from the study his data will be included in the final analysis. This will allow extrapolation of his post-intervention data using the SPSS missing values option. We anticipate that we will collect sufficient data to determine the effects of rehabilitation interventions on protein expression, mitochondrial enzymatic and ETC activities in individuals with SCI. Statistical analysis will be performed using SPSS version 23.0 (Chicago, IL) with a level of significance set at $p<0.05$.

Ethics and Dissemination

Ethical approval has been obtained from the institutional review boards at the McGuire VA Medical Center and Virginia Commonwealth University. All participants read and signed approved consent forms prior to baseline assessment. Results of the study will be published in peer-reviewed journal and presented at national and international conferences.

Data Monitoring

The research staff oversaw and monitored the study to ensure data quality and participant compliance. There were no adverse events. Only members of the research team will have access to data.

Discussion

Individuals with SCI experience profound skeletal muscle atrophy, deterioration in body composition and abnormal metabolic profile. Within few weeks of injury, there is a significant decrease in whole body FFM, particularly lower extremity skeletal muscle mass, and subsequent increase in FM. These changes predispose this population to the risk of glucose intolerance, insulin resistance, dyslipidemia and the development of type II diabetes and cardiovascular disease. The main purpose of this study is to investigate the effects of 16 weeks of evoked RT+TRT or TRT alone on body composition (muscle CSA, IMF, VAT, %FM, FFM) and metabolic profile (glucose, lipid and BMR) in individuals with motor complete SCI.

Ectopic adipose tissue accumulation, IMF and VAT, has been strongly associated with altered metabolic profile after SCI [19, 20]. IMF has been determined to account for a 70% reduction in glucose tolerance in individuals with complete SCI [19]. VAT is independently associated with impairment in glucose tolerance, insulin resistance and dyslipidemia after SCI [20]. Edwards et al noted significant positive association between VAT and insulin resistance and a negative correlation between VAT:SAT ratio and HDL-C [13]. Increase in VAT is also related to leptin and plasminogen activator inhibitor-1 concentrations [14]. It is possible that increases in FM are associated with inflammatory biomarkers that trigger metabolic syndrome after SCI [22]. Ectopic adipose tissue has been demonstrated to secrete pro-inflammatory cytokines, including IL-6 and TNF-α. This stimulates hepatic production of CRP which is suggestive of vascular inflammation [15-19].

RT is an important type of exercise that has been shown to induce positive physiological adaptations such as increasing lean mass and reducing the incidence of metabolic disorders in other clinical populations. Previous work suggests that twice weekly NMES-RT can induce

favorable body composition and metabolic adaptations. Twelve weeks of NMES-RT has shown to increase thigh muscle CSA by 35-40% as measured by MRI [59]. Moreover, there was a reduction in %leg FM and a trend towards decrease in VAT CSA after 12 weeks of NMES-RT [64]. The favorable adaptations in body composition were associated with decrease in plasma insulin area under the curve and plasma triglycerides [59]. These findings were attributed to an increase in plasma IGF-1. However, the effect of training appears to be limited to the trained muscle and only modestly impacted whole body composition. It is unclear whether a RT program longer than 12 weeks may provide additional benefits to individuals with SCI.

The cellular changes underlying the alterations in skeletal muscle glucose utilization and energy metabolism after SCI are unclear. Previous work indicated that expression of AMP-activated protein kinase (AMPK), a key regulator of energy homeostasis for lipid and carbohydrate utilization, was altered in persons with SCI compared to BMI matched able bodied controls [79]. Another study revealed decreased expression of genes involved in glucose and lipid metabolism [80]. Despite these abnormalities, one study reported that leg glucose uptake during cycling was increased in individuals with SCI compared to able-bodied controls [61]. Another study showed similar glucose uptake of isolated muscle fibers from SCI and able bodied individuals *in vitro* [81]. Benefits of other forms of functional electrical stimulation lower extremity cycling (FES-LEC) have included improvements in body composition, carbohydrate- and lipid metabolism and muscle fiber type composition [60-63, 82-84]. Similarly, we have recently shown that 16 weeks of FES-LEC increased the protein abundance of GLUT-4, PGC-1α and AMPK by 3.8, 2.3 and 3.4 fold, respectively, in the vastus lateralis muscle in persons with motor complete SCI [85].

TRT has been shown to increase skeletal muscle mass in hypogonadal men, men with chronic illness, and older men [53-55]. Androgen deficiency in men is associated with a loss of FFM and an increase in FM [53]. In epidemiologic studies, men with decreased free T had lower appendicular skeletal muscle mass than those with normal T levels [86]. Previous work documented that TRT increases muscle mass with a reciprocal decrease in total body FM [53, 54]. This reciprocal action has been suggested due to a switch from adipogenic toward myogenic differentiation of mesenchymal stem cells [86]. In a randomized controlled double blinded clinical trial, TRT was shown to improve insulin sensitivity, CRP and reduce VAT [54]. Sixty percent of men with SCI have low T level and levels are associated with time since injury [49, 50]. TRT has also been reported to increase IGF-1 and several molecular mechanisms related to the protective pathways have been recently elucidated [47, 48, 51, 87]. Therefore, enhancing the decline in anabolic homeostasis by providing TRT may provide additional benefits as was previously demonstrated by increasing lean mass and metabolic rate in individuals with SCI.

It was very important to highlight that this protocol has 3 different phases including four weeks of delayed entry, 16 weeks of intervention and 16 weeks of detraining. The delayed entry period was included to allow each participant to serve as his own control. Moreover, we were successful in retaining 12 participants (n=6/group) to complete the detraining phase. This means that we had participants 6 participants that agreed to stick to our exercise program and the use of TRT patches up to 9 months. This may reflect on the study protocol frequency that ensured long-term adherence despite the length of the study. A very important point that is worth highlighting is that our study protocol was designed to include 3 levels of outcome measurements including body composition, metabolic profile and cellular changes. This design

is likely to provide mechanistic explanations to changes that occur at the body composition and metabolic levels

Limitations

The current study was limited to those who were less than or equal to 50 years old. Because of advances in healthcare, many individuals with SCI have a near-normal life span which may make the results of this study less generalizable. However, this age limit was implemented because the effect of TRT on cardiovascular health has been controversial. There are data showing that hypogonadism is a risk for cardiovascular disease [88]. Some replacement studies show increased risk, but another study showed decreased mortality in men receiving testosterone [89]. Another study in older men reported that injectable testosterone may be associated with increased cardiovascular risk but topical testosterone was not [90]. Therefore, we set the inclusion criteria of less than or equal to 50 years old to reduce the likelihood of developing cardiovascular complications.

Currently NMES-RT is not readily available to the majority of SCI patients. Women were not included because administering TRT is not either appropriate or safe, because women are at risk of virulization by testosterone. Thus, the trial was limited only to males with SCI. Moreover, only a small percentage of individuals with motor complete SCI are women.

In summary, we anticipate that this trial will provide important insights into the body composition and metabolic benefits of 16 weeks of evoked RT+TRT or TRT. If beneficial, this may be a feasible strategy for the rehabilitation of individuals with chronic SCI and increase the health of this and other clinical populations. Additionally, TRT alone may provide an alternative intervention for those who cannot benefit from training using surface NMES, because of lower motor neuron denervation or intolerance to applications of electrical stimulation. The study will

also shed light on several molecular pathways that have been suggested to influence both body composition and metabolic profile.

Trial Status: Enrollment into the study started in July 2012 and as of April 2016 all participants have completed the study. Data collection and data analysis are expected to be completed in December 2016. The study is expected to be closed in June 2017.

Contributions: ASG supervised all aspects of the trial including all interventions and measurements and secured funding for the trial procedures. ASG, REK, LCO drafted the manuscript. REK, JS, RK, and LCO contributed to data collection and analysis. RG, TL, TC, DXC, CC, RA, EJL and DRG are research physicians that contributed to patient monitoring and study design. DXC and RA will provide guidance during data analysis and manuscript preparation. All authors affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Competing interests: The authors have no competing interests to declare

Funding: This work was supported by VA-RRD CDA2 to Ashraf S. Gorgey, clinical trial registration number NCT01652040.

Acknowledgements: We would like to thank Rodney Wade, Sakita Sistrun, Liron Segal for helping in the processes of data capturing and analysis as well as the study participants for their time and effort. We would also like to thank Hunter Holmes McGuire Research Institute and Spinal Cord Injury Services and Disorders for providing the environment to conduct clinical human research trials. VCU Clinical Research Service for providing space and staff to perform the blood work procedures. The work is supported by the Department of Veteran Affairs, Veteran Health Administration, Rehabilitation Research and Development Service (B7867-W).

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Table 1. Randomization of individuals with motor complete SCI into either RT+TRT (n=13) or TRT (n =13) using n Query software with a treatment ratio 1:1.

Subject ID	Assignment	Baseline 1	Baseline 2	Post-Intervention 1	Post-Intervention 2
10001	RT+TRT	C	C	C	C
10002	TRT	C	C	C	C
10003	TRT	C	C	C	C
10004	RT+TRT	C	C	C	C
10005	RT+TRT	C	C	C	C
10006	TRT	C	C	C	X
10007	RT+TRT	C	C	C	C
10008	TRT	C	C	C	C
10009	RT+TRT	C	withdraw	X	X
10010	TRT	NA	NA	NA	NA
10011	RT+TRT	C	withdraw	x	x
10012	TRT	C	C	C	C
10013	TRT	C	C	C	x
10014	RT+TRT	C	C	C	withdraw
10015	TRT	C	C	C	x
10016	TRT	C	C	C	C
10017	RT+TRT	C	C	C	x
10018	TRT	C	C	C	x
10019	RT+TRT	C	C	C	C
10020	RT+TRT	withdraw	x	x	x
10021	RT+TRT	C	C	C	x
10022	TRT	C	C	C	C
10023	RT+TRT	C	C	C	C
10024	TRT	C	withdraw	x	x
10025	RT+TRT	C	C	C	C
10026	RT+TRT	C	C	withdraw	x
10027	TRT	C	C	C	x

C: completed; **NA**: not assigned for #10. Baseline 1 was followed by 4 weeks of no intervention for all the participants. Prior to baseline 2, randomization was performed into RT+TRT or TRT groups. Post-intervention 1 (n=22) was conducted following 16 weeks of intervention. Post-intervention 2 (n= 13) was conducted following 16 weeks of RT+TRT (n=6) or no intervention (n=6).

Table 2. Metabolic health variables measured at baseline 1, baseline 2, post-intervention 1 and post-intervention 2

	Quantity	Special handling	Techniques of Analysis
Insulin and Glucose	2 ml	Potassium oxalate/sodium fluoride tube (Grey Top)	ELISA and biochemistry analyzer
HBA1C		SST	Standard Procedure
Testosterone every 4 weeks	4 ml	SST	Liquid chromatography with isotope dilution mass spectrometry detection
Albumin*		SST	Standard Procedure
SHBG*		SST	Standard Procedure
IGF-1, IGFBP-1 and 3	4 ml	SST	ELISA
Inflammatory biomarkers (CRP, IL-6, TNFα)	4 ml	SST	ELISA
Free fatty acids	2 ml	Potassium oxalate/sodium fluoride tube (Grey Top)	Enzymatic colorimetric quantification
Triglycerides, total cholesterol, HDL, LDL	4 ml	SST	Enzymatic colorimetric quantification

*, Only at baseline 2 and post-intervention 1 to calculate free testosterone. ELISA, enzyme-linked immunosorbent assay; HBA1C, hemoglobin A1c; SHBG, sex hormone binding globulin; SST, serum separator tube; IGF-1, insulin-like growth factor 1; IGF-BP, insulin-like growth factor binding protein; CRP, C-reactive protein; IL-6, interleukin 6; TNFα, tumor necrosis factor alpha

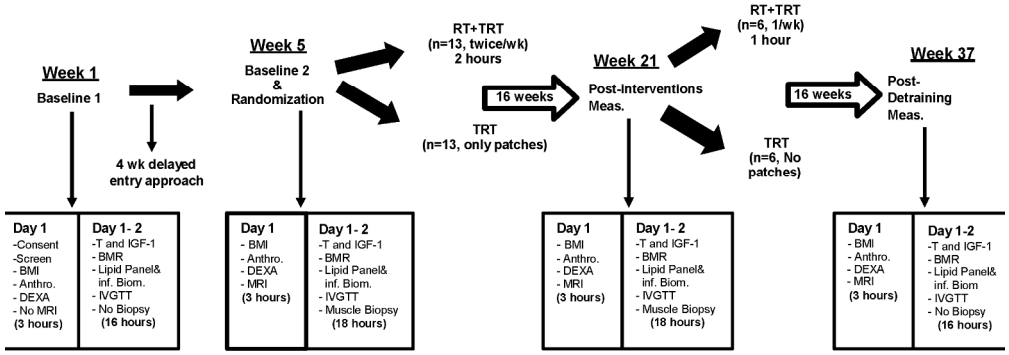
Figure Legends

Figure 1. Timeline and main procedures of the TEREX-SCI trial for both the RT+TRT and TRT groups during baseline 1, baseline 2, post-intervention 1 and post-intervention 26 for persons with motor complete SCI.

Figure 2. A person with T4 motor complete SCI undergoing both electrically evoked RT (left panel) and TRT using transdermal patches (right panel) as a part of a 16 week intervention.

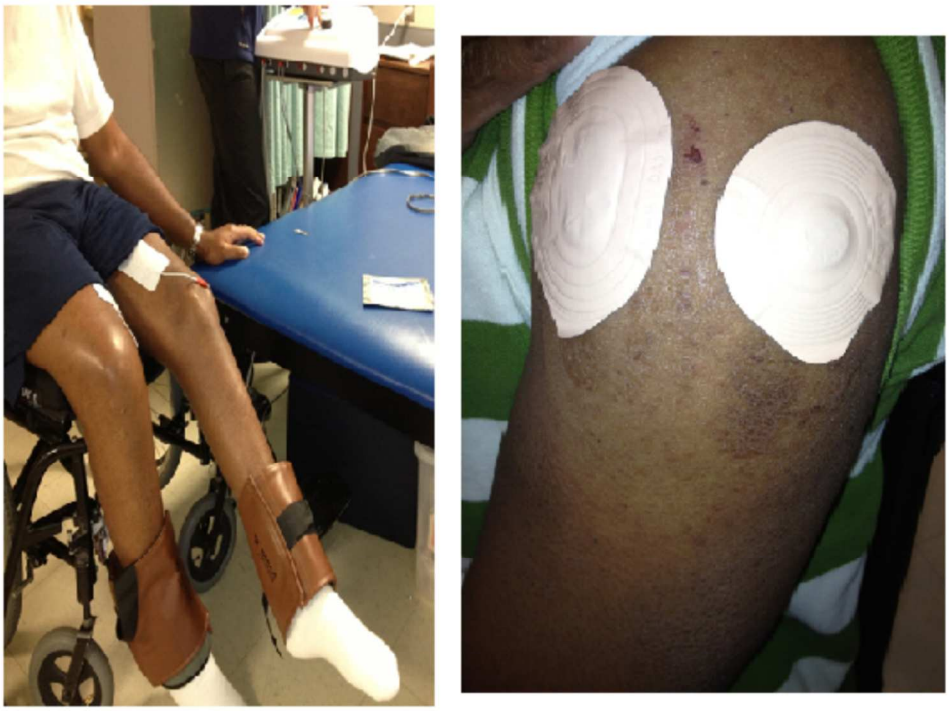
Figure 3. Schematic diagram showing the process of recruitment over the 3 year period of the TEREX trial.

Figure 4. A representative figure of analysis for IVGTT in a person with SCI after infusion of dextrose followed by insulin 20 minutes later. The dextrose concentration is calculated by multiplying 0.3g x body weight (kg) in 50% solution. Insulin concentration is determined by multiplying 0.02 units x body weight (kg). Blue line represents fasting glucose concentration, resting, following infusion, and over 120 minutes. Red line represents the line of best fit of glucose concentration. Green line represents fasting insulin level, spike following 20 minutes of dextrose infusion and over 120 minutes. Black line represents the line of best fit for insulin concentration.



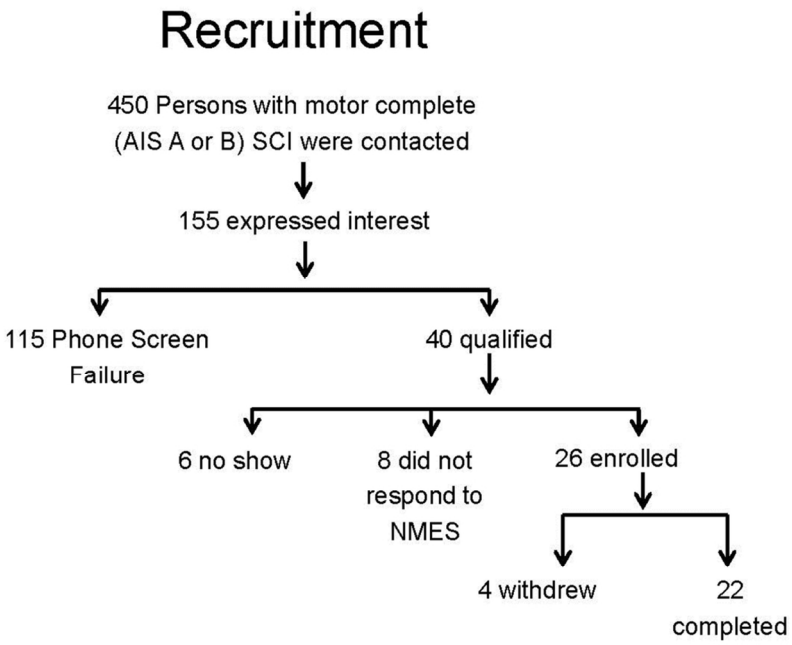
Timeline and main procedures of the TEREX-SCI trial for both the RT+TRT and TRT groups during baseline 1, baseline 2, post-intervention 1 and post-intervention 26 for persons with motor complete SCI.

215x77mm (300 x 300 DPI)



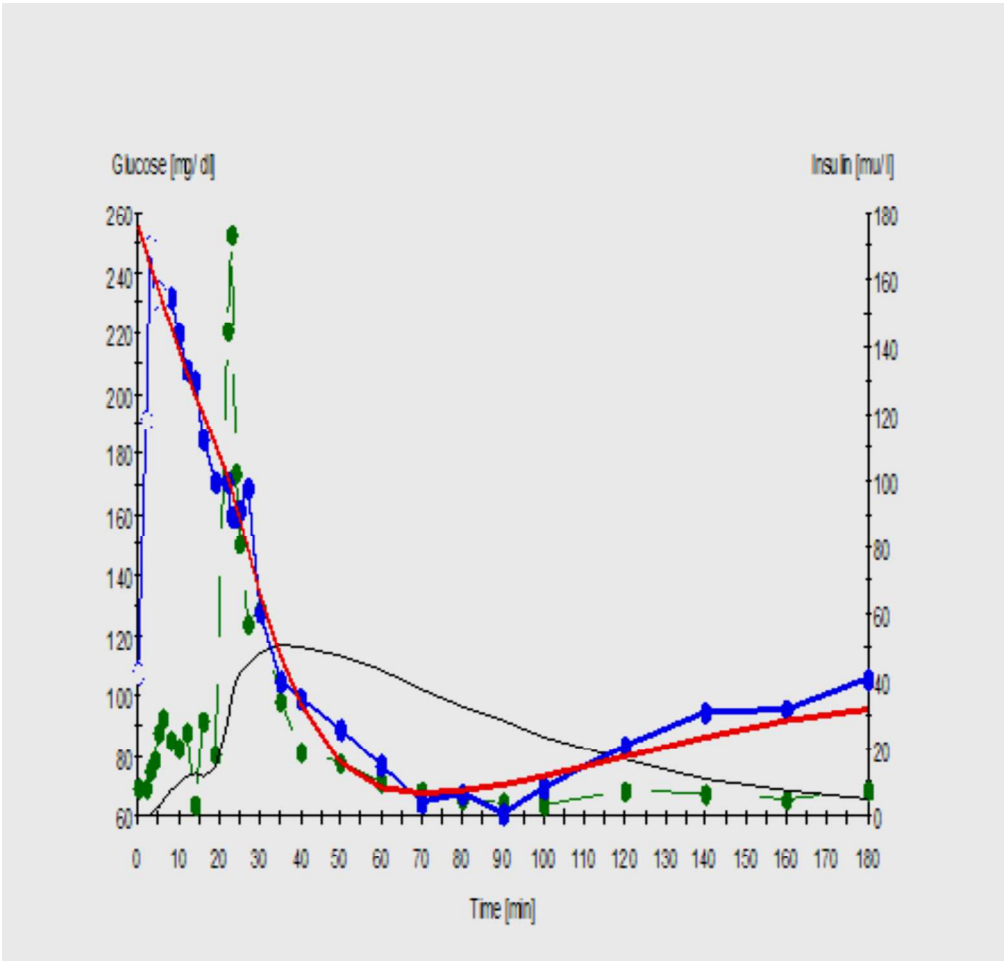
A person with T4 motor complete SCI undergoing both electrically evoked RT (left panel) and TRT using transdermal patches (right panel) as a part of a 16 week intervention.

121x92mm (300 x 300 DPI)



Schematic diagram showing the process of recruitment over the 3 year period of the TEREX trial.

114x76mm (300 x 300 DPI)



A representative figure of analysis for IVGTT in a person with SCI after infusion of dextrose followed by insulin 20 minutes later. The dextrose concentration is calculated by multiplying 0.3g x body weight (kg) in 50% solution. Insulin concentration is determined by multiplying 0.02 units x body weight (kg). Blue line represents fasting glucose concentration, resting, following infusion, and over 120 minutes. Red line represents the line of best fit of glucose concentration. Green line represents fasting insulin level, spike following 20 minutes of dextrose infusion and over 120 minutes. Black line represents the line of best fit for insulin concentration.

126x120mm (300 x 300 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3, 7
	2b	All items from the World Health Organization Trial Registration Data Set	3, 7
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	20
	5b	Name and contact information for the trial sponsor	21
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
	6b	Explanation for choice of comparators	5-6
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-16
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7, Fig 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	____16-17_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____7-8_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	_____7-8_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_____7-8_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____8_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____9-10_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_____10-16_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	_____17_____

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	16-17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	17
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	3, 7, 17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	3, 17
	31b	Authorship eligibility guidelines and any intended use of professional writers	20
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	attached
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Effects of Testosterone and Evoked Resistance Exercise after Spinal Cord Injury [TEREX-SCI]: A Randomized Clinical Trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-014125.R2
Article Type:	Protocol
Date Submitted by the Author:	17-Jan-2017
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Primary Subject Heading:	Rehabilitation medicine
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	SPINAL CORD INJURY, TESTOSTERONE, RESISTANCE TRAINING, BODY COMPOSITION and METABOLISM, MITOCHONDRIA, INFLAMMATORY BIOMARKERS

Effects of Testosterone and Evoked Resistance Exercise after Spinal Cord Injury [TEREX-SCI]: study protocol for a randomized controlled trial

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Abstract

Introduction: Individuals with spinal cord injury (SCI) are at a lifelong risk of obesity and chronic metabolic disorders including insulin resistance and dyslipidemia. Within few weeks of injury, there is a significant decline in whole body fat-free mass, particularly lower extremity skeletal muscle mass, and subsequent increase in fat mass (FM). This is accompanied by a decrease in anabolic hormones including testosterone. Testosterone replacement therapy (TRT) has been shown to increase skeletal muscle mass and improve metabolic profiles. Additionally, resistance training (RT) has been shown to increase lean mass and reduce metabolic disturbances in SCI and other clinical populations.

Methods and analysis: Twenty-six individuals with chronic, motor complete SCI between 18-50 years old were randomly assigned to a RT+TRT group (n = 13) or a TRT group (n = 13). Twenty-two participants completed the initial 16-week training phase of the study and 4 participants withdrew. Twelve participants out of the 22 completed 16 weeks of detraining. The TRT was provided via transdermal testosterone patches (4-6 mg/day). The RT+TRT group had 16 weeks of supervised unilateral progressive RT using surface neuromuscular electrical stimulation with ankle weights. This study will investigate the effects of evoked RT+TRT or TRT alone on body composition (muscle cross sectional area, visceral adipose tissue, %FM) and metabolic profile (glucose and lipid metabolisms) in individuals with motor complete SCI. Findings from this study may help in designing exercise therapies to alleviate the deterioration in body composition after SCI and decrease the incidence of metabolic disorders in this clinical population.

Ethics and Dissemination: The study is currently approved by the McGuire VA Medical Center and Virginia Commonwealth University. All participants read and signed approved consent

forms. Results will be submitted to peer-reviewed journals and presented at national and international conferences.

Trial Registration: NCT01652040

Keywords: RESISTANCE TRAINING, SPINAL CORD INJURY, BODY COMPOSITION, METABOLISM, TESTOSTERONE, MITOCHONDRIA, INFLAMMATORY BIOMARKERS

Strengths and limitations

- The trial will investigate the use of surface neuromuscular electrical stimulation induced resistance training (NMES-RT) to restore muscle size after spinal cord injury (SCI)
- The trial will provide evidence on the effectiveness of testosterone replacement therapy (TRT) to restore muscle size and lean mass and serve as an alternative approach for those who cannot benefit from NMES.
- The trial will determine how restoring muscle size and lean mass by RT+TRT or TRT can benefit the metabolic profile after SCI.
- The study is only limited to men with complete SCI
- Surface NMES may not benefit those with full sensation or lower motor neuron denervation

Introduction

There are approximately 11,000-12,000 new cases of spinal cord injury (SCI) in the United States annually with an overall prevalence of 250,000-400,000 [1, 2]. Persons with motor complete injury have loss of both sensation and motor function below the level of injury, while incomplete injury is characterized by preserved motor or sensory function. Chronic SCI, defined as one year post injury, is associated with dramatic skeletal muscle atrophy [3-5], increase of fat mass (FM) [6-8] and decrease of fat free mass (FFM) [6, 7]. Collectively, these factors put individuals with SCI at risk for metabolic disorders such as type II diabetes and cardiovascular disease.

Previous studies reveal that 60% of individuals with SCI in the United States are either overweight or obese [2, 9-11]. Despite a low body mass index (BMI) in 50% of the SCI population, individuals are likely to have more than 30% of their body mass as FM. Furthermore, person with SCI are 13% fatter per unit BMI than able-bodied individuals. Individuals with SCI also have a redistribution of adipose tissue, with greater trunk FM and visceral adipose tissue (VAT) compared to age and waist circumference matched able-bodied controls [12-14]. Adipose tissue, particularly VAT, secretes proinflammatory cytokines including interleukin-6 (IL-6) and tumor-necrosis factor-α (TNF-α). Therefore, the increase in VAT after SCI may contribute to metabolic syndrome by stimulating the hepatic production of C-reactive protein (CRP), which is tied to vascular inflammation [15-18]. Another type of ectopic adipose tissue, intramuscular fat (IMF), is increased after SCI and has been correlated with reduced insulin sensitivity [19, 20].

Metabolic changes also accompany SCI, with previous studies finding that more than 50% of individuals with SCI are glucose intolerant, while one out of five is diabetic [2, 9-11, 21].

Other studies report that 55% of individuals with SCI are at risk of developing metabolic syndrome [21-23]. Individuals with complete tetraplegia are more likely to experience decreased glucose and carbohydrate tolerance and have a higher prevalence of heart disease than those with incomplete injuries [24, 25]. Likewise, depressed HDL-C (<35 $mg \cdot dL^{-1}$) and a higher total cholesterol/HDL-C ratio, predictors of coronary heart disease, were noted in those with chronic SCI compared with able bodied controls [24, 26]. These are not universal findings, however, as a systematic review of carbohydrate and lipid disorders in persons with SCI did not find strong evidence of increased risk beyond that of the general population [27].

While previous studies have shown a link between body composition and metabolic profile after SCI, the cellular mechanisms remain unknown. Mitochondria are the site of oxygen consumption and energy production from glucose and lipid metabolism. Unfortunately, mitochondrial function is impaired in a number of diseases including neurodegenerative disease, atherosclerosis, hypertension and cancer [28-31]. Fewer and smaller mitochondria are found in skeletal muscle of insulin resistant, obese and type II diabetic individuals [32]. Previous studies found decreased muscle oxidative capacity and succinate dehydrogenase activity, complex II of the electron transport chain, after SCI [33-35].

One hypothesis is that skeletal muscle mitochondrial function is decreased in metabolic disorders, leading to decreased fatty acid oxidation which contributes to insulin resistance [36, 37]; however, this is controversial [37] . Mitochondria are dynamic organelles and undergo biogenesis, remodeling, and degradation. Mitochondrial biogenesis is driven in part through the action of peroxisome proliferator-activated receptor (PPAR) coactivator 1 alpha (PGC-1α) [38, 39]. In addition, PGC-1α integrates insulin signaling and lipogenesis in skeletal muscle [40, 41]. PGC-1α is decreased in animal and human models following denervation [42, 43]. Decreased

mitochondrial biogenesis and reduced mitochondrial mass may result in decreased energy production and therefore play a significant role in the altered metabolic profile following SCI [44].

Body composition and metabolic changes after SCI may be further exacerbated by reduced anabolic hormones including testosterone (T), growth hormone and the growth hormone second messenger insulin like growth factor-1 (IGF-1) [45, 46]. Previous studies have shown that 60% of men with SCI have low T and that testosterone replacement therapy (TRT) increases IGF-1 in men [47-50]. In rodent models of SCI, TRT attenuates the loss of muscle [51, 52]. TRT decreases total body fat, increases lean mass [53, 54] and increases the number of proliferating skeletal muscle satellite cells in in men [55]. These findings suggest that TRT may provide metabolic benefits to individuals with SCI.

Resistance training (RT) improves insulin sensitivity and increases fatty acid and carbohydrate metabolism as well as attenuates sarcopenia in the elderly and after SCI [56-63]. Moreover, RT has been shown to influence body composition by increasing lean mass, decreasing FM and reducing VAT, suggesting that the benefits of RT could overcome the risk of developing insulin resistance [56-59]. Functional electrical stimulation (FES) has been shown to improve fatty acid kinetics, carbohydrate metabolism and vascular health after SCI [60-63]. Electrically evoked RT using neuromuscular electrical stimulation (NMES-RT) and ankle weights is another form that has been shown to be effective in inducing muscle hypertrophy in individuals with chronic SCI [59, 64]. One study showed a 40% increase in skeletal muscle size and improved glucose tolerance after 12 weeks of training [59]. Another study showed that following 12 weeks of NMES-RT, whole thigh, knee extensor and flexor cross sectional areas (CSAs) increased by 28%, 35% and 16%, respectively. Moreover, the ratio of leg FFM to whole

body FFM increased by 20% following intervention. There was 32% decrease in glucose area under the curve adjusted to muscle CSA following 12 weeks of NMES-RT. However, there were only modest effects on whole body composition as well as a non-significant decrease in VAT [64]. It is possible that the limited effects of NMES-RT on parameters of body composition and VAT can be possibly explained by depressed T-level in persons with SCI. Supplementing exogenous T may optimize the outcomes of NMES-RT on parameters of body composition and metabolic profile such as increase basal metabolic rate (BMR).

TRT may be an effective therapy to counterbalance the growing rate of obesity, type II diabetes and cardiovascular disease among individuals with SCI. Moreover, results from the current trial may provide evidence that TRT is an effective intervention for those who cannot effectively benefit from NMES because of lower motor neuron denervation or intolerance to electrical stimulation. Therefore, the primary hypothesis is that the addition of TRT will maximize the benefits of electrically evoked RT on parameters of body composition and metabolic profile in men with chronic complete SCI. We, hereby, report the design of a study for which the major research goal is to investigate the effects of 16 weeks of evoked RT+TRT vs. TRT on body composition (primary outcome variables; muscle CSA, VAT, %FM) and metabolic profiles (secondary outcome variables; glucose and lipid metabolism) in individuals with motor complete SCI.

Methods and analysis

Study design

A randomized controlled study was undertaken in which individuals with SCI were randomized to receive RT+TRT or TRT alone for 16 weeks. The study was approved by the McGuire Veteran Affairs Investigation Research Board and the Virginia Commonwealth

University (VCU) Office of Research and Innovation. The trial has been registered at clinicaltrials.gov (NCT01652040). A member of the research team explained the study and obtained written informed consent. After informed consent each subject underwent a detailed physical examination at the Hunter Holmes McGuire VA Medical Center (VAMC) by a physiatrist board certified in SCI medicine. This exam included a neurological assessment according to the International Standards for Neurological Classification of SCI (ISNCSCI), including the American Spinal Injury Association (ASIA) Impairment Scale (AIS) [65].

The study design and procedures are presented in Figures 1 and 2. The study visits included estimation of body composition, anthropometry, and dual x-ray absorptiometry (DEXA; baselines 1 and 2 and post-interventions 1 and 2). Additionally, MRI scans were obtained for trunk adipose tissue and lower extremity skeletal muscles and IMF CSA (baseline 2, post-interventions 1 and 2). Participants were then escorted to the VCU-CRS unit (VCU Clinical Research Unit) for dinner, and remained in the VCU-CRS unit overnight for the four study visits. Muscle biopsies were obtained at baseline 2 and post intervention 1.

Recruitment and Randomization

The recruitment process started in July 2012 and ended in June 2015. Data analysis is currently being performed. Recruitment details and randomization are presented in Table 1 and Figure 3. Prior to the start of the study, numbers 1-26 were randomized using the n-Query software program by the principal investigator. At the end of the two-day assessment period (Baseline 1), the allocation of subjects into either group was performed in a blinded fashion by drawing a folded paper with a number (1-26) by the research coordinator. This number was matched with the assignment from the randomization procedure.

Twenty-six participants were recruited to participate in the study. A four week delayed entry period was included to obtain baseline measurements, stabilize body weight and educate participants on how to monitor their dietary intake. This allowed participants to serve as their own controls. Four participants withdrew from the trial. At baseline 2, two participants failed to comply with study protocol and withdrew from the study because lack of interest. Nurses failed to locate the veins on the third participant to conduct IVGTT and he was asked to withdraw from the trial. The fourth participant was diagnosed with a grade IV pressure ulcer after being admitted into the trial. Following the delayed entry period, participants were randomly assigned to a RT+TRT group (n = 13) or TRT group (n = 13). TRT patches (2-6 mg/day) were replaced daily on alternating shoulders at bedtime for 16 weeks. The RT+TRT group received 16 weeks of supervised unilateral progressive RT using surface NMES and ankle weights. Following the intervention the two-day assessment period was repeated (Figure 1).

Participants and eligibility criteria

Participants were men between 18-50 years old with a BMI of $\leq$ 30 kg/m^2. The upper limit of age was set to 50 years to avoid unanticipated side effects that may result from TRT. Participants had motor complete SCI C5-L2, ASIA A or B. Participants with pre-existing medical conditions were excluded. These included cardiovascular disease, uncontrolled type II diabetes and those on insulin, pressures sores stage 2 or greater, supra-physiological T level, hematocrit above 50% and urinary tract infection or symptoms.

I. Interventions

Resistance training

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 weight was added. Surface NMES was applied to the knee extensor muscles via surface electrodes (Figure 2). One electrode was placed 2–3 cm above the superior aspect of the patella over the vastus medialis muscle, and the other lateral to and 30 cm above the patella over vastus lateralis muscle. Current from the stimulator was manually increased in 5-second intervals to evoke full knee extension with a 3-minute rest between sets, 30 Hz, 450 μs pulses and a current sufficient to evoke full knee extension as previously described [64, 66, 67]. Four sets of 10 repetitions was performed twice weekly for 16 weeks.

Testosterone replacement therapy (TRT)

Following baseline measurements, T was administered by patches (Androderm, Watson Pharma. Inc, Parsippany, NJ) that delivered between 2-6 mg/day [53] (Figure 2). Serum T concentration was measured and reviewed in a blinded fashion weekly for the first month and then every 4 weeks by an endocrinologist. Baseline dose was prescribed according to the initial T level. A dose of 6, 4 or 2 mg/day was initially prescribed if the serum baseline T-level was less than 300, 300-600 or above 600, respectively. During the course of the study, the dose was decreased to 2 mg/day if the serum T concentration was more than 1000 ng/dL (34.7 nmol/L) and the participant was reeducated about use of the patch if the concentration was less than 250 ng/dL (8.7nmol/L) above the pretreatment concentration. Patches were returned after use to ensure adherence to the intervention protocol. Participants were instructed to place patches at bedtime and only remove them during showering. If skin irritation became an issue, participants were initially advised to move patches up or down on the shoulder muscles from the irritation site and if the situation was not resolved, a hydrocortisone cream was prescribed.

Detraining after 16-week intervention

Six participants from each group were followed for 16 weeks after the initial intervention. The RT+TRT group continued training once weekly using the same training approach for an additional16 weeks. For the first week, the maximum ankle weights attained during the training phase were used. The weights were then gradually decreased by two pounds per week until the lowest weight was attained (2 pounds). TRT dose was set at 2 mg/day for the entire detraining phase. Participants from the TRT group were followed for additional 16 weeks without intervention. Following the detraining phase, the two-day assessment period was repeated without performing skeletal muscle biopsy (Figure 1). The rationale of the detraining phase is to determine whether once weekly training can maintain skeletal muscle hypertrophy, body composition and metabolic improvements incurred by the 16 week intervention.

II. Primary Outcomes

Data will remain confidential at all times and any patient identifiers will be removed prior to data analysis. Analysis for all study procedures will be performed in a blinded fashion ensuring full concealment until complete data analysis.

Anthropometrics and Body Composition Assessments

Height of each participant was determined while lying in the supine position. Two smooth wooden boards were placed at the participant's head and heels and the distance between them was measured to the nearest cm. Measurement of waist circumference was determined in triplicate by identifying the narrowest region of the trunk from sitting and supine positions. Three-site skin fold assessment was conducted in triplicate for suprailiac, abdominal and thigh.

A Lunar Prodigy Advance (Lunar Inc., Madison, WI) bone densitometer was used to measure total body and regional (lumbar spine, proximal femur, and forearm) FM and FFM.

Testing was performed after lower extremity elevation for at least 20 minutes to minimize fluid shift. The subject was assisted to lie on a padded table and both legs were strapped proximal to the knees and ankles. The arms and legs were positioned to ensure proper alignment.

Magnetic resonance imaging (MRI)

MRI was performed at the VAMC Hospital using a 1.5 Tesla magnet (GE) as previously described [4, 19, 59, 68, 69]. Transaxial images, 10 mm thick and 10 mm apart, were taken from the hip joint to the knee joint and from knee to the ankle using the whole body coil. The location of the scan was identified by placing a mark 6 inches proximal to and distal to the patella and matched on follow up scans. To analyze VAT and subcutaneous adipose tissue (SAT) transverse slices (0.8 cm thickness) were acquired every 0.4 cm gap from the xyphoid process to the femoral heads. Images were acquired in series of two stacks with L4-L5 used as a separating point. TRT patches were removed 48-72 hours prior to MRI scans to avoid skin burn.

Analyses will be performed using commercial available software (X-vessel) as previously described [4, 19, 68, 69]. Briefly, the thigh and leg images will be segmented into fat (high intensity), skeletal muscle (mid intensity) and background/bone (low intensity). Manual selection of a pixel of skeletal muscle will highlight all skeletal muscle pixels and provide the total number of skeletal muscle pixels while excluding fat. VAT and SAT will be measured by manually tracing around the anatomical borders. The number of pixels in the highlighted region will be multiplied by the matrix size to measure VAT and SAT CSA (cm^2).

Skeletal muscle torque and specific tension

Torque of the knee extensor muscle group was evaluated using a Biodex isokinetic dynamometer (Shirely, NY). Measurements were done 72 hours after the muscle biopsy to prevent acute effects on protein expression. Participants were seated with both the trunk-thigh

angle and the knee-thigh angle at 90º. Each participant was securely strapped to the test chair by a crossover shoulder harnesses and a belt across the hip joint. The axis of the dynamometer was aligned to the anatomical knee axis and the lever arm was attached 2-3 cm above the lateral malleolus. Before measuring isometric torque, passive tension of the right knee extensor muscle group was measured at 5, 30, 60, 90,180, 270 degrees/sec as an index of spasticity. Isometric torque was measured using current amplitude of 50 and 100 mA at a frequency of 30 Hz and pulse duration 450 μs. Isokinetic torque was measured at 60, 90 and 180 degrees/sec using the same stimulation protocol.

Serum and plasma analysis

All metabolic profile analysis is presented in Table 2. Blood samples were collected after an overnight fast. Total T was measured by liquid chromatography with isotope dilution mass spectrometry detection after supported liquid extraction. Free T concentration was calculated using sex hormone binding globulin and albumin concentrations (www.issam.ch/freetesto.htm) [70]. Serum IGF-I concentration was measured by an immunoluminometric assay (Quest Diagnostics, Madison, NJ). Fasting lipid profiles (HDL-C, LDL-C, total cholesterol, and triglycerides) were determined as previously described [8, 20]. Inflammatory biomarkers CRP, IL-6, TNF-α , and free fatty acids were determined by commercially available enzyme-linked immunosorbent assay kits (ALPCO; Salem, NH).

Energy Expenditure

After an overnight fast for 10-12 hours, participants were kept in a dark room for 20-30 minutes to attain a resting state during which BMR was measured as previously described [8]. Briefly, while in a supine position a canopy was placed over the subject's head. Each subject was allowed 2-3 minutes before starting the test to ensure no signs of apnea or claustrophobic

episodes. All subjects were instructed to stay awake during the entire test and to breathe normally. The canopy was then attached to a vacuum to draw the expired gases to the flowmeter of the metabolic unit (COSMED KB42). Prior to the test, the metabolic unit was calibrated using the standard procedures identified by the manufacturer. Carbon dioxide and oxygen output was used to calculate the respiratory exchange ratio and BMR (kcal/day) was calculated using the average of the last 15 minutes of the test. This was used to measure the percentage of substrate utilization (% fat vs. % carbohydrate) [8, 71].

To determine whether NMES-RT improves exercise performance, testing was performed using a functional electrical stimulation bike (Restorative Therapies, RTI-300) against progressive resistance protocol until fatigue. The protocol started with 3 minutes resting, 3 minute warm-up (35-37 RPM) using the servomotor and then a two minute incremental progressive resistance protocol (1 Nm, 3 Nm, 5 Nm, etc.) until fatigue. After fatigue, a one minute cool down period was allowed followed by 5 minutes of rest. Energy expenditure and cardiovascular performance [VO_2 (l/min), blood pressure and heart rate] was collected at baseline 2 and post-interventions 1 and 2.

Each participant met with a dietician at the start of the study and was asked to maintain a 5 day food dietary log monitoring their caloric intake for the duration of the study. Participants were instructed to record all liquid and food consumption and no nutritional advice was given on the size or the portion of the food. Dietary logs were analyzed on a weekly basis using a nutritional software package (Nutrition Data System for Research version 2014) under the supervision of a registered dietitian. After analysis was completed, the average caloric intake (kcal) and percentage macronutrients (carbohydrates, fat and protein) were calculated. Each participant received monthly feedback via phone call with the registered dietician on how to

maintain appropriate dietary habits based on his BMR and percentage macronutrients (45% carbohydrates, 30% fat and 25% protein). Every effort was made to balance the dietary habits between the RT+TRT and the TRT group.

Intravenous Glucose Tolerance Test (IVGTT)

An IVGTT was used to determine insulin sensitivity and glucose effectiveness before training and 48 hours after the last exercise bout. After an overnight fast, an intravenous line was placed to facilitate infusion of glucose and blood sampling. Blood samples were taken every 2-3 minutes before and 30 minutes after glucose injection (0.3 gm/kg IV over 30 seconds), followed by 5-10 minutes sampling ending at 180 minutes. Twenty minutes after the glucose injection a bolus of insulin (0.02 U/kg) was injected to determine insulin sensitivity. Plasma glucose was measured by the Autoanalyzer glucose oxidase method and plasma insulin concentrations were determined by commercial radioimmunoassay (Table 2). The glucose disposal rate per unit of secreted insulin per unit time and glucose mediated glucose disposal rate were calculated from a least-squares fitting of the temporal pattern of glucose and insulin throughout the IVGTT using the MINMOD program [72]. A representative analysis of IVGTT is presented in Figure 4. The acute insulin response to IV glucose was calculated as the mean rise in plasma insulin above baseline at 3, 4 and 5 minutes after IV glucose administration. KG, a measure of glucose tolerance, was calculated as the least square slope of the natural log of absolute glucose concentration between 5 and 20 minutes after the glucose bolus [73]. The homeostatic model of assessment of insulin resistance (HOMA-IR) was calculated and insulin sensitivity was determined using Matsuda and Defronzo formula [73, 74].

III. Secondary Outcomes

Skeletal muscle biopsy

Biopsy samples of vastus lateralis muscle (~50-100 mg wet weight total) were obtained by a 14 gauge tru-cut biopsy needle, immediately prior-to and 72 hours after the 16 weeks of RT+TRT or TRT interventions. The 72 hours post-intervention was to avoid any acute effects from the last training bout on muscle protein expression to ensure that changes are due training effect. There was no muscle biopsy during the detraining phase. The biopsy samples were quickly frozen in liquid nitrogen and stored at -80°C until further analysis. One sample was split into two halves and used for measuring activities of mitochondrial enzymes. The second sample was used for Western blot analysis. The third sample was used for immunohistochemistry.

Mitochondrial Electron Transport Chain activities

Electron transport chain enzyme activities were measured spectrophotometrically in skeletal muscle homogenates as previously described [75]. Rotenone-sensitive NADH cytochrome c reductase measured complexes I and III. Decylubiquinol-cytochrome c oxidoreductase was measured as the antimycin-sensitive reductase to assess complex III. Citrate synthase was measured as an estimate of mitochondrial mass as previously described [75].

Protein content

Proteins were resolved by SDS-PAGE then transferred to a PVDF membrane (Trans-blot; Bio-Rad). Equal protein loading was confirmed with a Ponceau S stain after the transfer. After blocking for one hour, membranes were incubated overnight at 4 ˚C with primary antibody diluted 1:1000. Primary antibodies included glucose transpoter-4, focal adhesion kinase, PGC-1α (Santa Cruz Biotechnology), total protein kinase B (AKT), phosphorylated AKT, total mammalian target of rapamycin (mTOR), phosphorylated mTOR, (Cell Signaling). Membranes

were rinsed and placed in a 1:2000 solution of a horseradish peroxidase-conjugated secondary antibody (Cell Signaling) for 1 hour at room temperature. Membranes were then rinsed and incubated with a horseradish peroxidase chemiluminescence detection reagent (GE Healthcare) for 5 minutes. Proteins were visualized using an Amersham Imager 600 (GE Healthcare). Optical densities were measured using iQuant software and all samples were normalized to the baseline values for that participant.

Histological Analysis

Immediately after muscle biopsy, samples were mounted on tongue blades by using a medium of OCT compound and tragacanth gum and stored at -70°C until analysis. Serial cross sections (8-10 μM) were collected on glass slides and frozen at -20°C until analysis. Fiber type and CSA will be determined by histochemical staining for myosin ATPase (preincubation at pH 4.3 or 9.4) as previously described [76]. Type I fibers will be identified by dark staining after acid preincubation, type II fibers light staining, and type IIB intermediate. At pH 9.4 the staining pattern was the opposite. Haematoxylin & Eosin (H&E) staining was performed according to conventional histological procedures. Mitochondrial complex II and IV activity was estimated by the activity of succinate dehydrogenase and cytochrome c oxidase activity, respectively, as previously described [77, 78]. Stained muscle sections will be observed using an Olympus BX-51 fluorescent microscope (Olympus, Tokyo, Japan) and analyzed using ImageJ software.

Statistical Analyses

Paired t-tests will be used to determine differences in body composition and metabolic profile between baseline 1 and baseline 2. To determine the effect of interventions and detraining, a repeated measures ANOVA will be used. Simple linear and multivariate regression analyses will be used to examine the relationship between body composition and metabolic

profile variables. For protein expression, we will use paired t-tests to examine the effects of each intervention.

The effect size was calculated based on the effects of RT on body composition and metabolic profiles that were previously published [62]. The number of subjects necessary to find statistical differences in the major variables (muscle size, VAT and insulin concentration) of this study was found to be 10 participants per group. One participant from the RT+TRT group withdrew after developing side effects to TRT patches and the medical monitor personnel recommended him to withdraw from the trial in week 8. However, we will perform intent to treat analysis on his data, which means that despite his early withdrawal from the study his data will be included in the final analysis. This will allow extrapolation of his post-intervention data using the SPSS missing values option. We anticipate that we will collect sufficient data to determine the effects of rehabilitation interventions on protein expression, mitochondrial enzymatic and ETC activities in individuals with SCI. Statistical analysis will be performed using SPSS version 23.0 (Chicago, IL) with a level of significance set at $p<0.05$.

Ethics and Dissemination

Ethical approval has been obtained from the institutional review boards at the McGuire VA Medical Center and Virginia Commonwealth University. All participants read and signed approved consent forms prior to baseline assessment. Results of the study will be published in peer-reviewed journal and presented at national and international conferences.

Data Monitoring

The research staff oversaw and monitored the study to ensure data quality and participant compliance. There were no adverse events. Only members of the research team will have access to data.

Discussion

Individuals with SCI experience profound skeletal muscle atrophy, deterioration in body composition and abnormal metabolic profile. Within few weeks of injury, there is a significant decrease in whole body FFM, particularly lower extremity skeletal muscle mass, and subsequent increase in FM. These changes predispose this population to the risk of glucose intolerance, insulin resistance, dyslipidemia and the development of type II diabetes and cardiovascular disease. The main purpose of this study is to investigate the effects of 16 weeks of evoked RT+TRT or TRT alone on body composition (muscle CSA, IMF, VAT, %FM, FFM) and metabolic profile (glucose, lipid and BMR) in individuals with motor complete SCI.

Ectopic adipose tissue accumulation, IMF and VAT, has been strongly associated with altered metabolic profile after SCI [19, 20]. IMF has been determined to account for a 70% reduction in glucose tolerance in individuals with complete SCI [19]. VAT is independently associated with impairment in glucose tolerance, insulin resistance and dyslipidemia after SCI [20]. Edwards et al noted significant positive association between VAT and insulin resistance and a negative correlation between VAT:SAT ratio and HDL-C [13]. Increase in VAT is also related to leptin and plasminogen activator inhibitor-1 concentrations [14]. It is possible that increases in FM are associated with inflammatory biomarkers that trigger metabolic syndrome after SCI [22]. Ectopic adipose tissue has been demonstrated to secrete pro-inflammatory cytokines, including IL-6 and TNF-α. This stimulates hepatic production of CRP which is suggestive of vascular inflammation [15-19].

RT is an important type of exercise that has been shown to induce positive physiological adaptations such as increasing lean mass and reducing the incidence of metabolic disorders in other clinical populations. Previous work suggests that twice weekly NMES-RT can induce

favorable body composition and metabolic adaptations. Twelve weeks of NMES-RT has shown to increase thigh muscle CSA by 35-40% as measured by MRI [59]. Moreover, there was a reduction in %leg FM and a trend towards decrease in VAT CSA after 12 weeks of NMES-RT [64]. The favorable adaptations in body composition were associated with decrease in plasma insulin area under the curve and plasma triglycerides [59]. These findings were attributed to an increase in plasma IGF-1. However, the effect of training appears to be limited to the trained muscle and only modestly impacted whole body composition. It is unclear whether a RT program longer than 12 weeks may provide additional benefits to individuals with SCI.

The cellular changes underlying the alterations in skeletal muscle glucose utilization and energy metabolism after SCI are unclear. Previous work indicated that expression of AMP-activated protein kinase (AMPK), a key regulator of energy homeostasis for lipid and carbohydrate utilization, was altered in persons with SCI compared to BMI matched able bodied controls [79]. Another study revealed decreased expression of genes involved in glucose and lipid metabolism [80]. Despite these abnormalities, one study reported that leg glucose uptake during cycling was increased in individuals with SCI compared to able-bodied controls [61]. Another study showed similar glucose uptake of isolated muscle fibers from SCI and able bodied individuals *in vitro* [81]. Benefits of other forms of functional electrical stimulation lower extremity cycling (FES-LEC) have included improvements in body composition, carbohydrate- and lipid metabolism and muscle fiber type composition [60-63, 82-84]. Similarly, we have recently shown that 16 weeks of FES-LEC increased the protein abundance of GLUT-4, PGC-1α and AMPK by 3.8, 2.3 and 3.4 fold, respectively, in the vastus lateralis muscle in persons with motor complete SCI [85].

TRT has been shown to increase skeletal muscle mass in hypogonadal men, men with chronic illness, and older men [53-55]. Androgen deficiency in men is associated with a loss of FFM and an increase in FM [53]. In epidemiologic studies, men with decreased free T had lower appendicular skeletal muscle mass than those with normal T levels [86]. Previous work documented that TRT increases muscle mass with a reciprocal decrease in total body FM [53, 54]. This reciprocal action has been suggested due to a switch from adipogenic toward myogenic differentiation of mesenchymal stem cells [86]. In a randomized controlled double blinded clinical trial, TRT was shown to improve insulin sensitivity, CRP and reduce VAT [54]. Sixty percent of men with SCI have low T level and levels are associated with time since injury [49, 50]. TRT has also been reported to increase IGF-1 and several molecular mechanisms related to the protective pathways have been recently elucidated [47, 48, 51, 87]. Therefore, enhancing the decline in anabolic homeostasis by providing TRT may provide additional benefits as was previously demonstrated by increasing lean mass and metabolic rate in individuals with SCI.

It was very important to highlight that this protocol has 3 different phases including four weeks of delayed entry, 16 weeks of intervention and 16 weeks of detraining. The delayed entry period was included to allow each participant to serve as his own control. Moreover, we were successful in retaining 12 participants (n=6/group) to complete the detraining phase. This means that we had participants 6 participants that agreed to stick to our exercise program and the use of TRT patches up to 9 months. This may reflect on the study protocol frequency that ensured long-term adherence despite the length of the study. A very important point that is worth highlighting is that our study protocol was designed to include 3 levels of outcome measurements including body composition, metabolic profile and cellular changes. This design

is likely to provide mechanistic explanations to changes that occur at the body composition and metabolic levels

Limitations

The current study was limited to those who were less than or equal to 50 years old. Because of advances in healthcare, many individuals with SCI have a near-normal life span which may make the results of this study less generalizable. However, this age limit was implemented because the effect of TRT on cardiovascular health has been controversial. There are data showing that hypogonadism is a risk for cardiovascular disease [88]. Some replacement studies show increased risk, but another study showed decreased mortality in men receiving testosterone [89]. Another study in older men reported that injectable testosterone may be associated with increased cardiovascular risk but topical testosterone was not [90]. Therefore, we set the inclusion criteria of less than or equal to 50 years old to reduce the likelihood of developing cardiovascular complications.

Currently NMES-RT is not readily available to the majority of SCI patients. Women were not included because administering TRT is not either appropriate or safe, because women are at risk of virulization by testosterone. Thus, the trial was limited only to males with SCI. Moreover, only 21% of individuals with motor complete SCI are women based on the Model System Data.

In summary, we anticipate that this trial will provide important insights into the body composition and metabolic benefits of 16 weeks of evoked RT+TRT or TRT. If beneficial, this may be a feasible strategy for the rehabilitation of individuals with chronic SCI and increase the health of this and other clinical populations. Additionally, TRT alone may provide an alternative intervention for those who cannot benefit from training using surface NMES, because of lower

motor neuron (LMN) denervation or intolerance to applications of electrical stimulation. Although the current study did not include any cauda equina participants, further research is warranted to examine the effects of TRT on muscle atrophy following LMN denervation. This is important because skeletal muscle following LMN denervation may respond differently than innervated muscle. The study will also shed light on several molecular pathways that have been suggested to influence both body composition and metabolic profile.

Trial Status: Enrollment into the study started in July 2012 and as of April 2016 all participants have completed the study. Data collection and data analysis are expected to be completed in December 2016. The study is expected to be closed in June 2017.

Contributions: ASG supervised all aspects of the trial including all interventions and measurements and secured funding for the trial procedures. ASG, REK, LCO drafted the manuscript. REK, JS, RK, and LCO contributed to data collection and analysis. RG, TL, TC, DXC, CC, RA, EJL and DRG are research physicians that contributed to patient monitoring and study design. DXC and RA will provide guidance during data analysis and manuscript preparation. All authors affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Competing interests: The authors have no competing interests to declare

Funding: This work was supported by VA-RRD CDA2 to Ashraf S. Gorgey, clinical trial registration number NCT01652040.

Acknowledgements: We would like to thank Rodney Wade, Sakita Sistrun, Liron Segal for helping in the processes of data capturing and analysis as well as the study participants for their time and effort. We would also like to thank Hunter Holmes McGuire Research Institute and Spinal Cord Injury Services and Disorders for providing the environment to conduct clinical human research trials. VCU Clinical Research Service for providing space and staff to perform the blood work procedures. The work is supported by the Department of Veteran Affairs, Veteran Health Administration, Rehabilitation Research and Development Service (B7867-W).

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Table 1. Randomization of individuals with motor complete SCI into either RT+TRT (n=13) or TRT (n =13) using n Query software with a treatment ratio 1:1.

Subject ID	Assignment	Baseline 1	Baseline 2	Post-Intervention 1	Post-Intervention 2
10001	RT+TRT	C	C	C	C
10002	TRT	C	C	C	C
10003	TRT	C	C	C	C
10004	RT+TRT	C	C	C	C
10005	RT+TRT	C	C	C	C
10006	TRT	C	C	C	X
10007	RT+TRT	C	C	C	C
10008	TRT	C	C	C	C
10009	RT+TRT	C	withdraw	X	X
10010	TRT	NA	NA	NA	NA
10011	RT+TRT	C	withdraw	x	x
10012	TRT	C	C	C	C
10013	TRT	C	C	C	x
10014	RT+TRT	C	C	C	withdraw
10015	TRT	C	C	C	x
10016	TRT	C	C	C	C
10017	RT+TRT	C	C	C	x
10018	TRT	C	C	C	x
10019	RT+TRT	C	C	C	C
10020	RT+TRT	withdraw	x	x	x
10021	RT+TRT	C	C	C	x
10022	TRT	C	C	C	C
10023	RT+TRT	C	C	C	C
10024	TRT	C	withdraw	x	x
10025	RT+TRT	C	C	C	C
10026	RT+TRT	C	C	withdraw	x
10027	TRT	C	C	C	x

C: completed; **NA**: not assigned for #10. Baseline 1 was followed by 4 weeks of no intervention for all the participants. Prior to baseline 2, randomization was performed into RT+TRT or TRT groups. Post-intervention 1 (n=22) was conducted following 16 weeks of intervention. Post-intervention 2 (n= 13) was conducted following 16 weeks of RT+TRT (n=6) or no intervention (n=6).

Table 2. Metabolic health variables measured at baseline 1, baseline 2, post-intervention 1 and post-intervention 2

	Quantity	Special handling	Techniques of Analysis
Insulin and Glucose	2 ml	Potassium oxalate/sodium fluoride tube (Grey Top)	ELISA and biochemistry analyzer
HBA1C		SST	Standard Procedure
Testosterone every 4 weeks	4 ml	SST	Liquid chromatography with isotope dilution mass spectrometry detection
Albumin*		SST	Standard Procedure
SHBG*		SST	Standard Procedure
IGF-1, IGFBP-1 and 3	4 ml	SST	ELISA
Inflammatory biomarkers (CRP, IL-6, TNFα)	4 ml	SST	ELISA
Free fatty acids	2 ml	Potassium oxalate/sodium fluoride tube (Grey Top)	Enzymatic colorimetric quantification
Triglycerides, total cholesterol, HDL, LDL	4 ml	SST	Enzymatic colorimetric quantification

*, Only at baseline 2 and post-intervention 1 to calculate free testosterone. ELISA, enzyme-linked immunosorbent assay; HBA1C, hemoglobin A1c; SHBG, sex hormone binding globulin; SST, serum separator tube; IGF-1, insulin-like growth factor 1; IGF-BP, insulin-like growth factor binding protein; CRP, C-reactive protein; IL-6, interleukin 6; TNFα, tumor necrosis factor alpha

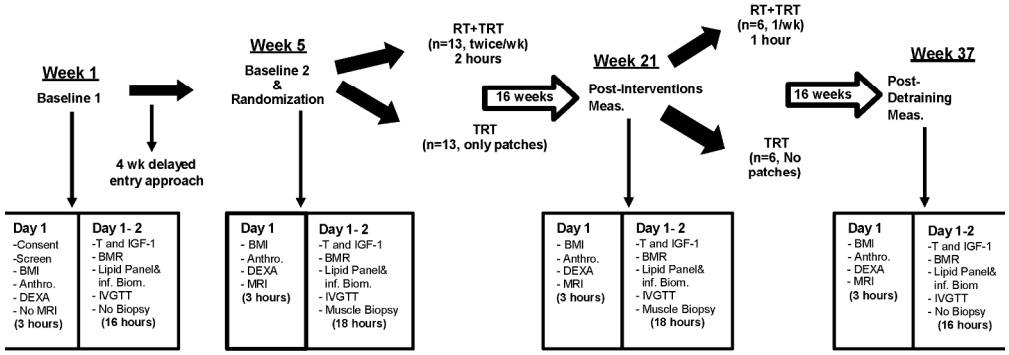
Figure Legends

Figure 1. Timeline and main procedures of the TEREX-SCI trial for both the RT+TRT and TRT groups during baseline 1, baseline 2, post-intervention 1 and post-intervention 26 for persons with motor complete SCI.

Figure 2. A person with T4 motor complete SCI undergoing both electrically evoked RT (left panel) and TRT using transdermal patches (right panel) as a part of a 16 week intervention.

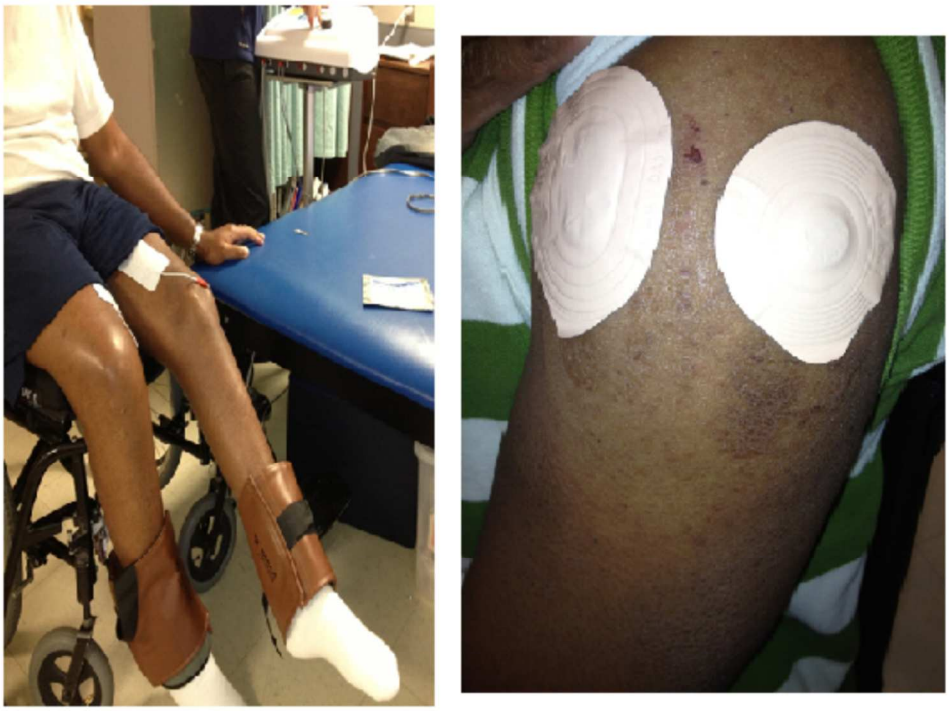
Figure 3. Schematic diagram showing the process of recruitment over the 3 year period of the TEREX trial.

Figure 4. A representative figure of analysis for IVGTT in a person with SCI after infusion of dextrose followed by insulin 20 minutes later. The dextrose concentration is calculated by multiplying 0.3g x body weight (kg) in 50% solution. Insulin concentration is determined by multiplying 0.02 units x body weight (kg). Blue line represents fasting glucose concentration, resting, following infusion, and over 120 minutes. Red line represents the line of best fit of glucose concentration. Green line represents fasting insulin level, spike following 20 minutes of dextrose infusion and over 120 minutes. Black line represents the line of best fit for insulin concentration.



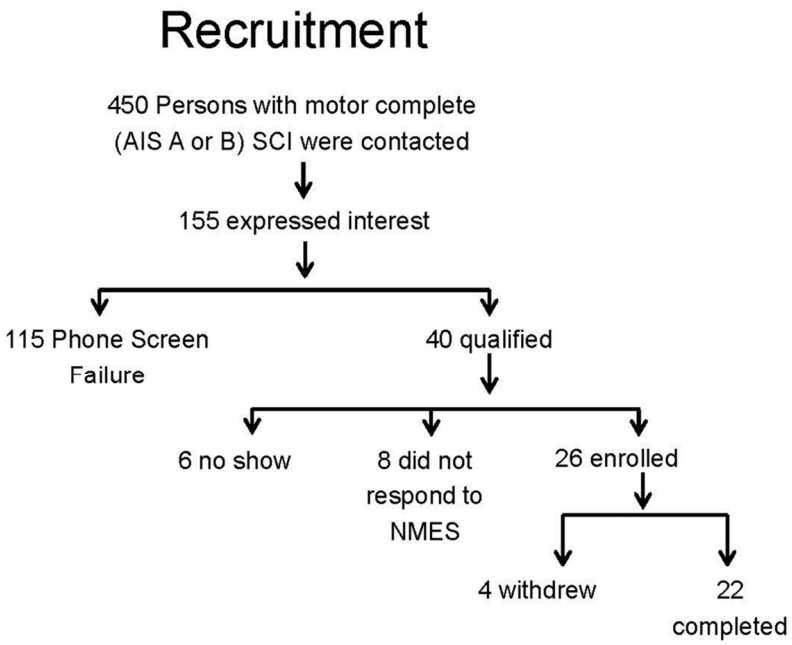
Timeline and main procedures of the TEREX-SCI trial for both the RT+TRT and TRT groups during baseline 1, baseline 2, post-intervention 1 and post-intervention 26 for persons with motor complete SCI.

215x77mm (300 x 300 DPI)



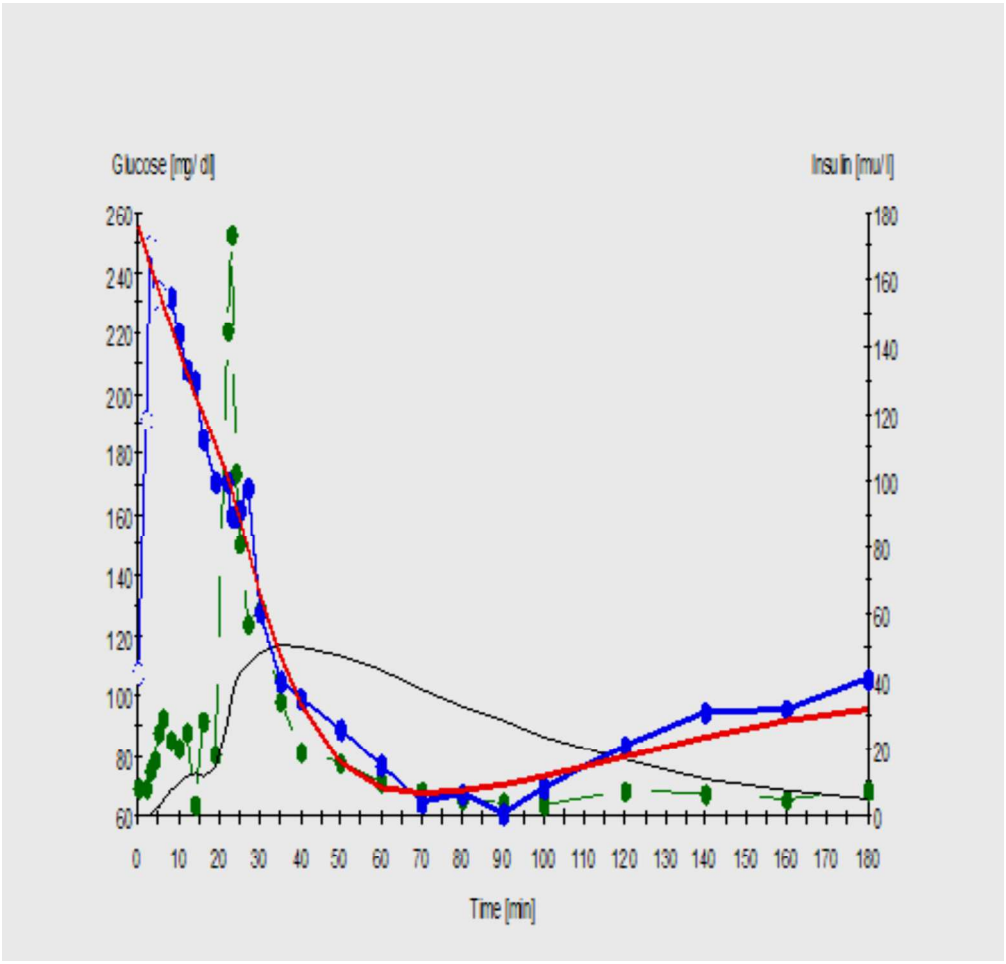
A person with T4 motor complete SCI undergoing both electrically evoked RT (left panel) and TRT using transdermal patches (right panel) as a part of a 16 week intervention.

121x92mm (300 x 300 DPI)



Schematic diagram showing the process of recruitment over the 3 year period of the TEREX trial.

114x76mm (300 x 300 DPI)



A representative figure of analysis for IVGTT in a person with SCI after infusion of dextrose followed by insulin 20 minutes later. The dextrose concentration is calculated by multiplying 0.3g x body weight (kg) in 50% solution. Insulin concentration is determined by multiplying 0.02 units x body weight (kg). Blue line represents fasting glucose concentration, resting, following infusion, and over 120 minutes. Red line represents the line of best fit of glucose concentration. Green line represents fasting insulin level, spike following 20 minutes of dextrose infusion and over 120 minutes. Black line represents the line of best fit for insulin concentration.

126x120mm (300 x 300 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	2
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4, 9
	2b	All items from the World Health Organization Trial Registration Data Set	4, 9
Protocol version	3	Date and version identifier	9
Funding	4	Sources and types of financial, material, and other support	24
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	24
	5b	Name and contact information for the trial sponsor	24
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	24
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-8
	6b	Explanation for choice of comparators	6-8
Objectives	7	Specific objectives or hypotheses	8
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	2, 8
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8-9
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12-18
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9, Fig 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	19
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9-10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-18
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	18-19

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18-19
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	19
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	19
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	19
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	3, 8-9, 19
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	19
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	19
	31b	Authorship eligibility guidelines and any intended use of professional writers	24
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	attached
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	17-18

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.