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

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1 **Abstract**

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

10 **Methods and analysis:** Twenty-six individuals with chronic, motor complete SCI between 18-
11 50 years old were randomly assigned to a RT+TRT group (n = 13) or a TRT group (n = 13).
12 Twenty-two participants completed the initial 16-week training phase of the study and 4
13 participants withdrew. Thirteen participants out of the 22 completed the second 16 week of the
14 detraining phase. The TRT was provided via transdermal testosterone patches (4-6 mg/day). The
15 RT+TRT group had 16 weeks of supervised unilateral progressive RT using surface
16 neuromuscular electrical stimulation with ankle weights. This study will investigate the effects of
17 evoked RT+TRT or TRT alone on body composition (muscle cross sectional area, visceral
18 adipose tissue, %FM) and metabolic profile (glucose and lipid metabolisms) in individuals with
19 motor complete SCI. Findings from this study may help in designing exercise therapies to
20 alleviate the deterioration in body composition after SCI and decrease the incidence of metabolic
21 disorders in this clinical population.

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

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

3 **Trial Registration:** NCT01652040

4
5 **Keywords:** RESISTANCE TRAINING, SPINAL CORD INJURY, BODY COMPOSITION,
6 METABOLISM, TESTOSTERONE, MITOCHONDRIA, INFLAMMATORY BIOMARKERS

7 8 **Strengths and limitations**

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

1 Introduction

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

9 Previous studies reveal that 60% of individuals with SCI are either overweight or obese
10 [2, 9-11]. Individuals with SCI also have a redistribution of adipose tissue, with greater trunk FM
11 and visceral adipose tissue (VAT) compared to age and waist circumference matched able-
12 bodied controls [12-14]. Adipose tissue, particularly VAT, secretes proinflammatory cytokines
13 including interleukin-6 (IL-6) and tumor-necrosis factor- α (TNF- α). Therefore, the increase in
14 VAT after SCI may contribute to metabolic syndrome by stimulating the hepatic production of
15 C-reactive protein (CRP) which is tied to vascular inflammation [15-18]. Another type of ectopic
16 adipose tissue, intramuscular fat (IMF), is increased after SCI and has been correlated with
17 reduced insulin sensitivity [19, 20].

18 Metabolic changes also accompany SCI, with previous studies finding that more than
19 50% of individuals with SCI are glucose intolerant, while one out of five is diabetic [2, 9-11, 21].
20 Other studies report that 55% of individuals with SCI are at risk of developing metabolic
21 syndrome [22, 23]. Individuals with complete tetraplegia are more likely to experience
22 decreased glucose and carbohydrate tolerance and have a higher prevalence of heart disease than

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

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

11 One hypothesis is that skeletal muscle mitochondrial function is decreased in metabolic
12 disorders, leading to decreased fatty acid oxidation which contributes to insulin resistance [34,
13 35]; however, this is controversial [35]. Mitochondria are dynamic organelles and undergo
14 biogenesis, remodeling, and degradation. Mitochondrial biogenesis is driven in part through the
15 action of peroxisome proliferator-activated receptor (PPAR) coactivator 1 alpha (PGC-1 α) [36,
16 37]. In addition, PGC-1 α integrates insulin signaling and lipogenesis in skeletal muscle [38, 39].
17 PGC-1 α is decreased in animal models following denervation [40, 41]. Decreased
18 mitochondrial biogenesis and reduced mitochondrial mass may result in decreased energy
19 production and therefore play a significant role in the altered metabolic profile following SCI
20 [42].

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

1 of men with SCI have low T and that TRT increases IGF-1 in men [45-48]. In a rodent model of
2 SCI, TRT attenuates the loss of muscle [49, 50]. TRT decreases total body fat, increases lean
3 mass [51, 52] and increases the number of proliferating skeletal muscle satellite cells in in men
4 [53]. These findings suggest that TRT may provide metabolic benefits to individuals with SCI.

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

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

1 **Methods and analysis**

2 **Study design**

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

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

13 The study design and procedures are presented in Figure 1. The study visits included
14 estimation of body composition, anthropometry, and dual x-ray absorptiometry (DXA; baselines
15 1 and 2 and post-interventions 1 and 2). Additionally, MRI scans were obtained for trunk
16 adipose tissue and lower extremity skeletal muscles and IMF CSA (baseline 2, post-interventions
17 1 and 2). Participants were then escorted to the VCU-CRS unit (VCU Clinical Research Unit)
18 for dinner, and remained in the VCU-CRS unit overnight for the 4 study visits.

19 **Recruitment and Randomization**

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

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

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

16 **Participants and eligibility criteria**

17 Participants were men between 18-50 years old with a body mass index (BMI) of ≤ 30
18 kg/m². The upper limit of age was set to 50 years to avoid unanticipated side effects that may
19 result from TRT applications. Participants had motor complete SCI C5-L2, ASIA A or B.
20 Participants with pre-existing medical conditions were excluded. These included cardiovascular
21 disease, uncontrolled type II diabetes and those on insulin, pressures sores stage 2 or greater,
22 supra-physiological T level, hematocrit above 50% and urinary tract infection or symptoms.

23

1 I. Interventions

2 Resistance training

3 The first week of RT was conducted with no ankle weights to ensure that the knee
4 extensor muscles could extend the weight of the lower leg against gravity. Once full knee
5 extension was achieved in a sitting position, two pounds were added on a weekly basis with the
6 criteria that full knee extension was achieved before more weight was added. Surface NMES was
7 applied to the knee extensor muscles via surface electrodes. One electrode was placed 2–3 cm
8 above the superior aspect of the patella over the vastus medialis muscle, and the other lateral to
9 and 30 cm above the patella over vastus lateralis muscle. Current from the stimulator was
10 manually increased in 5-second intervals to evoke full knee extension with a 3-minute rest
11 between sets, 30 Hz, 450µs pulses and a current sufficient to evoke full knee extension as
12 previously described [62-64]. Four sets of 10 repetitions was performed twice weekly for 16
13 weeks.

14 Testosterone replacement therapy (TRT)

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

22
23

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

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

3 **Magnetic resonance imaging (MRI)**

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

12 Analyses will be performed using commercial available software (X-vessel) as previously
13 described [4, 19, 65, 66]. Briefly, the thigh and leg images will be segmented into fat (high
14 intensity), skeletal muscle (mid intensity) and background/bone (low intensity). Manual
15 selection of a pixel of skeletal muscle will highlight all skeletal muscle pixels and provide the
16 total number of skeletal muscle pixels while excluding fat. VAT and SAT will be measured by
17 manually tracing around the anatomical borders. The number of pixels in the highlighted region
18 will be multiplied by the matrix size to measure VAT and SAT CSA (cm²).

19 **Skeletal muscle torque and specific tension**

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

1 shoulder harnesses and a belt across the hip joint. The axis of the dynamometer was aligned to
2 the anatomical knee axis and the lever arm was attached 2-3 cm above the lateral malleolus.
3 Before measuring isometric torque, passive tension of the right knee extensor muscle group was
4 measured at 5, 30, 60, 90, 180, 270 degrees/sec as an index of spasticity. Isometric torque was
5 measured using current amplitude of 50 and 100 mA at a frequency of 30 Hz and pulse duration
6 450 μ s. Isokinetic torque was measured at 60, 90 and 180 degrees/sec using the same
7 stimulation protocol.

8 **Serum and plasma analysis**

9 All metabolic profile analysis is presented in Table 2. Blood samples were collected after
10 an overnight fast. Total T was measured by liquid chromatography with isotope dilution mass
11 spectrometry detection after supported liquid extraction. Free T concentration was calculated
12 using sex hormone binding globulin and albumin concentrations (www.issam.ch/freetesto.htm)
13 [67]. Serum IGF-I concentration will be measured by an immunoluminometric assay (Quest
14 Diagnostics, Madison, NJ). Fasting lipid profiles (HDL-C, LDL-C, total cholesterol, and
15 triglycerides) will be determined as previously described [8, 20]. Inflammatory biomarkers CRP,
16 IL-6, TNF- α , and free fatty acids will be determined by commercially available enzyme-linked
17 immunosorbent assay kits (ALPCO; Salem, NH).

18 **Basal metabolic rate**

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

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

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

15 Each participant met with a dietician at the start of the study and was asked to maintain a
16 5 day food dietary log monitoring their caloric intake for the duration of the study. Participants
17 were instructed to record all liquid and food consumption and no nutritional advice was given on
18 the size or the portion of the food. Dietary logs were analyzed on a weekly basis using a
19 nutritional software package (Nutrition Data System for Research version 2014) under the
20 supervision of a registered dietitian. After analysis was completed, the average caloric intake
21 (kcal) and percentage macronutrients (carbohydrates, fat and protein) were calculated. Each
22 participant received monthly feedback via phone call with the registered dietician on how to
23 maintain appropriate dietary habits based on his basal metabolic rate and percentage

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

3 **Intravenous Glucose Tolerance Test (IVGTT)**

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

1 **III. Secondary Outcomes**

2 **Skeletal muscle biopsy**

3 Biopsy samples of vastus lateralis muscle (~100 mg wet weight total) were obtained by a
4 14 gauge tru-cut biopsy needle, before and 72 hours after the interventions. The biopsy samples
5 were quickly frozen in liquid nitrogen and stored at -80°C until further analysis. One sample was
6 split into two halves and used for measuring activities of mitochondrial enzymes. The second
7 sample was used for Western blot analysis. The third sample was used for
8 immunohistochemistry.

9 **Mitochondrial Electron Transport Chain activities**

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

15 **Protein content**

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

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

5 **Histological Analysis**

6 Immediately after muscle biopsy, samples were mounted on tongue blades by using a
7 medium of OCT compound and tragacanth gum and stored at -70°C until analysis. Serial cross
8 sections (8-10 μM) will be collected on glass slides and frozen at -20°C until analysis. Fiber type
9 and CSA will be determined by histochemical staining for myosin ATPase (preincubation at pH
10 4.3 or 9.4) as previously described [73]. Type I fibers will be identified by dark staining after
11 acid preincubation, type II fibers light staining, and type IIB intermediate. At pH 9.4 the staining
12 pattern was the opposite. Haematoxylin & Eosin (H&E) staining will be performed according to
13 conventional histological procedures. Mitochondrial complex II and IV activity will be estimated
14 by the activity of succinate dehydrogenase and cytochrome c oxidase activity, respectively, as
15 previously described [74, 75]. Stained muscle sections will be observed using an Olympus BX-
16 51 fluorescent microscope (Olympus, Tokyo, Japan) and analyzed using ImageJ software.

17 **Statistical Analyses**

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

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

13 **Ethics and Dissemination**

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

18 **Data Monitoring**

19 The research staff will oversee and monitor the study to ensure data quality and
20 participant compliance. Adverse events, regardless of severity, will be reported to the McGuire
21 institutional review board. Only members of the research team will have access to data.
22
23

1 Discussion

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

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

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

1 populations. Previous work suggests that twice weekly NMES-RT can induce favorable body
2 composition and metabolic adaptations. Twelve weeks of NMES-RT has shown to increase
3 thigh muscle CSA by 35-40% as measured by MRI [57]. Moreover, there was a reduction in
4 %leg FM and a trend towards decrease in VAT CSA after 12 weeks of NMES-RT [62]. The
5 favorable adaptations in body composition were associated with decrease in plasma insulin area
6 under the curve and plasma triglycerides [57]. These findings were attributed to an increase in
7 plasma IGF-1. However, the effect of training appears to be limited to the trained muscle and
8 only modestly impacted whole body composition. It is unclear whether a longer RT program
9 greater than 12 weeks would provide additional benefits to individuals with SCI.

10 TRT has been shown to increase skeletal muscle mass in hypogonadal men, men with
11 chronic illness, and older men [51-53]. Androgen deficiency in men is associated with a loss of
12 FFM and an increase in FM [51]. In epidemiologic studies, men with decreased free T-index had
13 lower appendicular skeletal muscle mass than those with normal T levels [76]. Previous work
14 documented that TRT increases muscle mass with a reciprocal decrease in total body FM [51,
15 52]. This reciprocal action has been suggested due to a switch from adipogenic toward myogenic
16 differentiation of mesenchymal stem cells [76]. In a randomized controlled double blinded
17 clinical trial, TRT was shown to improve insulin sensitivity, CRP and reduce VAT [52]. Sixty
18 percent of men with SCI have low T level and levels are associated with time since injury [47,
19 48]. TRT has also been reported to increase IGF-1 and several molecular mechanisms related to
20 the protective pathways have been recently elucidated [45, 46, 49, 77]. Therefore, enhancing the
21 decline in anabolic homeostasis by providing TRT may provide additional benefits as was
22 previously demonstrated by increasing lean mass and resting metabolic rate in individuals with
23 SCI.

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3 1 In summary, we anticipate that this trial will provide important insights into the body
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5 2 composition and metabolic benefits following 16 weeks of evoked RT+TRT or TRT. If
6
7 3 beneficial, this may be a feasible strategy for the rehabilitation of individuals with chronic SCI
8
9 4 and increase the health of this and other clinical populations. Additionally, TRT alone may
10
11 5 provide an alternative intervention for those who cannot benefit from training using surface
12
13 6 NMES, because of lower motor neuron denervation or intolerance to applications of electrical
14
15 7 stimulation. The study will also shed light on several molecular pathways that have been
16
17 8 suggested to influence both body composition and metabolic profile.
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24 10 **Trial Status:** Enrollment into the study started in July 2012 and as of April 2016 all participants
25
26 11 have completed the study. Data collection and data analysis are expected to be completed in
27
28 12 December 2016. The study is expected to be closed in June 2017.
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34 14 **Contributions:** ASG supervised all aspects of the trial including all interventions and
35
36 15 measurements and secured funding for the trial procedures. ASG, REK, LCO drafted the
37
38 16 manuscript. REK, JS, RK, and LCO contributed to data collection and analysis. RG, TL, TC,
39
40 17 DXC, CC, RA, EJM and DRG are research physicians that contributed to patient monitoring and
41
42 18 study design. DXC and RA will provide guidance during data analysis and manuscript
43
44 19 preparation. All authors affirm that the manuscript is an honest, accurate, and transparent account
45
46 20 of the study being reported; that no important aspects of the study have been omitted; and that
47
48 21 any discrepancies from the study as planned have been explained.
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1 **Competing interests:** The authors have no competing interests to declare

2
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13

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1 References

- 2 1. Center, N.S.C.I.S., *Spinal cord injury facts and figures at a glance*. J Spinal Cord Med, 2013. **36**(1): p. 1-2.
- 3 2. DeVivo, M.J., B.K. Go, and A.B. Jackson, *Overview of the national spinal cord injury statistical center database*. J Spinal Cord Med, 2002. **25**(4): p. 335-8.
- 4 3. Castro, M.J., et al., *Influence of complete spinal cord injury on skeletal muscle cross-sectional area within the first 6 months of injury*. Eur J Appl Physiol Occup Physiol, 1999. **80**(4): p. 373-8.
- 5 4. Gorgey, A.S. and G.A. Dudley, *Skeletal muscle atrophy and increased intramuscular fat after incomplete spinal cord injury*. Spinal Cord, 2007. **45**(4): p. 304-9.
- 6 5. Wielopolski, L., et al., *Measuring partial body potassium in the legs of patients with spinal cord injury: a new approach*. J Appl Physiol (1985), 2009. **106**(1): p. 268-73.
- 7 6. Spungen, A.M., et al., *Soft tissue body composition differences in monozygotic twins discordant for spinal cord injury*. J Appl Physiol (1985), 2000. **88**(4): p. 1310-5.
- 8 7. Spungen, A.M., et al., *Factors influencing body composition in persons with spinal cord injury: a cross-sectional study*. J Appl Physiol (1985), 2003. **95**(6): p. 2398-407.
- 9 8. Gorgey, A.S., et al., *Relationship of spasticity to soft tissue body composition and the metabolic profile in persons with chronic motor complete spinal cord injury*. J Spinal Cord Med, 2010. **33**(1): p. 6-15.
- 10 9. Gater, D.R., Jr., *Obesity after spinal cord injury*. Phys Med Rehabil Clin N Am, 2007. **18**(2): p. 333-51, vii.
- 11 10. Weaver, F.M., et al., *Prevalence of obesity and high blood pressure in veterans with spinal cord injuries and disorders: a retrospective review*. Am J Phys Med Rehabil, 2007. **86**(1): p. 22-9.
- 12 11. Lavela, S.L., et al., *Diabetes mellitus in individuals with spinal cord injury or disorder*. J Spinal Cord Med, 2006. **29**(4): p. 387-95.
- 13 12. Mojtahedi, M.C., et al., *The association between regional body composition and metabolic outcomes in athletes with spinal cord injury*. Spinal Cord, 2008. **46**(3): p. 192-7.
- 14 13. Edwards, L.A., J.M. Bugaresti, and A.C. Buchholz, *Visceral adipose tissue and the ratio of visceral to subcutaneous adipose tissue are greater in adults with than in those without spinal cord injury, despite matching waist circumferences*. Am J Clin Nutr, 2008. **87**(3): p. 600-7.
- 15 14. Maruyama, Y., et al., *Serum leptin, abdominal obesity and the metabolic syndrome in individuals with chronic spinal cord injury*. Spinal Cord, 2008. **46**(7): p. 494-9.
- 16 15. Manns, P.J., J.A. McCubbin, and D.P. Williams, *Fitness, inflammation, and the metabolic syndrome in men with paraplegia*. Arch Phys Med Rehabil, 2005. **86**(6): p. 1176-81.
- 17 16. Matsuzawa, Y., *White adipose tissue and cardiovascular disease*. Best Pract Res Clin Endocrinol Metab, 2005. **19**(4): p. 637-47.
- 18 17. Kern, P.A., et al., *Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance*. Am J Physiol Endocrinol Metab, 2001. **280**(5): p. E745-51.
- 19 18. Blake, G.J. and P.M. Ridker, *Novel clinical markers of vascular wall inflammation*. Circ Res, 2001. **89**(9): p. 763-71.

19. Elder, C.P., et al., *Intramuscular fat and glucose tolerance after spinal cord injury--a cross-sectional study*. Spinal Cord, 2004. **42**(12): p. 711-6.
20. Gorgey, A.S., K.J. Mather, and D.R. Gater, *Central adiposity associations to carbohydrate and lipid metabolism in individuals with complete motor spinal cord injury*. Metabolism, 2011. **60**(6): p. 843-51.
21. Bauman, W.A. and A.M. Spungen, *Disorders of carbohydrate and lipid metabolism in veterans with paraplegia or quadriplegia: a model of premature aging*. Metabolism, 1994. **43**(6): p. 749-56.
22. Grundy, S.M., et al., *Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition*. Circulation, 2004. **109**(3): p. 433-8.
23. Nelson, M.D., et al., *Metabolic syndrome in adolescents with spinal cord dysfunction*. J Spinal Cord Med, 2007. **30 Suppl 1**: p. S127-39.
24. Bauman, W.A. and A.M. Spungen, *Coronary heart disease in individuals with spinal cord injury: assessment of risk factors*. Spinal Cord, 2008. **46**(7): p. 466-76.
25. Lee, C.S., et al., *Evaluating the prevalence of silent coronary artery disease in asymptomatic patients with spinal cord injury*. Int Heart J, 2006. **47**(3): p. 325-30.
26. Itoh, K., et al., *Mitochondrial dynamics in neurodegeneration*. Trends Cell Biol, 2013. **23**(2): p. 64-71.
27. Chan, D.C., *Mitochondria: dynamic organelles in disease, aging, and development*. Cell, 2006. **125**(7): p. 1241-52.
28. Zhao, J., et al., *Mitochondrial dynamics regulates migration and invasion of breast cancer cells*. Oncogene, 2013. **32**(40): p. 4814-24.
29. Phielix, E. and M. Mensink, *Type 2 diabetes mellitus and skeletal muscle metabolic function*. Physiol Behav, 2008. **94**(2): p. 252-8.
30. Ritov, V.B., et al., *Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity*. Am J Physiol Endocrinol Metab, 2010. **298**(1): p. E49-58.
31. Erickson, M.L., et al., *Near-infrared assessments of skeletal muscle oxidative capacity in persons with spinal cord injury*. Eur J Appl Physiol, 2013. **113**(9): p. 2275-83.
32. Martin, T.P., et al., *Influence of electrical stimulation on the morphological and metabolic properties of paralyzed muscle*. J Appl Physiol (1985), 1992. **72**(4): p. 1401-6.
33. Grimby, G., et al., *Muscle fiber composition in patients with traumatic cord lesion*. Scand J Rehabil Med, 1976. **8**(1): p. 37-42.
34. Goodpaster, B.H., *Mitochondrial deficiency is associated with insulin resistance*. Diabetes, 2013. **62**(4): p. 1032-5.
35. Holloszy, J.O., *"Deficiency" of mitochondria in muscle does not cause insulin resistance*. Diabetes, 2013. **62**(4): p. 1036-40.
36. Scarpulla, R.C., *Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network*. Biochim Biophys Acta, 2011. **1813**(7): p. 1269-78.
37. Arany, Z., *PGC-1 coactivators and skeletal muscle adaptations in health and disease*. Curr Opin Genet Dev, 2008. **18**(5): p. 426-34.
38. Summermatter, S., et al., *Peroxisome proliferator-activated receptor {gamma} coactivator 1{alpha} (PGC-1{alpha}) promotes skeletal muscle lipid refueling in vivo by activating de novo lipogenesis and the pentose phosphate pathway*. J Biol Chem, 2010. **285**(43): p. 32793-800.

- 1
2
3 1 39. Pagel-Langenickel, I., et al., *PGC-1alpha integrates insulin signaling, mitochondrial*
4 2 *regulation, and bioenergetic function in skeletal muscle*. J Biol Chem, 2008. **283**(33): p.
5 3 22464-72.
6 3
7 4 40. Sacheck, J.M., et al., *Rapid disuse and denervation atrophy involve transcriptional*
8 5 *changes similar to those of muscle wasting during systemic diseases*. FASEB J, 2007.
9 6 **21**(1): p. 140-55.
10 7 41. Adhihetty, P.J., et al., *Effect of denervation on mitochondrially mediated apoptosis in*
11 8 *skeletal muscle*. J Appl Physiol (1985), 2007. **102**(3): p. 1143-51.
12 8
13 9 42. O'Brien, L.C.G., A.S., *Skeletal muscle mitochondrial health and spinal cord injury*.
14 10 World J Orthop., 2016(In press).
15 11 43. Tsitouras, P.D., et al., *Serum testosterone and growth hormone/insulin-like growth*
16 12 *factor-I in adults with spinal cord injury*. Horm Metab Res, 1995. **27**(6): p. 287-92.
17 13 44. Bauman, W.A., et al., *Blunted growth hormone response to intravenous arginine in*
18 14 *subjects with a spinal cord injury*. Horm Metab Res, 1994. **26**(3): p. 152-6.
19 14
20 15 45. Bhasin, S., *Regulation of body composition by androgens*. J Endocrinol Invest, 2003.
21 16 **26**(9): p. 814-22.
22 17 46. Bhasin, S. and J.G. Buckwalter, *Testosterone supplementation in older men: a rational*
23 18 *idea whose time has not yet come*. J Androl, 2001. **22**(5): p. 718-31.
24 18
25 19 47. Clark, M.J., et al., *Testosterone levels among men with spinal cord injury: relationship*
26 20 *between time since injury and laboratory values*. Am J Phys Med Rehabil, 2008. **87**(9): p.
27 21 758-67.
28 22 48. Kostovski, E., et al., *Decreased levels of testosterone and gonadotrophins in men with*
29 23 *long-standing tetraplegia*. Spinal Cord, 2008. **46**(8): p. 559-64.
30 23
31 24 49. Zhao, W., et al., *Testosterone protects against dexamethasone-induced muscle atrophy,*
32 25 *protein degradation and MAFbx upregulation*. J Steroid Biochem Mol Biol, 2008. **110**(1-
33 26 2): p. 125-9.
34 27 50. Gregory, C.M., et al., *Effects of testosterone replacement therapy on skeletal muscle after*
35 28 *spinal cord injury*. Spinal Cord, 2003. **41**(1): p. 23-8.
36 28
37 29 51. Isidori, A.M., et al., *Effects of testosterone on body composition, bone metabolism and*
38 30 *serum lipid profile in middle-aged men: a meta-analysis*. Clin Endocrinol (Oxf), 2005.
39 31 **63**(3): p. 280-93.
40 32 52. Aversa, A., et al., *Effects of testosterone undecanoate on cardiovascular risk factors and*
41 33 *atherosclerosis in middle-aged men with late-onset hypogonadism and metabolic*
42 34 *syndrome: results from a 24-month, randomized, double-blind, placebo-controlled study*.
43 35 J Sex Med, 2010. **7**(10): p. 3495-503.
44 35
45 36 53. Sinha-Hikim, I., et al., *Effects of testosterone supplementation on skeletal muscle fiber*
46 37 *hypertrophy and satellite cells in community-dwelling older men*. J Clin Endocrinol
47 38 Metab, 2006. **91**(8): p. 3024-33.
48 39 54. Ryan, A.S., et al., *Skeletal muscle hypertrophy and muscle myostatin reduction after*
49 40 *resistive training in stroke survivors*. Stroke, 2011. **42**(2): p. 416-20.
50 40
51 41 55. Ibanez, J., et al., *Twice-weekly progressive resistance training decreases abdominal fat*
52 42 *and improves insulin sensitivity in older men with type 2 diabetes*. Diabetes Care, 2005.
53 43 **28**(3): p. 662-7.
54 43
55 44 56. Chromiak, J.A., et al., *Effect of a 10-week strength training program and recovery drink*
56 45 *on body composition, muscular strength and endurance, and anaerobic power and*
57 46 *capacity*. Nutrition, 2004. **20**(5): p. 420-7.
58
59
60

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2
3
4
5
6
7
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43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- 1 57. Mahoney, E.T., et al., *Changes in skeletal muscle size and glucose tolerance with electrically stimulated resistance training in subjects with chronic spinal cord injury*. Arch Phys Med Rehabil, 2005. **86**(7): p. 1502-4.
- 2
3
4 58. Mohr, T., et al., *Insulin action and long-term electrically induced training in individuals with spinal cord injuries*. Med Sci Sports Exerc, 2001. **33**(8): p. 1247-52.
- 5
6 59. Kjaer, M., et al., *Fatty acid kinetics and carbohydrate metabolism during electrical exercise in spinal cord-injured humans*. Am J Physiol Regul Integr Comp Physiol, 2001. **281**(5): p. R1492-8.
- 7
8
9 60. Crameri, R.M., et al., *Effects of electrical stimulation-induced leg training on skeletal muscle adaptability in spinal cord injury*. Scand J Med Sci Sports, 2002. **12**(5): p. 316-22.
- 10
11
12 61. Gerrits, H.L., et al., *Peripheral vascular changes after electrically stimulated cycle training in people with spinal cord injury*. Arch Phys Med Rehabil, 2001. **82**(6): p. 832-9.
- 13
14 62. Gorgey, A.S., et al., *Effects of resistance training on adiposity and metabolism after spinal cord injury*. Med Sci Sports Exerc, 2012. **44**(1): p. 165-74.
- 15
16 63. Gorgey, A.S., et al., *Effects of neuromuscular electrical stimulation parameters on specific tension*. Eur J Appl Physiol, 2006. **97**(6): p. 737-44.
- 17
18 64. Gorgey, A.S., et al., *Effects of electrical stimulation parameters on fatigue in skeletal muscle*. J Orthop Sports Phys Ther, 2009. **39**(9): p. 684-92.
- 19
20 65. Gorgey, A.S., et al., *Influence of motor complete spinal cord injury on visceral and subcutaneous adipose tissue measured by multi-axial magnetic resonance imaging*. J Spinal Cord Med, 2011. **34**(1): p. 99-109.
- 21
22 66. Gorgey, A.S. and C. Shepherd, *Skeletal muscle hypertrophy and decreased intramuscular fat after unilateral resistance training in spinal cord injury: case report*. J Spinal Cord Med, 2010. **33**(1): p. 90-5.
- 23
24
25 67. Vermeulen, A., L. Verdonck, and J.M. Kaufman, *A critical evaluation of simple methods for the estimation of free testosterone in serum*. J Clin Endocrinol Metab, 1999. **84**(10): p. 3666-72.
- 26
27
28 68. Collins, E.G., et al., *Energy cost of physical activities in persons with spinal cord injury*. Med Sci Sports Exerc, 2010. **42**(4): p. 691-700.
- 29
30 69. Bergman, R.N., *Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach*. Diabetes, 1989. **38**(12): p. 1512-27.
- 31
32 70. Matthews, D.R., et al., *Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man*. Diabetologia, 1985. **28**(7): p. 412-9.
- 33
34
35 71. Matsuda, M. and R.A. DeFronzo, *Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp*. Diabetes Care, 1999. **22**(9): p. 1462-70.
- 36
37
38 72. Brass, E.P., et al., *Decreased NADH dehydrogenase and ubiquinol-cytochrome c oxidoreductase in peripheral arterial disease*. Am J Physiol Heart Circ Physiol, 2001. **280**(2): p. H603-9.
- 39
40
41 73. Brooke, M.H. and K.K. Kaiser, *Three "myosin adenosine triphosphatase" systems: the nature of their pH lability and sulfhydryl dependence*. J Histochem Cytochem, 1970. **18**(9): p. 670-2.

- 1
2
3 1 74. Nachlas, M.M., et al., *Cytochemical demonstration of succinic dehydrogenase by the use*
4 2 *of a new p-nitrophenyl substituted ditetrazole*. J Histochem Cytochem, 1957. **5**(4): p.
5 3 420-36.
6 4 75. Wong-Riley, M., *Changes in the visual system of monocularly sutured or enucleated cats*
7 5 *demonstrable with cytochrome oxidase histochemistry*. Brain Res, 1979. **171**(1): p. 11-28.
8 6 76. Herbst, K.L. and S. Bhasin, *Testosterone action on skeletal muscle*. Curr Opin Clin Nutr
9 7 Metab Care, 2004. **7**(3): p. 271-7.
10 8 77. Wu, Y., et al., *Identification of androgen response elements in the insulin-like growth*
11 9 *factor I upstream promoter*. Endocrinology, 2007. **148**(6): p. 2984-93.
12
13
14
15 10
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17 11
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21
22 13
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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, TNF α)	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; TNF α , tumor necrosis factor alpha

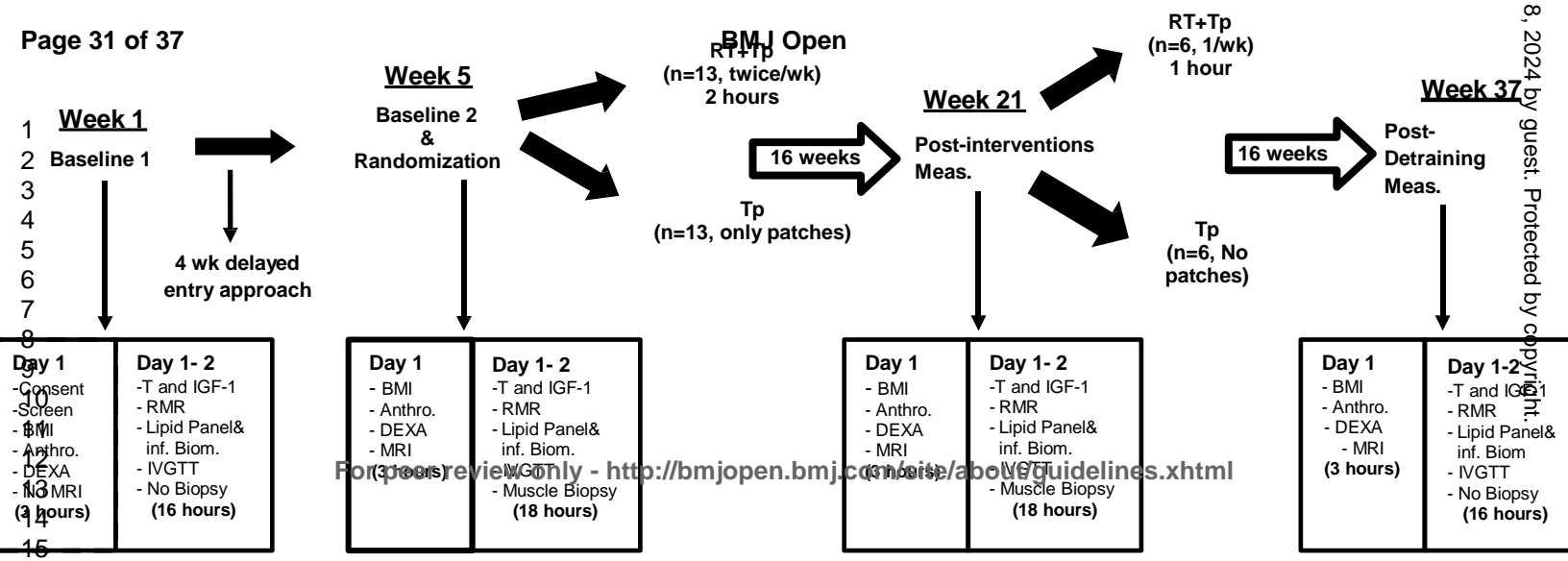
1 **Figure Legends**

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

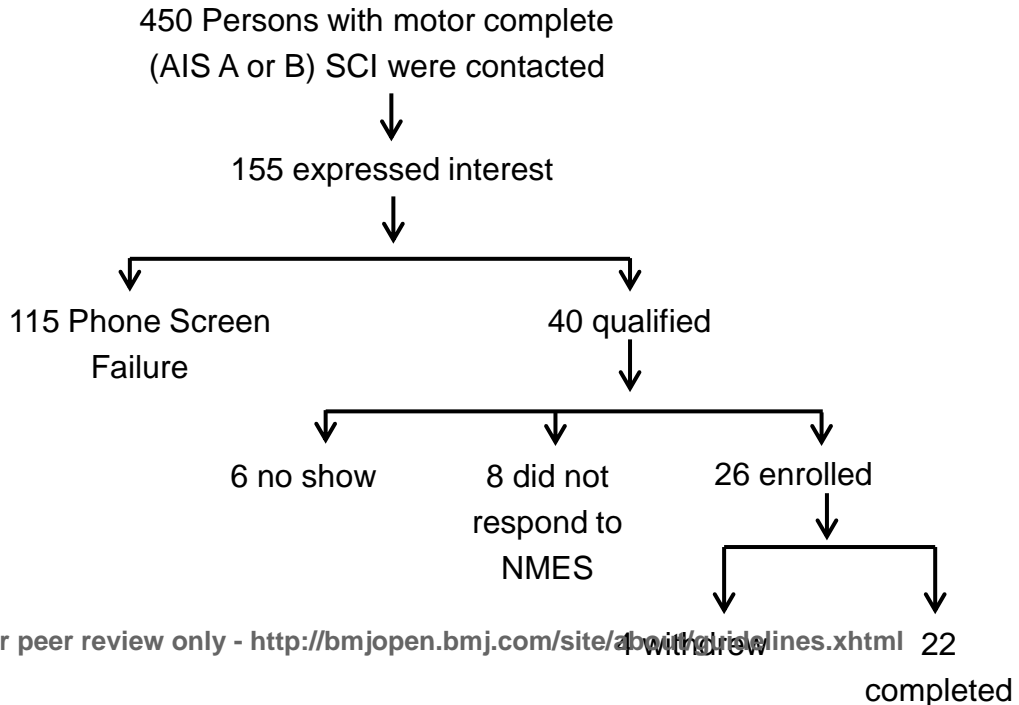
5 **Figure 2.** Schematic diagram showing the process of recruitment over the 3 year period of the
6 TEREX trial.

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

15



Recruitment



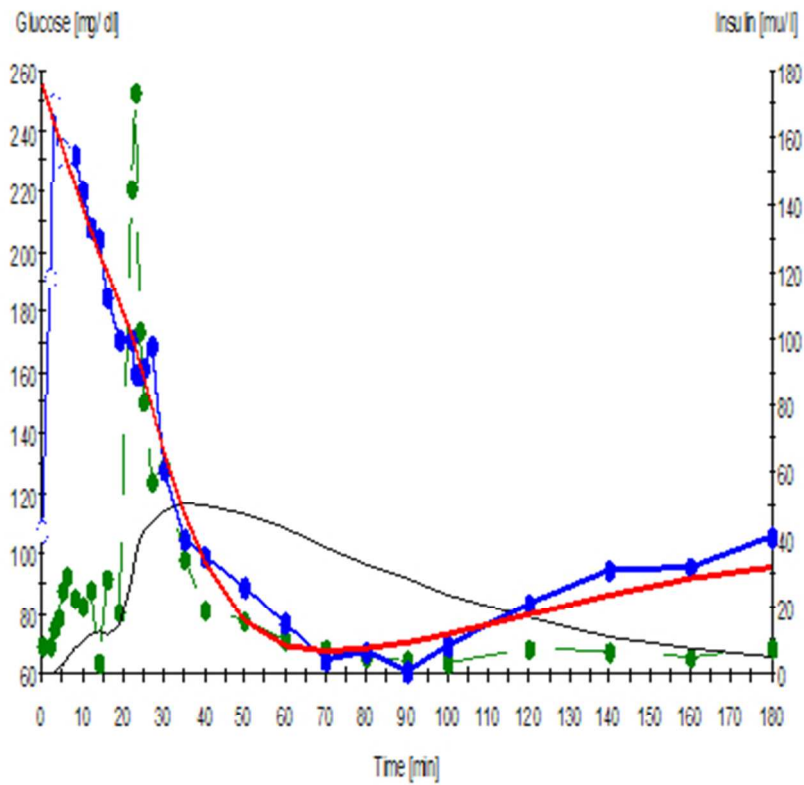


Figure 3. A representative figure of analysis for IVGTT in a person with SCI after infusion of dextrose followed by insulin 20 minutes later. The dextrose concentration is calculated by multiplying $0.3g \times$ body weight (kg) in 50% solution. Insulin concentration is determined by multiplying 0.02 units \times 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 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

1				
2				
3	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____
4				
5				
6	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	____7-8____
7				

8 **Methods: Assignment of interventions (for controlled trials)**

9 Allocation:

10				
11				
12	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____
13				
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17				
18	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____
19				
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22	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____8____
23				
24				
25	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____9-10____
26				
27				
28		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____
29				
30				
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32 **Methods: Data collection, management, and analysis**

33				
34	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____
35				
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39		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____
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3	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 ___
4				
5				
6				
7	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 ___
8				
9				
10		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	___ N/A ___
11				
12		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 ___
13				
14				
15				
16	Methods: Monitoring			
17				
18	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 ___
19				
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22				
23		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	_____
24				
25				
26	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 ___
27				
28				
29	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
30				
31				
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33	Ethics and dissemination			
34				
35	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	___ 3, 7, 17 ___
36				
37				
38	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)	_____
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3	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	___7___
4				
5				
6		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	___N/A___
7				
8				
9	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___
10				
11				
12	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	___21___
13				
14				
15	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___
16				
17				
18	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	_____
19				
20				
21	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___
22				
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26		31b	Authorship eligibility guidelines and any intended use of professional writers	___20___
27				
28		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
29				
30	Appendices			
31				
32	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	___attached___
33				
34				
35	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___
36				
37				

*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

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

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

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44 **Abstract**

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

53 **Methods and analysis:** Twenty-six individuals with chronic, motor complete SCI between 18-
54 50 years old were randomly assigned to a RT+TRT group (n = 13) or a TRT group (n = 13).
55 Twenty-two participants completed the initial 16-week training phase of the study and 4
56 participants withdrew. Twelve participants out of the 22 completed 16 weeks of detraining. The
57 TRT was provided via transdermal testosterone patches (4-6 mg/day). The RT+TRT group had
58 16 weeks of supervised unilateral progressive RT using surface neuromuscular electrical
59 stimulation with ankle weights. This study will investigate the effects of evoked RT+TRT or
60 TRT alone on body composition (muscle cross sectional area, visceral adipose tissue, %FM) and
61 metabolic profile (glucose and lipid metabolisms) in individuals with motor complete SCI.
62 Findings from this study may help in designing exercise therapies to alleviate the deterioration in
63 body composition after SCI and decrease the incidence of metabolic disorders in this clinical
64 population.

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

1
2
3 67 forms. Results will be submitted to peer-reviewed journals and presented at national and
4
5 68 international conferences.
6
7

8 **Trial Registration:** NCT01652040
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10

11
12 **Keywords:** RESISTANCE TRAINING, SPINAL CORD INJURY, BODY COMPOSITION,
13
14 METABOLISM, TESTOSTERONE, MITOCHONDRIA, INFLAMMATORY BIOMARKERS
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20 **Strengths and limitations**
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- 22
23 75 ➤ The trial will investigate the use of surface neuromuscular electrical stimulation induced
24
25 76 resistance training (NMES-RT) to restore muscle size after spinal cord injury (SCI)
26
27
28 77 ➤ The trial will provide evidence on the effectiveness of testosterone replacement therapy
29
30 78 (TRT) to restore muscle size and lean mass and serve as an alternative approach for those
31
32 79 who cannot benefit from NMES.
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35 80 ➤ The trial will determine how restoring muscle size and lean mass by RT+TRT or TRT can
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37 81 benefit the metabolic profile after SCI.
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40 82 ➤ The study is only limited to men with complete SCI
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42 83 ➤ Surface NMES may not benefit those with full sensation or lower motor neuron denervation
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90 Introduction

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

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

111 Metabolic changes also accompany SCI, with previous studies finding that more than
112 50% of individuals with SCI are glucose intolerant, while one out of five is diabetic [2, 9-11, 21].

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3 113 Other studies report that 55% of individuals with SCI are at risk of developing metabolic
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5 114 syndrome [21-23]. Individuals with complete tetraplegia are more likely to experience decreased
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8 115 glucose and carbohydrate tolerance and have a higher prevalence of heart disease than those with
9
10 116 incomplete injuries [24, 25]. Likewise, depressed HDL-C ($<35 \text{ mg} \cdot \text{dL}^{-1}$) and a higher total
11
12 117 cholesterol/HDL-C ratio, predictors of coronary heart disease, were noted in those with chronic
13
14 118 SCI compared with able bodied controls [24, 26]. These are not universal findings, however, as a
15
16 119 systematic review of carbohydrate and lipid disorders in persons with SCI did not find strong
17
18 120 evidence of increased risk beyond that of the general population [27].
19

20
21
22 121 While previous studies have shown a link between body composition and metabolic
23
24 122 profile after SCI, the cellular mechanisms remain unknown. Mitochondria are the site of oxygen
25
26 123 consumption and energy production from glucose and lipid metabolism. Unfortunately,
27
28 124 mitochondrial function is impaired in a number of diseases including neurodegenerative disease,
29
30 125 atherosclerosis, hypertension and cancer [28-31]. Fewer and smaller mitochondria are found in
31
32 126 skeletal muscle of insulin resistant, obese and type II diabetic individuals [32]. Previous studies
33
34 127 found decreased muscle oxidative capacity and succinate dehydrogenase activity, complex II of
35
36 128 the electron transport chain, after SCI [33-35].
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41 129 One hypothesis is that skeletal muscle mitochondrial function is decreased in metabolic
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43 130 disorders, leading to decreased fatty acid oxidation which contributes to insulin resistance [36,
44
45 131 37]; however, this is controversial [37]. Mitochondria are dynamic organelles and undergo
46
47 132 biogenesis, remodeling, and degradation. Mitochondrial biogenesis is driven in part through the
48
49 133 action of peroxisome proliferator-activated receptor (PPAR) coactivator 1 alpha (PGC-1 α) [38,
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51 134 39]. In addition, PGC-1 α integrates insulin signaling and lipogenesis in skeletal muscle [40, 41].
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53 135 PGC-1 α is decreased in animal models following denervation [42, 43]. Decreased
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3 136 mitochondrial biogenesis and reduced mitochondrial mass may result in decreased energy
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6 137 production and therefore play a significant role in the altered metabolic profile following SCI
7
8 138 [44].
9

10 139 Body composition and metabolic changes after SCI may be further exacerbated by
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12
13 140 reduced anabolic hormones including testosterone (T), growth hormone and the growth hormone
14
15 141 second messenger insulin like growth factor-1 (IGF-1) [45, 46]. Previous studies have shown that
16
17 142 60% of men with SCI have low T and that testosterone replacement therapy (TRT) increases
18
19 143 IGF-1 in men [47-50]. In rodent models of SCI, TRT attenuates the loss of muscle [51, 52]. TRT
20
21 144 decreases total body fat, increases lean mass [53, 54] and increases the number of proliferating
22
23 145 skeletal muscle satellite cells in in men [55]. These findings suggest that TRT may provide
24
25 146 metabolic benefits to individuals with SCI.
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28

29 147 Resistance training (RT) improves insulin sensitivity and increases fatty acid and
30
31 148 carbohydrate metabolism as well as attenuates sarcopenia in the elderly and after SCI [56-63].
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33 149 Moreover, RT has been shown to influence body composition by increasing lean mass,
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35 150 decreasing FM and reducing VAT, suggesting that the benefits of RT could overcome the risk of
36
37 151 developing insulin resistance [56-59]. Functional electrical stimulation (FES) has been shown to
38
39 152 improve fatty acid kinetics, carbohydrate metabolism and vascular health after SCI [60-63].
40
41 153 Electrically evoked RT using neuromuscular electrical stimulation (NMES-RT) and ankle
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43 154 weights is another form that has been shown to be effective in inducing muscle hypertrophy in
44
45 155 individuals with chronic SCI [59, 64]. One study showed a 40% increase in skeletal muscle size
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47 156 and improved glucose tolerance after 12 weeks of training [59]. Another study showed that
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49 157 following 12 weeks of NMES-RT, whole thigh, knee extensor and flexor cross sectional areas
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51 158 (CSAs) increased by 28%, 35% and 16%, respectively. Moreover, the ratio of leg FFM to whole
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3 159 body FFM increased by 20% following intervention. There was 32% decrease in glucose area
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5
6 160 under the curve adjusted to muscle CSA following 12 weeks of NMES-RT. However, there
7
8 161 were only modest effects on whole body composition as well as a non-significant decrease in
9
10 162 VAT [64]. It is possible that the limited effects of NMES-RT on parameters of body
11
12 163 composition and VAT can be possibly explained by depressed T-level in persons with SCI.
13
14 164 Supplementing exogenous T may optimize the outcomes of NMES-RT on parameters of body
15
16 165 composition and metabolic profile such as increase basal metabolic rate (BMR).
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19

20 166 TRT may be an effective therapy to counterbalance the growing rate of obesity, type II
21
22 167 diabetes and cardiovascular disease among individuals with SCI. Moreover, results from the
23
24 168 current trial may provide evidence that TRT is an effective intervention for those who cannot
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26 169 effectively benefit from NMES because of lower motor neuron denervation or intolerance to
27
28 170 electrical stimulation. Therefore, the primary hypothesis is that the addition of TRT will
29
30 171 maximize the benefits of electrically evoked RT on parameters of body composition and
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32 172 metabolic profile in men with chronic complete SCI. We, hereby, report the design of an study
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34 173 for which the major research goal is to investigate the effects of 16 weeks of evoked RT+TRT
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36 174 vs. TRT on body composition (primary outcome variables; muscle CSA, VAT, %FM) and
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38 175 metabolic profiles (secondary outcome variables; glucose and lipid metabolism) in individuals
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40 176 with motor complete SCI.
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46 177 **Methods and analysis**

47 48 178 **Study design**

49
50 179 A randomized controlled study was undertaken in which individuals with SCI were
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52 180 randomized to receive RT+TRT or TRT alone for 16 weeks. The study was approved by the
53
54 181 McGuire Veteran Affairs Investigation Research Board and the Virginia Commonwealth
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3 182 University (VCU) Office of Research and Innovation. The trial has been registered at
4
5 183 clinicaltrials.gov (NCT01652040). A member of the research team explained the study and
6
7
8 184 obtained written informed consent. After informed consent each subject underwent a detailed
9
10 185 physical examination at the Hunter Holmes McGuire VA Medical Center (VAMC) by a
11
12 186 physiatrist board certified in SCI medicine. This exam included a neurological assessment
13
14 187 according to the International Standards for Neurological Classification of SCI (ISNCSCI),
15
16 188 including the American Spinal Injury Association (ASIA) Impairment Scale (AIS) [65].
17
18
19

20 189 The study design and procedures are presented in Figures 1 and 2. The study visits
21
22 190 included estimation of body composition, anthropometry, and dual x-ray absorptiometry (DEXA;
23
24 191 baselines 1 and 2 and post-interventions 1 and 2). Additionally, MRI scans were obtained for
25
26 192 trunk adipose tissue and lower extremity skeletal muscles and IMF CSA (baseline 2, post-
27
28 193 interventions 1 and 2). Participants were then escorted to the VCU-CRS unit (VCU Clinical
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30 194 Research Unit) for dinner, and remained in the VCU-CRS unit overnight for the four study visits.
31
32
33 195 Muscle biopsies were obtained at baseline 2 and post intervention 1.
34
35

36 196 **Recruitment and Randomization**

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38
39 197 The recruitment process started in July 2012 and ended in June 2015. Data analysis is
40
41 198 currently being performed. Recruitment details and randomization are presented in Table 1 and
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43 199 Figure 3. Prior to the start of the study, numbers 1-26 were randomized using the n-Query
44
45 200 software program by the principal investigator. At the end of the two-day assessment period
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47 201 (Baseline 1), the allocation of subjects into either group was performed in a blinded fashion by
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49 202 drawing a folded paper with a number (1-26) by the research coordinator. This number was
50
51 203 matched with the assignment from the randomization procedure.
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204 Twenty-six participants were recruited to participate in the study. A four week delayed
205 entry period was included to obtain baseline measurements, stabilize body weight and educate
206 participants on how to monitor their dietary intake. This allowed participants to serve as their
207 own controls. Four participants withdrew from the trial. At baseline 2, two participants failed to
208 comply with study protocol and withdrew from the study because lack of interest. Nurses failed
209 to locate the veins on the third participant to conduct IVGTT and he was asked to withdraw from
210 the trial. The fourth participant was diagnosed with a grade IV pressure ulcer after being
211 admitted into the trial. Following the delayed entry period, participants were randomly assigned
212 to a RT+TRT group (n = 13) or TRT group (n = 13). TRT patches (2-6 mg/day) were replaced
213 daily on alternating shoulders at bedtime for 16 weeks. The RT+TRT group received 16 weeks
214 of supervised unilateral progressive RT using surface NMES and ankle weights. Following the
215 intervention the two-day assessment period was repeated (Figure 1).

216 **Participants and eligibility criteria**

217 Participants were men between 18-50 years old with a BMI of ≤ 30 kg/m². The upper
218 limit of age was set to 50 years to avoid unanticipated side effects that may result from TRT.
219 Participants had motor complete SCI C5-L2, ASIA A or B. Participants with pre-existing
220 medical conditions were excluded. These included cardiovascular disease, uncontrolled type II
221 diabetes and those on insulin, pressures sores stage 2 or greater, supra-physiological T level,
222 hematocrit above 50% and urinary tract infection or symptoms.

223 **I. Interventions**

224 **Resistance training**

225 The first week of RT was conducted with no ankle weights to ensure that the knee
226 extensor muscles could extend the weight of the lower leg against gravity. Once full knee

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3 227 extension was achieved in a sitting position, two pounds were added on a weekly basis with the
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5 228 criteria that full knee extension was achieved before more weight was added. Surface NMES was
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8 229 applied to the knee extensor muscles via surface electrodes (Figure 2). One electrode was placed
9
10 230 2–3 cm above the superior aspect of the patella over the vastus medialis muscle, and the other
11
12 231 lateral to and 30 cm above the patella over vastus lateralis muscle. Current from the stimulator
13
14 232 was manually increased in 5-second intervals to evoke full knee extension with a 3-minute rest
15
16 233 between sets, 30 Hz, 450 μ s pulses and a current sufficient to evoke full knee extension as
17
18 234 previously described [64, 66, 67]. Four sets of 10 repetitions was performed twice weekly for 16
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20 235 weeks.
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24 236 **Testosterone replacement therapy (TRT)**

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26
27 237 Following baseline measurements, T was administered by patches (Androderm, Watson
28
29 238 Pharma. Inc, Parsippany, NJ) that delivered between 2-6 mg/day [53] (Figure 2). Serum T
30
31 239 concentration was measured and reviewed in a blinded fashion weekly for the first month and
32
33 240 then every 4 weeks by an endocrinologist. Baseline dose was prescribed according to the initial T
34
35 241 level. A dose of 6, 4 or 2 mg/day was prescribed if the serum T-level was less than 300, 300-600
36
37 242 or above 600, respectively. The dose was decreased to 2 mg/day if the serum T concentration
38
39 243 was more than 1000 ng/dL (34.7 nmol/L) and the participant was reeducated about use of the
40
41 244 patch if the concentration was less than 250 ng/dL (8.7nmol/L) above the pretreatment
42
43 245 concentration. Patches were returned after use to ensure adherence to the intervention protocol.
44
45
46 246 Participants were instructed to place patches at bedtime and only remove them during showering.
47
48 247 If skin irritation became an issue, participants were initially advised to move patches up or down
49
50 248 on the shoulder muscles from the irritation site and if the situation was not resolved, a
51
52 249 hydrocortisone cream was prescribed.
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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 additional 16 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.

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2
3 273 Testing was performed after lower extremity elevation for at least 20 minutes to minimize fluid
4
5
6 274 shift. The subject was assisted to lie on a padded table and both legs were strapped proximal to
7
8 275 the knees and ankles. The arms and legs were positioned to ensure proper alignment.
9

10 276 **Magnetic resonance imaging (MRI)**

11
12
13 277 MRI was performed at the VAMC Hospital using a 1.5 Tesla magnet (GE) as previously
14
15 278 described [4, 19, 59, 68, 69]. Transaxial images, 10 mm thick and 10 mm apart, were taken from
16
17 279 the hip joint to the knee joint and from knee to the ankle using the whole body coil. The location
18
19
20 280 of the scan was identified by placing a mark 6 inches proximal to and distal to the patella and
21
22 281 matched on follow up scans. To analyze VAT and subcutaneous adipose tissue (SAT) transverse
23
24 282 slices (0.8 cm thickness) were acquired every 0.4 cm gap from the xyphoid process to the
25
26 283 femoral heads. Images were acquired in series of two stacks with L4-L5 used as a separating
27
28 284 point. TRT patches were removed 48-72 hours prior to MRI scans to avoid skin burn.
29
30

31
32 285 Analyses will be performed using commercial available software (X-vessel) as previously
33
34 286 described [4, 19, 68, 69]. Briefly, the thigh and leg images will be segmented into fat (high
35
36 287 intensity), skeletal muscle (mid intensity) and background/bone (low intensity). Manual
37
38 288 selection of a pixel of skeletal muscle will highlight all skeletal muscle pixels and provide the
39
40 289 total number of skeletal muscle pixels while excluding fat. VAT and SAT will be measured by
41
42 290 manually tracing around the anatomical borders. The number of pixels in the highlighted region
43
44 291 will be multiplied by the matrix size to measure VAT and SAT CSA (cm²).
45
46
47

48 292 **Skeletal muscle torque and specific tension**

49
50 293 Torque of the knee extensor muscle group was evaluated using a Biodex isokinetic
51
52 294 dynamometer (Shirely, NY). Measurements were done 72 hours after the muscle biopsy to
53
54 295 prevent acute effects on protein expression. Participants were seated with both the trunk-thigh
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1
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3 296 angle and the knee-thigh angle at 90°. Each participant was securely strapped to the test chair by
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5
6 297 a crossover shoulder harnesses and a belt across the hip joint. The axis of the dynamometer was
7
8 298 aligned to the anatomical knee axis and the lever arm was attached 2-3 cm above the lateral
9
10 299 malleolus. Before measuring isometric torque, passive tension of the right knee extensor muscle
11
12 300 group was measured at 5, 30, 60, 90, 180, 270 degrees/sec as an index of spasticity. Isometric
13
14 301 torque was measured using current amplitude of 50 and 100 mA at a frequency of 30 Hz and
15
16 302 pulse duration 450 μ s. Isokinetic torque was measured at 60, 90 and 180 degrees/sec using the
17
18 303 same stimulation protocol.
19

20 304 **Serum and plasma analysis**

21
22 305 All metabolic profile analysis is presented in Table 2. Blood samples were collected after
23
24 306 an overnight fast. Total T was measured by liquid chromatography with isotope dilution mass
25
26 307 spectrometry detection after supported liquid extraction. Free T concentration was calculated
27
28 308 using sex hormone binding globulin and albumin concentrations (www.issam.ch/freetesto.htm)
29
30 309 [70]. Serum IGF-I concentration was measured by an immunoluminometric assay (Quest
31
32 310 Diagnostics, Madison, NJ). Fasting lipid profiles (HDL-C, LDL-C, total cholesterol, and
33
34 311 triglycerides) were determined as previously described [8, 20]. Inflammatory biomarkers CRP,
35
36 312 IL-6, TNF- α , and free fatty acids were determined by commercially available enzyme-linked
37
38 313 immunosorbent assay kits (ALPCO; Salem, NH).
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46 314 **Energy Expenditure**

47
48 315 After an overnight fast for 10-12 hours, participants were kept in a dark room for 20-30
49
50 316 minutes to attain a resting state during which BMR was measured as previously described [8].
51
52 317 Briefly, while in a supine position a canopy was placed over the subject's head. Each subject was
53
54 318 allowed 2-3 minutes before starting the test to ensure no signs of apnea or claustrophobic
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3 319 episodes. All subjects were instructed to stay awake during the entire test and to breathe
4
5
6 320 normally. The canopy was then attached to a vacuum to draw the expired gases to the flowmeter
7
8 321 of the metabolic unit (COSMED KB42). Prior to the test, the metabolic unit was calibrated
9
10 322 using the standard procedures identified by the manufacturer. Carbon dioxide and oxygen output
11
12 323 was used to calculate the respiratory exchange ratio and BMR (kcal/day) was calculated using
13
14 324 the average of the last 15 minutes of the test. This was used to measure the percentage of
15
16 325 substrate utilization (% fat vs. % carbohydrate) [8, 71].
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20 326 To determine whether NMES-RT improves exercise performance, testing was performed
21
22 327 using a functional electrical stimulation bike (Restorative Therapies, RTI-300) against
23
24 328 progressive resistance protocol until fatigue. The protocol started with 3 minutes resting, 3
25
26 329 minute warm-up (35-37 RPM) using the servomotor and then a two minute incremental
27
28 330 progressive resistance protocol (1 Nm, 3 Nm, 5 Nm, etc.) until fatigue. After fatigue, a one
29
30 331 minute cool down period was allowed followed by 5 minutes of rest. Energy expenditure and
31
32 332 cardiovascular performance [VO_2 (l/min), blood pressure and heart rate] was collected at baseline
33
34 333 2 and post-interventions 1 and 2.
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39 334 Each participant met with a dietician at the start of the study and was asked to maintain a
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41 335 5 day food dietary log monitoring their caloric intake for the duration of the study. Participants
42
43 336 were instructed to record all liquid and food consumption and no nutritional advice was given on
44
45 337 the size or the portion of the food. Dietary logs were analyzed on a weekly basis using a
46
47 338 nutritional software package (Nutrition Data System for Research version 2014) under the
48
49 339 supervision of a registered dietitian. After analysis was completed, the average caloric intake
50
51 340 (kcal) and percentage macronutrients (carbohydrates, fat and protein) were calculated. Each
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53 341 participant received monthly feedback via phone call with the registered dietician on how to
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1
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3 342 maintain appropriate dietary habits based on his BMR and percentage macronutrients (45%
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5 343 carbohydrates, 30% fat and 25%). Every effort was made to balance the dietary habits between
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7
8 344 the RT+TRT and the TRT group.
9

10 345 **Intravenous Glucose Tolerance Test (IVGTT)**

11
12 346 An IVGTT was used to determine insulin sensitivity and glucose effectiveness before
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14 347 training and 48 hours after the last exercise bout. After an overnight fast, an intravenous line was
15
16 348 placed to facilitate infusion of glucose and blood sampling. Blood samples were taken every 2-3
17
18 349 minutes before and 30 minutes after glucose injection (0.3 gm/kg IV over 30 seconds), followed
19
20 350 by 5-10 minutes sampling ending at 180 minutes. Twenty minutes after the glucose injection a
21
22 351 bolus of insulin (0.02 U/kg) was injected to determine insulin sensitivity. Plasma glucose was
23
24 352 measured by the Autoanalyzer glucose oxidase method and plasma insulin concentrations were
25
26 353 determined by commercial radioimmunoassay (Table 2). The glucose disposal rate per unit of
27
28 354 secreted insulin per unit time and glucose mediated glucose disposal rate were calculated from a
29
30 355 least-squares fitting of the temporal pattern of glucose and insulin throughout the IVGTT using
31
32 356 the MINMOD program [72]. A representative analysis of IVGTT is presented in Figure 4. The
33
34 357 acute insulin response to IV glucose was calculated as the mean rise in plasma insulin above
35
36 358 baseline at 3, 4 and 5 minutes after IV glucose administration. KG, a measure of glucose
37
38 359 tolerance, was calculated as the least square slope of the natural log of absolute glucose
39
40 360 concentration between 5 and 20 minutes after the glucose bolus [73]. The homeostatic model of
41
42 361 assessment of insulin resistance (HOMA-IR) was calculated and insulin sensitivity was
43
44 362 determined using Matsuda and DeFronzo formula [73, 74].
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365 **III. Secondary Outcomes**

366 **Skeletal muscle biopsy**

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

375 **Mitochondrial Electron Transport Chain activities**

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

381 **Protein content**

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

1
2
3 388 were rinsed and placed in a 1:2000 solution of a horseradish peroxidase-conjugated secondary
4
5 389 antibody (Cell Signaling) for 1 hour at room temperature. Membranes were then rinsed and
6
7
8 390 incubated with a horseradish peroxidase chemiluminescence detection reagent (GE Healthcare)
9
10 391 for 5 minutes. Proteins were visualized using an Amersham Imager 600 (GE Healthcare). Optical
11
12 392 densities were measured using iQuant software and all samples were normalized to the baseline
13
14 393 values for that participant.

17 394 **Histological Analysis**

19
20 395 Immediately after muscle biopsy, samples were mounted on tongue blades by using a
21
22 396 medium of OCT compound and tragacanth gum and stored at -70°C until analysis. Serial cross
23
24 397 sections (8-10 µM) were collected on glass slides and frozen at -20°C until analysis. Fiber type
25
26
27 398 and CSA will be determined by histochemical staining for myosin ATPase (preincubation at pH
28
29 399 4.3 or 9.4) as previously described [76]. Type I fibers will be identified by dark staining after
30
31 400 acid preincubation, type II fibers light staining, and type IIB intermediate. At pH 9.4 the staining
32
33 401 pattern was the opposite. Haematoxylin & Eosin (H&E) staining was performed according to
34
35 402 conventional histological procedures. Mitochondrial complex II and IV activity was estimated by
36
37 403 the activity of succinate dehydrogenase and cytochrome c oxidase activity, respectively, as
38
39 404 previously described [77, 78]. Stained muscle sections will be observed using an Olympus BX-
40
41 405 51 fluorescent microscope (Olympus, Tokyo, Japan) and analyzed using ImageJ software.

46 406 **Statistical Analyses**

47
48 407 Paired t-tests will be used to determine differences in body composition and metabolic
49
50 408 profile between baseline 1 and baseline 2. To determine the effect of interventions and
51
52 409 detraining, a repeated measures ANOVA will be used. Simple linear and multivariate regression
53
54 410 analyses were used to examine the relationship between body composition and metabolic profile
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3 411 variables. For protein expression, we will use paired t-tests to examine the effects of each
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5 412 intervention.
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8 413 The effect size is calculated based on the effects of RT on body composition and
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10 414 metabolic profiles that were previously published [62]. The number of subjects necessary to find
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12 415 statistical differences in the major variables (muscle size, VAT and insulin concentration) of this
13
14 416 study was found to be 10 participants per group. One participant from the RT+TRT group
15
16 417 withdrew after developing side effects to TRT patches and the medical monitor personnel
17
18 418 recommended him to withdraw from the trial in week 8. However, we will perform intent to
19
20 419 treat analysis on his data, which means that despite his early withdrawal from the study his data
21
22 420 will be included in the final analysis. This will allow extrapolation of his post-intervention data
23
24 421 using the SPSS missing values option. We anticipate that we will collect sufficient data to
25
26 422 determine the effects of rehabilitation interventions on protein expression, mitochondrial
27
28 423 enzymatic and ETC activities in individuals with SCI. Statistical analysis will be performed
29
30 424 using SPSS version 23.0 (Chicago, IL) with a level of significance set at $p < 0.05$.
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36 425 **Ethics and Dissemination**

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38 426 Ethical approval has been obtained from the institutional review boards at the McGuire
39
40 427 VA Medical Center and Virginia Commonwealth University. All participants read and signed
41
42 428 approved consent forms prior to baseline assessment. Results of the study will be published in
43
44 429 peer-reviewed journal and presented at national and international conferences.
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48 430 **Data Monitoring**

49
50 431 The research staff oversaw and monitored the study to ensure data quality and participant
51
52 432 compliance. There were no adverse events. Only members of the research team will have access
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54 433 to data.
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434 Discussion

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

443 Ectopic adipose tissue accumulation, IMF and VAT, has been strongly associated with
444 altered metabolic profile after SCI [19, 20]. IMF has been determined to account for a 70%
445 reduction in glucose tolerance in individuals with complete SCI [19]. VAT is independently
446 associated with impairment in glucose tolerance, insulin resistance and dyslipidemia after SCI
447 [20]. Edwards et al noted significant positive association between VAT and insulin resistance
448 and a negative correlation between VAT:SAT ratio and HDL-C [13]. Increase in VAT is also
449 related to leptin and plasminogen activator inhibitor-1 concentrations [14]. It is possible that
450 increases in FM are associated with inflammatory biomarkers that trigger metabolic syndrome
451 after SCI [22]. Ectopic adipose tissue has been demonstrated to secrete pro-inflammatory
452 cytokines, including IL-6 and TNF- α . This stimulates hepatic production of CRP which is
453 suggestive of vascular inflammation [15-19].

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

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3 457 favorable body composition and metabolic adaptations. Twelve weeks of NMES-RT has shown
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5
6 458 to increase thigh muscle CSA by 35-40% as measured by MRI [59]. Moreover, there was a
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8 459 reduction in %leg FM and a trend towards decrease in VAT CSA after 12 weeks of NMES-RT
9
10 460 [64]. The favorable adaptations in body composition were associated with decrease in plasma
11
12 461 insulin area under the curve and plasma triglycerides [59]. These findings were attributed to an
13
14 462 increase in plasma IGF-1. However, the effect of training appears to be limited to the trained
15
16 463 muscle and only modestly impacted whole body composition. It is unclear whether a RT
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18 464 program longer than 12 weeks may provide additional benefits to individuals with SCI.
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21
22 465 The cellular changes underlying the alterations in skeletal muscle glucose utilization and
23
24 466 energy metabolism after SCI are unclear. Previous work indicated that expression of AMP-
25
26 467 activated protein kinase (AMPK), a key regulator of energy homeostasis for lipid and
27
28 468 carbohydrate utilization, was altered in persons with SCI compared to BMI matched able bodied
29
30 469 controls [79]. Another study revealed decreased expression of genes involved in glucose and
31
32 470 lipid metabolism [80]. Despite these abnormalities, one study reported that leg glucose uptake
33
34 471 during cycling was increased in individuals with SCI compared to able-bodied controls [61].
35
36 472 Another study showed similar glucose uptake of isolated muscle fibers from SCI and able bodied
37
38 473 individuals *in vitro* [81]. Benefits of other forms of functional electrical stimulation lower
39
40 474 extremity cycling (FES-LEC) have included improvements in body composition, carbohydrate-
41
42 475 and lipid metabolism and muscle fiber type composition [60-63, 82-84]. Similarly, we have
43
44 476 recently shown that 16 weeks of FES-LEC increased the protein abundance of GLUT-4, PGC-
45
46 477 1 α and AMPK by 3.8, 2.3 and 3.4 fold, respectively, in the vastus lateralis muscle in persons
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48 478 with motor complete SCI [85].
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3 479 TRT has been shown to increase skeletal muscle mass in hypogonadal men, men with
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6 480 chronic illness, and older men [53-55]. Androgen deficiency in men is associated with a loss of
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8 481 FFM and an increase in FM [53]. In epidemiologic studies, men with decreased free T had lower
9
10 482 appendicular skeletal muscle mass than those with normal T levels [86]. Previous work
11
12 483 documented that TRT increases muscle mass with a reciprocal decrease in total body FM [53,
13
14 484 54]. This reciprocal action has been suggested due to a switch from adipogenic toward myogenic
15
16 485 differentiation of mesenchymal stem cells [86]. In a randomized controlled double blinded
17
18 486 clinical trial, TRT was shown to improve insulin sensitivity, CRP and reduce VAT [54]. Sixty
19
20 487 percent of men with SCI have low T level and levels are associated with time since injury [49,
21
22 488 50]. TRT has also been reported to increase IGF-1 and several molecular mechanisms related to
23
24 489 the protective pathways have been recently elucidated [47, 48, 51, 87]. Therefore, enhancing the
25
26 490 decline in anabolic homeostasis by providing TRT may provide additional benefits as was
27
28 491 previously demonstrated by increasing lean mass and metabolic rate in individuals with SCI.
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34 492 It was very important to highlight that this protocol has 3 different phases including four
35
36 493 weeks of delayed entry, 16 weeks of intervention and 16 weeks of detraining. The delayed entry
37
38 494 period was included to allow each participant to serve as his own control. Moreover, we were
39
40 495 successful in retaining 12 participants (n=6/group) to complete the detraining phase. This
41
42 496 means that we had participants 6 participants that agreed to stick to our exercise program and the
43
44 497 use of TRT patches up to 9 months. This may reflect on the study protocol frequency that
45
46 498 ensured long-term adherence despite the length of the study. A very important point that is worth
47
48 499 highlighting is that our study protocol was designed to include 3 levels of outcome
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51 500 measurements including body composition, metabolic profile and cellular changes. This design
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3 501 is likely to provide mechanistic explanations to changes that occur at the body composition and
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5 502 metabolic levels
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8 503 **Limitations**
9

10 504 The current study was limited to those who were less than or equal to 50 years old.
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12 505 Because of advances in healthcare, many individuals with SCI have a near-normal life span
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14 506 which may make the results of this study less generalizable. However, this age limit was
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16 507 implemented because the effect of TRT on cardiovascular health has been controversial. There
17
18 508 are data showing that hypogonadism is a risk for cardiovascular disease [88]. Some replacement
19
20 509 studies show increased risk, but another study showed decreased mortality in men receiving
21
22 510 testosterone [89]. Another study in older men reported that injectable testosterone may be
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24 511 associated with increased cardiovascular risk but topical testosterone was not [90]. Therefore, we
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26 512 set the inclusion criteria of less than or equal to 50 years old to reduce the likelihood of
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28 513 developing cardiovascular complications.
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34 514 Currently NMES-RT is not readily available to the majority of SCI patients. Women
35
36 515 were not included because administering TRT is not either appropriate or safe, because women
37
38 516 are at risk of virulization by testosterone. Thus, the trial was limited only to males with SCI.
39
40 517 Moreover, only a small percentage of individuals with motor complete SCI are women.
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42

43 518 In summary, we anticipate that this trial will provide important insights into the body
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45 519 composition and metabolic benefits of 16 weeks of evoked RT+TRT or TRT. If beneficial, this
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47 520 may be a feasible strategy for the rehabilitation of individuals with chronic SCI and increase the
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49 521 health of this and other clinical populations. Additionally, TRT alone may provide an alternative
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51 522 intervention for those who cannot benefit from training using surface NMES, because of lower
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53 523 motor neuron denervation or intolerance to applications of electrical stimulation. The study will
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3 524 also shed light on several molecular pathways that have been suggested to influence both body
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5 525 composition and metabolic profile.
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8 **Trial Status:** Enrollment into the study started in July 2012 and as of April 2016 all participants
9
10 527 have completed the study. Data collection and data analysis are expected to be completed in
11
12 528 December 2016. The study is expected to be closed in June 2017.
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16
17 **Contributions:** ASG supervised all aspects of the trial including all interventions and
18 530 measurements and secured funding for the trial procedures. ASG, REK, LCO drafted the
19 531 manuscript. REK, JS, RK, and LCO contributed to data collection and analysis. RG, TL, TC,
20 532 DXC, CC, RA, EYL and DRG are research physicians that contributed to patient monitoring and
21 533 study design. DXC and RA will provide guidance during data analysis and manuscript
22 534 preparation. All authors affirm that the manuscript is an honest, accurate, and transparent account
23 535 of the study being reported; that no important aspects of the study have been omitted; and that
24 536 any discrepancies from the study as planned have been explained.
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42
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32 559 commercial. See: <http://creativecommons.org/licenses/by-nc/3.0/>.
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569 **References**

- 570 1. Center, N.S.C.I.S., *Spinal cord injury facts and figures at a glance*. J Spinal Cord Med, 2013. **36**(1): p. 1-2.
- 571
- 572 2. DeVivo, M.J., B.K. Go, and A.B. Jackson, *Overview of the national spinal cord injury statistical center database*. J Spinal Cord Med, 2002. **25**(4): p. 335-8.
- 573
- 574 3. Castro, M.J., et al., *Influence of complete spinal cord injury on skeletal muscle cross-sectional area within the first 6 months of injury*. Eur J Appl Physiol Occup Physiol, 1999. **80**(4): p. 373-8.
- 575
- 576
- 577 4. Gorgey, A.S. and G.A. Dudley, *Skeletal muscle atrophy and increased intramuscular fat after incomplete spinal cord injury*. Spinal Cord, 2007. **45**(4): p. 304-9.
- 578
- 579 5. Wielopolski, L., et al., *Measuring partial body potassium in the legs of patients with spinal cord injury: a new approach*. J Appl Physiol (1985), 2009. **106**(1): p. 268-73.
- 580
- 581 6. Spungen, A.M., et al., *Soft tissue body composition differences in monozygotic twins discordant for spinal cord injury*. J Appl Physiol (1985), 2000. **88**(4): p. 1310-5.
- 582
- 583 7. Spungen, A.M., et al., *Factors influencing body composition in persons with spinal cord injury: a cross-sectional study*. J Appl Physiol (1985), 2003. **95**(6): p. 2398-407.
- 584
- 585 8. Gorgey, A.S., et al., *Relationship of spasticity to soft tissue body composition and the metabolic profile in persons with chronic motor complete spinal cord injury*. J Spinal Cord Med, 2010. **33**(1): p. 6-15.
- 586
- 587
- 588 9. Gater, D.R., Jr., *Obesity after spinal cord injury*. Phys Med Rehabil Clin N Am, 2007. **18**(2): p. 333-51, vii.
- 589
- 590 10. Weaver, F.M., et al., *Prevalence of obesity and high blood pressure in veterans with spinal cord injuries and disorders: a retrospective review*. Am J Phys Med Rehabil, 2007. **86**(1): p. 22-9.
- 591
- 592
- 593 11. Lavela, S.L., et al., *Diabetes mellitus in individuals with spinal cord injury or disorder*. J Spinal Cord Med, 2006. **29**(4): p. 387-95.
- 594
- 595 12. Mojtahedi, M.C., et al., *The association between regional body composition and metabolic outcomes in athletes with spinal cord injury*. Spinal Cord, 2008. **46**(3): p. 192-7.
- 596
- 597
- 598 13. Edwards, L.A., J.M. Bugaresti, and A.C. Buchholz, *Visceral adipose tissue and the ratio of visceral to subcutaneous adipose tissue are greater in adults with than in those without spinal cord injury, despite matching waist circumferences*. Am J Clin Nutr, 2008. **87**(3): p. 600-7.
- 599
- 600
- 601
- 602 14. Maruyama, Y., et al., *Serum leptin, abdominal obesity and the metabolic syndrome in individuals with chronic spinal cord injury*. Spinal Cord, 2008. **46**(7): p. 494-9.
- 603
- 604 15. Manns, P.J., J.A. McCubbin, and D.P. Williams, *Fitness, inflammation, and the metabolic syndrome in men with paraplegia*. Arch Phys Med Rehabil, 2005. **86**(6): p. 1176-81.
- 605
- 606
- 607 16. Matsuzawa, Y., *White adipose tissue and cardiovascular disease*. Best Pract Res Clin Endocrinol Metab, 2005. **19**(4): p. 637-47.
- 608
- 609 17. Kern, P.A., et al., *Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance*. Am J Physiol Endocrinol Metab, 2001. **280**(5): p. E745-51.
- 610
- 611
- 612 18. Blake, G.J. and P.M. Ridker, *Novel clinical markers of vascular wall inflammation*. Circ Res, 2001. **89**(9): p. 763-71.
- 613

- 1
2
3 614 19. Elder, C.P., et al., *Intramuscular fat and glucose tolerance after spinal cord injury--a*
4 615 *cross-sectional study*. Spinal Cord, 2004. **42**(12): p. 711-6.
- 5 616 20. Gorgey, A.S., K.J. Mather, and D.R. Gater, *Central adiposity associations to*
6 617 *carbohydrate and lipid metabolism in individuals with complete motor spinal cord injury*.
7 618 Metabolism, 2011. **60**(6): p. 843-51.
- 8 619 21. Bauman, W.A. and A.M. Spungen, *Disorders of carbohydrate and lipid metabolism in*
9 620 *veterans with paraplegia or quadriplegia: a model of premature aging*. Metabolism,
10 621 1994. **43**(6): p. 749-56.
- 11 622 22. Grundy, S.M., et al., *Definition of metabolic syndrome: Report of the National Heart,*
12 623 *Lung, and Blood Institute/American Heart Association conference on scientific issues*
13 624 *related to definition*. Circulation, 2004. **109**(3): p. 433-8.
- 14 625 23. Nelson, M.D., et al., *Metabolic syndrome in adolescents with spinal cord dysfunction*. J
15 626 Spinal Cord Med, 2007. **30 Suppl 1**: p. S127-39.
- 16 627 24. Bauman, W.A. and A.M. Spungen, *Coronary heart disease in individuals with spinal*
17 628 *cord injury: assessment of risk factors*. Spinal Cord, 2008. **46**(7): p. 466-76.
- 18 629 25. Lee, C.S., et al., *Evaluating the prevalence of silent coronary artery disease in*
19 630 *asymptomatic patients with spinal cord injury*. Int Heart J, 2006. **47**(3): p. 325-30.
- 20 631 26. Bauman, W.A., et al., *Depressed serum high density lipoprotein cholesterol levels in*
21 632 *veterans with spinal cord injury*. Paraplegia, 1992. **30**(10): p. 697-703.
- 22 633 27. Wilt, T.J., et al., *Carbohydrate and lipid disorders and relevant considerations in persons*
23 634 *with spinal cord injury*. Evid Rep Technol Assess (Full Rep), 2008(163): p. 1-95.
- 24 635 28. Itoh, K., et al., *Mitochondrial dynamics in neurodegeneration*. Trends Cell Biol, 2013.
25 636 **23**(2): p. 64-71.
- 26 637 29. Chan, D.C., *Mitochondria: dynamic organelles in disease, aging, and development*. Cell,
27 638 2006. **125**(7): p. 1241-52.
- 28 639 30. Zhao, J., et al., *Mitochondrial dynamics regulates migration and invasion of breast*
29 640 *cancer cells*. Oncogene, 2013. **32**(40): p. 4814-24.
- 30 641 31. Phielix, E. and M. Mensink, *Type 2 diabetes mellitus and skeletal muscle metabolic*
31 642 *function*. Physiol Behav, 2008. **94**(2): p. 252-8.
- 32 643 32. Ritov, V.B., et al., *Deficiency of electron transport chain in human skeletal muscle*
33 644 *mitochondria in type 2 diabetes mellitus and obesity*. Am J Physiol Endocrinol Metab,
34 645 2010. **298**(1): p. E49-58.
- 35 646 33. Erickson, M.L., et al., *Near-infrared assessments of skeletal muscle oxidative capacity in*
36 647 *persons with spinal cord injury*. Eur J Appl Physiol, 2013. **113**(9): p. 2275-83.
- 37 648 34. Martin, T.P., et al., *Influence of electrical stimulation on the morphological and*
38 649 *metabolic properties of paralyzed muscle*. J Appl Physiol (1985), 1992. **72**(4): p. 1401-6.
- 39 650 35. Grimby, G., et al., *Muscle fiber composition in patients with traumatic cord lesion*. Scand
40 651 J Rehabil Med, 1976. **8**(1): p. 37-42.
- 41 652 36. Goodpaster, B.H., *Mitochondrial deficiency is associated with insulin resistance*.
42 653 Diabetes, 2013. **62**(4): p. 1032-5.
- 43 654 37. Holloszy, J.O., *"Deficiency" of mitochondria in muscle does not cause insulin resistance*.
44 655 Diabetes, 2013. **62**(4): p. 1036-40.
- 45 656 38. Scarpulla, R.C., *Metabolic control of mitochondrial biogenesis through the PGC-1 family*
46 657 *regulatory network*. Biochim Biophys Acta, 2011. **1813**(7): p. 1269-78.
- 47 658 39. Arany, Z., *PGC-1 coactivators and skeletal muscle adaptations in health and disease*.
48 659 Curr Opin Genet Dev, 2008. **18**(5): p. 426-34.

- 1
2
3 660 40. Summermatter, S., et al., *Peroxisome proliferator-activated receptor {gamma} coactivator 1{alpha} (PGC-1{alpha}) promotes skeletal muscle lipid refueling in vivo by activating de novo lipogenesis and the pentose phosphate pathway.* J Biol Chem, 2010. 661
662 **285**(43): p. 32793-800.
663
- 664 41. Pagel-Langenickel, I., et al., *PGC-1alpha integrates insulin signaling, mitochondrial regulation, and bioenergetic function in skeletal muscle.* J Biol Chem, 2008. **283**(33): p. 665
666 22464-72.
667
- 668 42. Sacheck, J.M., et al., *Rapid disuse and denervation atrophy involve transcriptional changes similar to those of muscle wasting during systemic diseases.* FASEB J, 2007. 669
670 **21**(1): p. 140-55.
671
- 672 43. Adhietty, P.J., et al., *Effect of denervation on mitochondrially mediated apoptosis in skeletal muscle.* J Appl Physiol (1985), 2007. **102**(3): p. 1143-51.
673
- 674 44. O'Brien, L.C.G., A.S., *Skeletal muscle mitochondrial health and spinal cord injury.* World J Orthop., 2016(In press).
675
- 676 45. Tsitouras, P.D., et al., *Serum testosterone and growth hormone/insulin-like growth factor-I in adults with spinal cord injury.* Horm Metab Res, 1995. **27**(6): p. 287-92.
677
- 678 46. Bauman, W.A., et al., *Blunted growth hormone response to intravenous arginine in subjects with a spinal cord injury.* Horm Metab Res, 1994. **26**(3): p. 152-6.
679
- 680 47. Bhasin, S., *Regulation of body composition by androgens.* J Endocrinol Invest, 2003. **26**(9): p. 814-22.
681
- 682 48. Bhasin, S. and J.G. Buckwalter, *Testosterone supplementation in older men: a rational idea whose time has not yet come.* J Androl, 2001. **22**(5): p. 718-31.
683
- 684 49. Clark, M.J., et al., *Testosterone levels among men with spinal cord injury: relationship between time since injury and laboratory values.* Am J Phys Med Rehabil, 2008. **87**(9): p. 685
686 758-67.
687
- 688 50. Kostovski, E., et al., *Decreased levels of testosterone and gonadotrophins in men with long-standing tetraplegia.* Spinal Cord, 2008. **46**(8): p. 559-64.
689
- 690 51. Zhao, W., et al., *Testosterone protects against dexamethasone-induced muscle atrophy, protein degradation and MAFbx upregulation.* J Steroid Biochem Mol Biol, 2008. **110**(1-2): p. 125-9.
691
- 692 52. Gregory, C.M., et al., *Effects of testosterone replacement therapy on skeletal muscle after spinal cord injury.* Spinal Cord, 2003. **41**(1): p. 23-8.
693
- 694 53. Isidori, A.M., et al., *Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis.* Clin Endocrinol (Oxf), 2005. **63**(3): p. 280-93.
695
- 696 54. Aversa, A., et al., *Effects of testosterone undecanoate on cardiovascular risk factors and atherosclerosis in middle-aged men with late-onset hypogonadism and metabolic syndrome: results from a 24-month, randomized, double-blind, placebo-controlled study.* J Sex Med, 2010. **7**(10): p. 3495-503.
697
- 698 55. Sinha-Hikim, I., et al., *Effects of testosterone supplementation on skeletal muscle fiber hypertrophy and satellite cells in community-dwelling older men.* J Clin Endocrinol Metab, 2006. **91**(8): p. 3024-33.
699
- 700 56. Ryan, A.S., et al., *Skeletal muscle hypertrophy and muscle myostatin reduction after resistive training in stroke survivors.* Stroke, 2011. **42**(2): p. 416-20.
701
702
703

- 1
2
3 704 57. Ibanez, J., et al., *Twice-weekly progressive resistance training decreases abdominal fat*
4 705 *and improves insulin sensitivity in older men with type 2 diabetes*. *Diabetes Care*, 2005.
5 706 **28**(3): p. 662-7.
6
7 707 58. Chromiak, J.A., et al., *Effect of a 10-week strength training program and recovery drink*
8 708 *on body composition, muscular strength and endurance, and anaerobic power and*
9 709 *capacity*. *Nutrition*, 2004. **20**(5): p. 420-7.
10 710 59. Mahoney, E.T., et al., *Changes in skeletal muscle size and glucose tolerance with*
11 711 *electrically stimulated resistance training in subjects with chronic spinal cord injury*.
12 712 *Arch Phys Med Rehabil*, 2005. **86**(7): p. 1502-4.
13 713 60. Mohr, T., et al., *Insulin action and long-term electrically induced training in individuals*
14 714 *with spinal cord injuries*. *Med Sci Sports Exerc*, 2001. **33**(8): p. 1247-52.
15 715 61. Kjaer, M., et al., *Fatty acid kinetics and carbohydrate metabolism during electrical*
16 716 *exercise in spinal cord-injured humans*. *Am J Physiol Regul Integr Comp Physiol*, 2001.
17 717 **281**(5): p. R1492-8.
18 718 62. Crameri, R.M., et al., *Effects of electrical stimulation-induced leg training on skeletal*
19 719 *muscle adaptability in spinal cord injury*. *Scand J Med Sci Sports*, 2002. **12**(5): p. 316-
20 720 22.
21 721 63. Gerrits, H.L., et al., *Peripheral vascular changes after electrically stimulated cycle*
22 722 *training in people with spinal cord injury*. *Arch Phys Med Rehabil*, 2001. **82**(6): p. 832-9.
23 723 64. Gorgey, A.S., et al., *Effects of resistance training on adiposity and metabolism after*
24 724 *spinal cord injury*. *Med Sci Sports Exerc*, 2012. **44**(1): p. 165-74.
25 725 65. Kirshblum, S.C., et al., *International standards for neurological classification of spinal*
26 726 *cord injury (revised 2011)*. *J Spinal Cord Med*, 2011. **34**(6): p. 535-46.
27 727 66. Gorgey, A.S., et al., *Effects of neuromuscular electrical stimulation parameters on*
28 728 *specific tension*. *Eur J Appl Physiol*, 2006. **97**(6): p. 737-44.
29 729 67. Gorgey, A.S., et al., *Effects of electrical stimulation parameters on fatigue in skeletal*
30 730 *muscle*. *J Orthop Sports Phys Ther*, 2009. **39**(9): p. 684-92.
31 731 68. Gorgey, A.S., et al., *Influence of motor complete spinal cord injury on visceral and*
32 732 *subcutaneous adipose tissue measured by multi-axial magnetic resonance imaging*. *J*
33 733 *Spinal Cord Med*, 2011. **34**(1): p. 99-109.
34 734 69. Gorgey, A.S. and C. Shepherd, *Skeletal muscle hypertrophy and decreased intramuscular*
35 735 *fat after unilateral resistance training in spinal cord injury: case report*. *J Spinal Cord*
36 736 *Med*, 2010. **33**(1): p. 90-5.
37 737 70. Vermeulen, A., L. Verdonck, and J.M. Kaufman, *A critical evaluation of simple methods*
38 738 *for the estimation of free testosterone in serum*. *J Clin Endocrinol Metab*, 1999. **84**(10): p.
39 739 3666-72.
40 740 71. Collins, E.G., et al., *Energy cost of physical activities in persons with spinal cord injury*.
41 741 *Med Sci Sports Exerc*, 2010. **42**(4): p. 691-700.
42 742 72. Bergman, R.N., *Lilly lecture 1989. Toward physiological understanding of glucose*
43 743 *tolerance. Minimal-model approach*. *Diabetes*, 1989. **38**(12): p. 1512-27.
44 744 73. Matthews, D.R., et al., *Homeostasis model assessment: insulin resistance and beta-cell*
45 745 *function from fasting plasma glucose and insulin concentrations in man*. *Diabetologia*,
46 746 1985. **28**(7): p. 412-9.
47 747 74. Matsuda, M. and R.A. DeFronzo, *Insulin sensitivity indices obtained from oral glucose*
48 748 *tolerance testing: comparison with the euglycemic insulin clamp*. *Diabetes Care*, 1999.
49 749 **22**(9): p. 1462-70.

- 1
2
3 750 75. Brass, E.P., et al., *Decreased NADH dehydrogenase and ubiquinol-cytochrome c*
4 751 *oxidoreductase in peripheral arterial disease*. Am J Physiol Heart Circ Physiol, 2001.
5 752 **280**(2): p. H603-9.
- 6
7 753 76. Brooke, M.H. and K.K. Kaiser, *Three "myosin adenosine triphosphatase" systems: the*
8 754 *nature of their pH lability and sulfhydryl dependence*. J Histochem Cytochem, 1970.
9 755 **18**(9): p. 670-2.
- 10
11 756 77. Nachlas, M.M., et al., *Cytochemical demonstration of succinic dehydrogenase by the use*
12 757 *of a new p-nitrophenyl substituted ditetrazole*. J Histochem Cytochem, 1957. **5**(4): p.
13 758 420-36.
- 14
15 759 78. Wong-Riley, M., *Changes in the visual system of monocularly sutured or enucleated cats*
16 760 *demonstrable with cytochrome oxidase histochemistry*. Brain Res, 1979. **171**(1): p. 11-28.
- 17
18 761 79. Kostovski, E., et al., *Altered content of AMP-activated protein kinase isoforms in skeletal*
19 762 *muscle from spinal cord injured subjects*. Am J Physiol Endocrinol Metab, 2013. **305**(9):
20 763 p. E1071-80.
- 21
22 764 80. Long, Y.C., et al., *Differential expression of metabolic genes essential for glucose and*
23 765 *lipid metabolism in skeletal muscle from spinal cord injured subjects*. J Appl Physiol
24 766 (1985), 2011. **110**(5): p. 1204-10.
- 25
26 767 81. Aksnes, A.K., et al., *Intact glucose transport in morphologically altered denervated*
27 768 *skeletal muscle from quadriplegic patients*. Am J Physiol, 1996. **271**(3 Pt 1): p. E593-
28 769 600.
- 29
30 770 82. Hjeltnes, N., et al., *Improved body composition after 8 wk of electrically stimulated leg*
31 771 *cycling in tetraplegic patients*. Am J Physiol, 1997. **273**(3 Pt 2): p. R1072-9.
- 32
33 772 83. Hjeltnes, N., et al., *Exercise-induced overexpression of key regulatory proteins involved*
34 773 *in glucose uptake and metabolism in tetraplegic persons: molecular mechanism for*
35 774 *improved glucose homeostasis*. FASEB J, 1998. **12**(15): p. 1701-12.
- 36
37 775 84. Mohr, T., et al., *Long-term adaptation to electrically induced cycle training in severe*
38 776 *spinal cord injured individuals*. Spinal Cord, 1997. **35**(1): p. 1-16.
- 39
40 777 85. Gorgey, A.S., et al., *Abundance in proteins expressed after functional electrical*
41 778 *stimulation cycling or arm cycling ergometry training in persons with chronic spinal*
42 779 *cord injury*. J Spinal Cord Med, 2016: p. 1-10.
- 43
44 780 86. Herbst, K.L. and S. Bhasin, *Testosterone action on skeletal muscle*. Curr Opin Clin Nutr
45 781 Metab Care, 2004. **7**(3): p. 271-7.
- 46
47 782 87. Wu, Y., et al., *Identification of androgen response elements in the insulin-like growth*
48 783 *factor I upstream promoter*. Endocrinology, 2007. **148**(6): p. 2984-93.
- 49
50 784 88. Araujo, A.B., et al., *Clinical review: Endogenous testosterone and mortality in men: a*
51 785 *systematic review and meta-analysis*. J Clin Endocrinol Metab, 2011. **96**(10): p. 3007-19.
- 52
53 786 89. Shores, M.M., et al., *Testosterone treatment and mortality in men with low testosterone*
54 787 *levels*. J Clin Endocrinol Metab, 2012. **97**(6): p. 2050-8.
- 55
56 788 90. Layton, J.B., et al., *Comparative Safety of Testosterone Dosage Forms*. JAMA Intern
57 789 Med, 2015. **175**(7): p. 1187-96.

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793 **Table 1.** Randomization of individuals with motor complete SCI into either RT+TRT (n=13) or
 794 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

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

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802 **Table 2.** Metabolic health variables measured at baseline 1, baseline 2, post-intervention 1 and
 803 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, TNF α)	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

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

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3 819 **Figure Legends**
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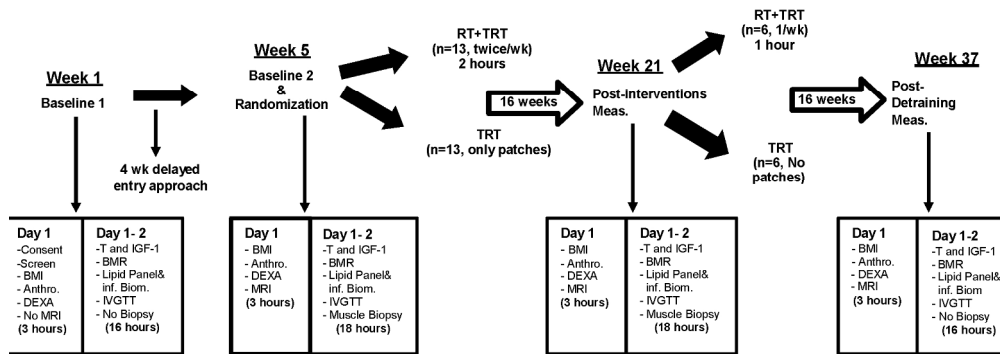
6 820 **Figure 1.** Timeline and main procedures of the TEREX-SCI trial for both the RT+TRT and TRT
7 821 groups during baseline 1, baseline 2, post-intervention 1 and post-intervention 26 for persons
8 822 with motor complete SCI.
9

10 823 **Figure 2.** A person with T4 motor complete SCI undergoing both electrically evoked RT (left
11 824 panel) and TRT using transdermal patches (right panel) as a part of a 16 week intervention.
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13 825 **Figure 3.** Schematic diagram showing the process of recruitment over the 3 year period of the
14 826 TEREX trial.
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16 827 **Figure 4.** A representative figure of analysis for IVGTT in a person with SCI after infusion of
17 828 dextrose followed by insulin 20 minutes later. The dextrose concentration is calculated by
18 829 multiplying 0.3g x body weight (kg) in 50% solution. Insulin concentration is determined by
19 830 multiplying 0.02 units x body weight (kg). Blue line represents fasting glucose concentration,
20 831 resting, following infusion, and over 120 minutes. Red line represents the line of best fit of
21 832 glucose concentration. Green line represents fasting insulin level, spike following 20 minutes of
22 833 dextrose infusion and over 120 minutes. Black line represents the line of best fit for insulin
23 834 concentration.
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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)

Peer review only

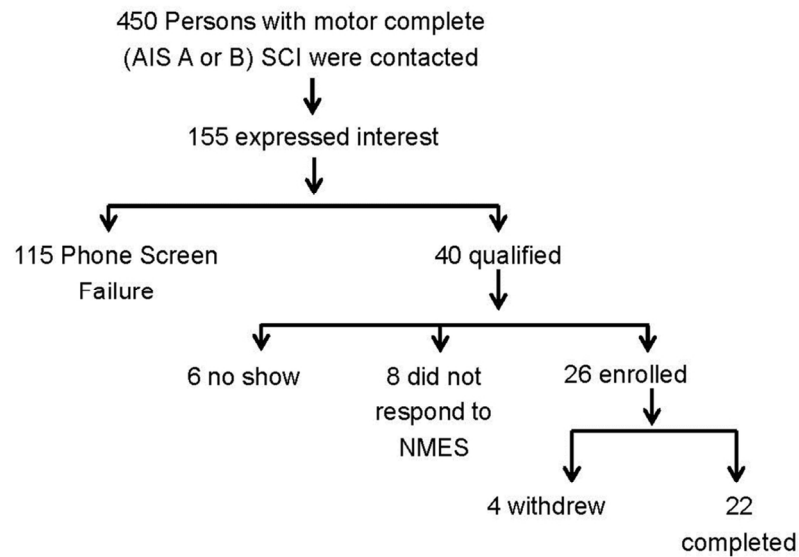
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A person with T4 motor complete SCI undergoing both electrically evoked RT (left panel) and TRT using transdermal patches (right panel) as a part of a 16 week intervention.

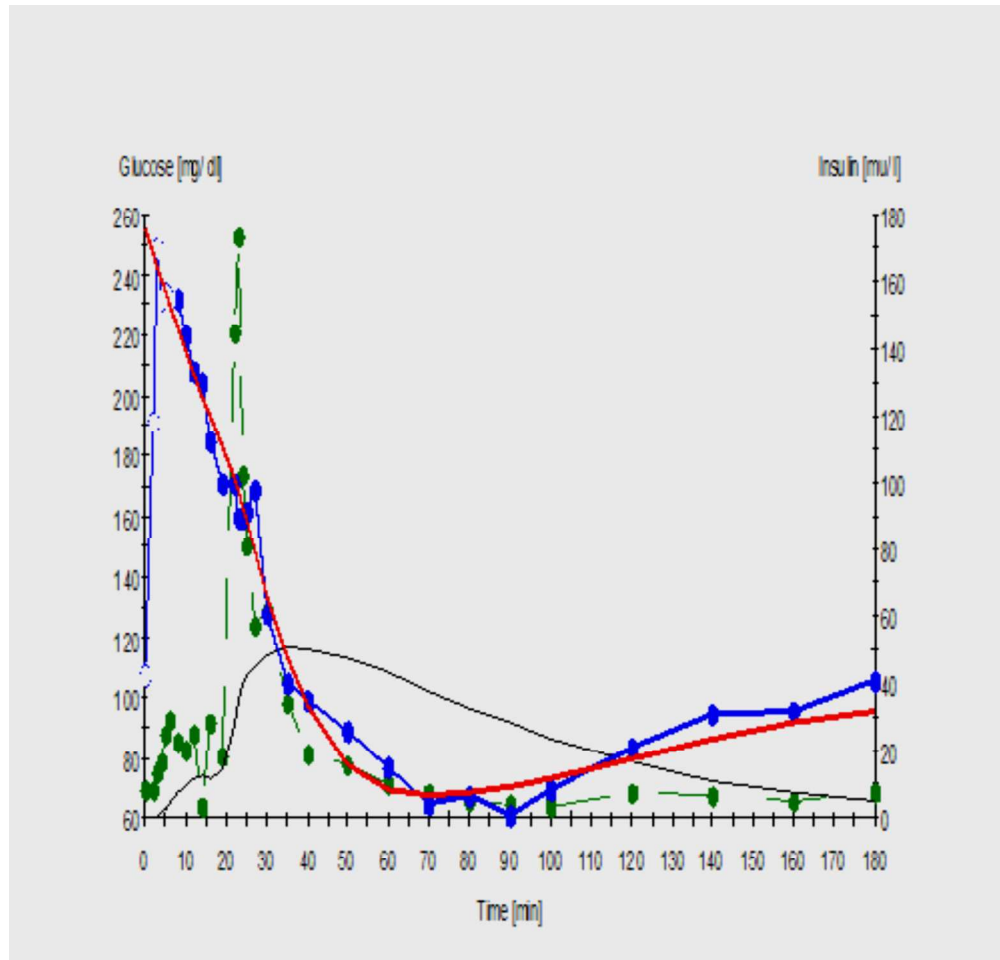
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Recruitment



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 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)	_____

1
2
3 **Introduction**
4

5	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	_____ 4-6 _____
6	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
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8		6b	Explanation for choice of comparators	_____ 5-6 _____
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10	Objectives	7	Specific objectives or hypotheses	_____ 6 _____
11				
12	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	
13			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	_____ 7 _____
14				

15
16 **Methods: Participants, interventions, and outcomes**
17

18	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	_____ 7 _____
19			be collected. Reference to where list of study sites can be obtained	
20				
21	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	_____ 8 _____
22			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
23				
24	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	_____ 9-10 _____
25			administered	
26				
27		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	_____ _____
28			change in response to harms, participant request, or improving/worsening disease)	
29				
30		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	_____ 9 _____
31			(eg, drug tablet return, laboratory tests)	
32				
33		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_____ 8 _____
34				
35	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	_____ 10-16 _____
36			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	
37			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
38			efficacy and harm outcomes is strongly recommended	
39				
40	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for	_____ 7, Fig 1 _____
41			participants. A schematic diagram is highly recommended (see Figure)	
42				
43				
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45				

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2				
3	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____
4				
5				
6	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	____7-8____
7				

8 **Methods: Assignment of interventions (for controlled trials)**

9 Allocation:

10				
11				
12	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____
13				
14				
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17				
18	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____
19				
20				
21				
22	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____8____
23				
24				
25	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____9-10____
26				
27				
28		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____
29				
30				
31				

32 **Methods: Data collection, management, and analysis**

33				
34	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____
35				
36				
37				
38				
39		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____
40				
41				
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3	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 ___
4				
5				
6				
7	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 ___
8				
9				
10		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	___ N/A ___
11				
12		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 ___
13				
14				
15				
16	Methods: Monitoring			
17				
18	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 ___
19				
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21				
22				
23		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	_____
24				
25				
26	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 ___
27				
28				
29	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
30				
31				
32				
33	Ethics and dissemination			
34				
35	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	___ 3, 7, 17 ___
36				
37				
38	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)	_____
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3	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	___7___
4				
5				
6		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	___N/A___
7				
8				
9	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___
10				
11				
12	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	___21___
13				
14				
15	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___
16				
17				
18	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	_____
19				
20				
21	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___
22				
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26		31b	Authorship eligibility guidelines and any intended use of professional writers	___20___
27				
28		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
29				
30	Appendices			
31				
32	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	___attached___
33				
34				
35	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___
36				
37				

*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

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

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

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44 **Abstract**

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

53 **Methods and analysis:** Twenty-six individuals with chronic, motor complete SCI between 18-
54 50 years old were randomly assigned to a RT+TRT group (n = 13) or a TRT group (n = 13).
55 Twenty-two participants completed the initial 16-week training phase of the study and 4
56 participants withdrew. Twelve participants out of the 22 completed 16 weeks of detraining. The
57 TRT was provided via transdermal testosterone patches (4-6 mg/day). The RT+TRT group had
58 16 weeks of supervised unilateral progressive RT using surface neuromuscular electrical
59 stimulation with ankle weights. This study will investigate the effects of evoked RT+TRT or
60 TRT alone on body composition (muscle cross sectional area, visceral adipose tissue, %FM) and
61 metabolic profile (glucose and lipid metabolisms) in individuals with motor complete SCI.
62 Findings from this study may help in designing exercise therapies to alleviate the deterioration in
63 body composition after SCI and decrease the incidence of metabolic disorders in this clinical
64 population.

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

1
2
3 67 forms. Results will be submitted to peer-reviewed journals and presented at national and
4
5 68 international conferences.
6
7

8 **Trial Registration:** NCT01652040
9
10

11
12 **Keywords:** RESISTANCE TRAINING, SPINAL CORD INJURY, BODY COMPOSITION,
13
14 METABOLISM, TESTOSTERONE, MITOCHONDRIA, INFLAMMATORY BIOMARKERS
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19
20 **Strengths and limitations**
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- 22
23 75 ➤ The trial will investigate the use of surface neuromuscular electrical stimulation induced
24
25 76 resistance training (NMES-RT) to restore muscle size after spinal cord injury (SCI)
26
27
28 77 ➤ The trial will provide evidence on the effectiveness of testosterone replacement therapy
29
30 78 (TRT) to restore muscle size and lean mass and serve as an alternative approach for those
31
32 79 who cannot benefit from NMES.
33
34
35 80 ➤ The trial will determine how restoring muscle size and lean mass by RT+TRT or TRT can
36
37 81 benefit the metabolic profile after SCI.
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40 82 ➤ The study is only limited to men with complete SCI
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42 83 ➤ Surface NMES may not benefit those with full sensation or lower motor neuron denervation
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90 Introduction

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

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

111 Metabolic changes also accompany SCI, with previous studies finding that more than
112 50% of individuals with SCI are glucose intolerant, while one out of five is diabetic [2, 9-11, 21].

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2
3 113 Other studies report that 55% of individuals with SCI are at risk of developing metabolic
4
5 114 syndrome [21-23]. Individuals with complete tetraplegia are more likely to experience decreased
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7
8 115 glucose and carbohydrate tolerance and have a higher prevalence of heart disease than those with
9
10 116 incomplete injuries [24, 25]. Likewise, depressed HDL-C ($<35 \text{ mg} \cdot \text{dL}^{-1}$) and a higher total
11
12 117 cholesterol/HDL-C ratio, predictors of coronary heart disease, were noted in those with chronic
13
14 118 SCI compared with able bodied controls [24, 26]. These are not universal findings, however, as a
15
16 119 systematic review of carbohydrate and lipid disorders in persons with SCI did not find strong
17
18 120 evidence of increased risk beyond that of the general population [27].
19

20
21
22 121 While previous studies have shown a link between body composition and metabolic
23
24 122 profile after SCI, the cellular mechanisms remain unknown. Mitochondria are the site of oxygen
25
26 123 consumption and energy production from glucose and lipid metabolism. Unfortunately,
27
28 124 mitochondrial function is impaired in a number of diseases including neurodegenerative disease,
29
30 125 atherosclerosis, hypertension and cancer [28-31]. Fewer and smaller mitochondria are found in
31
32 126 skeletal muscle of insulin resistant, obese and type II diabetic individuals [32]. Previous studies
33
34 127 found decreased muscle oxidative capacity and succinate dehydrogenase activity, complex II of
35
36 128 the electron transport chain, after SCI [33-35].
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40
41 129 One hypothesis is that skeletal muscle mitochondrial function is decreased in metabolic
42
43 130 disorders, leading to decreased fatty acid oxidation which contributes to insulin resistance [36,
44
45 131 37]; however, this is controversial [37]. Mitochondria are dynamic organelles and undergo
46
47 132 biogenesis, remodeling, and degradation. Mitochondrial biogenesis is driven in part through the
48
49 133 action of peroxisome proliferator-activated receptor (PPAR) coactivator 1 alpha (PGC-1 α) [38,
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51 134 39]. In addition, PGC-1 α integrates insulin signaling and lipogenesis in skeletal muscle [40, 41].
52
53 135 PGC-1 α is decreased in animal and human models following denervation [42, 43]. Decreased
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3 136 mitochondrial biogenesis and reduced mitochondrial mass may result in decreased energy
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6 137 production and therefore play a significant role in the altered metabolic profile following SCI
7
8 138 [44].
9

10 139 Body composition and metabolic changes after SCI may be further exacerbated by
11
12
13 140 reduced anabolic hormones including testosterone (T), growth hormone and the growth hormone
14
15 141 second messenger insulin like growth factor-1 (IGF-1) [45, 46]. Previous studies have shown that
16
17 142 60% of men with SCI have low T and that testosterone replacement therapy (TRT) increases
18
19 143 IGF-1 in men [47-50]. In rodent models of SCI, TRT attenuates the loss of muscle [51, 52]. TRT
20
21 144 decreases total body fat, increases lean mass [53, 54] and increases the number of proliferating
22
23 145 skeletal muscle satellite cells in in men [55]. These findings suggest that TRT may provide
24
25 146 metabolic benefits to individuals with SCI.
26
27
28

29 147 Resistance training (RT) improves insulin sensitivity and increases fatty acid and
30
31 148 carbohydrate metabolism as well as attenuates sarcopenia in the elderly and after SCI [56-63].
32
33 149 Moreover, RT has been shown to influence body composition by increasing lean mass,
34
35 150 decreasing FM and reducing VAT, suggesting that the benefits of RT could overcome the risk of
36
37 151 developing insulin resistance [56-59]. Functional electrical stimulation (FES) has been shown to
38
39 152 improve fatty acid kinetics, carbohydrate metabolism and vascular health after SCI [60-63].
40
41 153 Electrically evoked RT using neuromuscular electrical stimulation (NMES-RT) and ankle
42
43 154 weights is another form that has been shown to be effective in inducing muscle hypertrophy in
44
45 155 individuals with chronic SCI [59, 64]. One study showed a 40% increase in skeletal muscle size
46
47 156 and improved glucose tolerance after 12 weeks of training [59]. Another study showed that
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49 157 following 12 weeks of NMES-RT, whole thigh, knee extensor and flexor cross sectional areas
50
51 158 (CSAs) increased by 28%, 35% and 16%, respectively. Moreover, the ratio of leg FFM to whole
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3 159 body FFM increased by 20% following intervention. There was 32% decrease in glucose area
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5
6 160 under the curve adjusted to muscle CSA following 12 weeks of NMES-RT. However, there
7
8 161 were only modest effects on whole body composition as well as a non-significant decrease in
9
10 162 VAT [64]. It is possible that the limited effects of NMES-RT on parameters of body
11
12 163 composition and VAT can be possibly explained by depressed T-level in persons with SCI.
13
14 164 Supplementing exogenous T may optimize the outcomes of NMES-RT on parameters of body
15
16 165 composition and metabolic profile such as increase basal metabolic rate (BMR).
17
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19

20 166 TRT may be an effective therapy to counterbalance the growing rate of obesity, type II
21
22 167 diabetes and cardiovascular disease among individuals with SCI. Moreover, results from the
23
24 168 current trial may provide evidence that TRT is an effective intervention for those who cannot
25
26 169 effectively benefit from NMES because of lower motor neuron denervation or intolerance to
27
28 170 electrical stimulation. Therefore, the primary hypothesis is that the addition of TRT will
29
30 171 maximize the benefits of electrically evoked RT on parameters of body composition and
31
32 172 metabolic profile in men with chronic complete SCI. We, hereby, report the design of a study for
33
34 173 which the major research goal is to investigate the effects of 16 weeks of evoked RT+TRT vs.
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36 174 TRT on body composition (primary outcome variables; muscle CSA, VAT, %FM) and metabolic
37
38 175 profiles (secondary outcome variables; glucose and lipid metabolism) in individuals with motor
39
40 176 complete SCI.
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45 177 **Methods and analysis**

46 178 **Study design**

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48
49 179 A randomized controlled study was undertaken in which individuals with SCI were
50
51 180 randomized to receive RT+TRT or TRT alone for 16 weeks. The study was approved by the
52
53 181 McGuire Veteran Affairs Investigation Research Board and the Virginia Commonwealth
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3 182 University (VCU) Office of Research and Innovation. The trial has been registered at
4
5 183 clinicaltrials.gov (NCT01652040). A member of the research team explained the study and
6
7
8 184 obtained written informed consent. After informed consent each subject underwent a detailed
9
10 185 physical examination at the Hunter Holmes McGuire VA Medical Center (VAMC) by a
11
12 186 physiatrist board certified in SCI medicine. This exam included a neurological assessment
13
14 187 according to the International Standards for Neurological Classification of SCI (ISNCSCI),
15
16 188 including the American Spinal Injury Association (ASIA) Impairment Scale (AIS) [65].
17
18
19

20 189 The study design and procedures are presented in Figures 1 and 2. The study visits
21
22 190 included estimation of body composition, anthropometry, and dual x-ray absorptiometry (DEXA);
23
24 191 baselines 1 and 2 and post-interventions 1 and 2). Additionally, MRI scans were obtained for
25
26 192 trunk adipose tissue and lower extremity skeletal muscles and IMF CSA (baseline 2, post-
27
28 193 interventions 1 and 2). Participants were then escorted to the VCU-CRS unit (VCU Clinical
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30 194 Research Unit) for dinner, and remained in the VCU-CRS unit overnight for the four study visits.
31
32
33 195 Muscle biopsies were obtained at baseline 2 and post intervention 1.
34
35

36 196 **Recruitment and Randomization**

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38
39 197 The recruitment process started in July 2012 and ended in June 2015. Data analysis is
40
41 198 currently being performed. Recruitment details and randomization are presented in Table 1 and
42
43 199 Figure 3. Prior to the start of the study, numbers 1-26 were randomized using the n-Query
44
45 200 software program by the principal investigator. At the end of the two-day assessment period
46
47 201 (Baseline 1), the allocation of subjects into either group was performed in a blinded fashion by
48
49 202 drawing a folded paper with a number (1-26) by the research coordinator. This number was
50
51 203 matched with the assignment from the randomization procedure.
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204 Twenty-six participants were recruited to participate in the study. A four week delayed
205 entry period was included to obtain baseline measurements, stabilize body weight and educate
206 participants on how to monitor their dietary intake. This allowed participants to serve as their
207 own controls. Four participants withdrew from the trial. At baseline 2, two participants failed to
208 comply with study protocol and withdrew from the study because lack of interest. Nurses failed
209 to locate the veins on the third participant to conduct IVGTT and he was asked to withdraw from
210 the trial. The fourth participant was diagnosed with a grade IV pressure ulcer after being
211 admitted into the trial. Following the delayed entry period, participants were randomly assigned
212 to a RT+TRT group (n = 13) or TRT group (n = 13). TRT patches (2-6 mg/day) were replaced
213 daily on alternating shoulders at bedtime for 16 weeks. The RT+TRT group received 16 weeks
214 of supervised unilateral progressive RT using surface NMES and ankle weights. Following the
215 intervention the two-day assessment period was repeated (Figure 1).

216 **Participants and eligibility criteria**

217 Participants were men between 18-50 years old with a BMI of ≤ 30 kg/m². The upper
218 limit of age was set to 50 years to avoid unanticipated side effects that may result from TRT.
219 Participants had motor complete SCI C5-L2, ASIA A or B. Participants with pre-existing
220 medical conditions were excluded. These included cardiovascular disease, uncontrolled type II
221 diabetes and those on insulin, pressures sores stage 2 or greater, supra-physiological T level,
222 hematocrit above 50% and urinary tract infection or symptoms.

223 **I. Interventions**

224 **Resistance training**

225 The first week of RT was conducted with no ankle weights to ensure that the knee
226 extensor muscles could extend the weight of the lower leg against gravity. Once full knee

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3 227 extension was achieved in a sitting position, two pounds were added on a weekly basis with the
4
5 228 criteria that full knee extension was achieved before more weight was added. Surface NMES was
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7
8 229 applied to the knee extensor muscles via surface electrodes (Figure 2). One electrode was placed
9
10 230 2–3 cm above the superior aspect of the patella over the vastus medialis muscle, and the other
11
12 231 lateral to and 30 cm above the patella over vastus lateralis muscle. Current from the stimulator
13
14 232 was manually increased in 5-second intervals to evoke full knee extension with a 3-minute rest
15
16 233 between sets, 30 Hz, 450 μ s pulses and a current sufficient to evoke full knee extension as
17
18 234 previously described [64, 66, 67]. Four sets of 10 repetitions was performed twice weekly for 16
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20 235 weeks.
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23

24 236 **Testosterone replacement therapy (TRT)**

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26
27 237 Following baseline measurements, T was administered by patches (Androderm, Watson
28
29 238 Pharma. Inc, Parsippany, NJ) that delivered between 2-6 mg/day [53] (Figure 2). Serum T
30
31 239 concentration was measured and reviewed in a blinded fashion weekly for the first month and
32
33 240 then every 4 weeks by an endocrinologist. Baseline dose was prescribed according to the initial T
34
35 241 level. A dose of 6, 4 or 2 mg/day was initially prescribed if the serum baseline T-level was less
36
37 242 than 300, 300-600 or above 600, respectively. During the course of the study, the dose was
38
39 243 decreased to 2 mg/day if the serum T concentration was more than 1000 ng/dL (34.7 nmol/L)
40
41 244 and the participant was reeducated about use of the patch if the concentration was less than 250
42
43 245 ng/dL (8.7nmol/L) above the pretreatment concentration. Patches were returned after use to
44
45 246 ensure adherence to the intervention protocol. Participants were instructed to place patches at
46
47 247 bedtime and only remove them during showering. If skin irritation became an issue, participants
48
49 248 were initially advised to move patches up or down on the shoulder muscles from the irritation
50
51 249 site and if the situation was not resolved, a hydrocortisone cream was prescribed.
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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 additional 16 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.

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2
3 273 Testing was performed after lower extremity elevation for at least 20 minutes to minimize fluid
4
5
6 274 shift. The subject was assisted to lie on a padded table and both legs were strapped proximal to
7
8 275 the knees and ankles. The arms and legs were positioned to ensure proper alignment.
9

10 276 **Magnetic resonance imaging (MRI)**

11
12
13 277 MRI was performed at the VAMC Hospital using a 1.5 Tesla magnet (GE) as previously
14
15 278 described [4, 19, 59, 68, 69]. Transaxial images, 10 mm thick and 10 mm apart, were taken from
16
17 279 the hip joint to the knee joint and from knee to the ankle using the whole body coil. The location
18
19
20 280 of the scan was identified by placing a mark 6 inches proximal to and distal to the patella and
21
22 281 matched on follow up scans. To analyze VAT and subcutaneous adipose tissue (SAT) transverse
23
24 282 slices (0.8 cm thickness) were acquired every 0.4 cm gap from the xyphoid process to the
25
26 283 femoral heads. Images were acquired in series of two stacks with L4-L5 used as a separating
27
28
29 284 point. TRT patches were removed 48-72 hours prior to MRI scans to avoid skin burn.
30

31
32 285 Analyses will be performed using commercial available software (X-vessel) as previously
33
34 286 described [4, 19, 68, 69]. Briefly, the thigh and leg images will be segmented into fat (high
35
36 287 intensity), skeletal muscle (mid intensity) and background/bone (low intensity). Manual
37
38 288 selection of a pixel of skeletal muscle will highlight all skeletal muscle pixels and provide the
39
40 289 total number of skeletal muscle pixels while excluding fat. VAT and SAT will be measured by
41
42 290 manually tracing around the anatomical borders. The number of pixels in the highlighted region
43
44 291 will be multiplied by the matrix size to measure VAT and SAT CSA (cm²).
45
46
47

48 292 **Skeletal muscle torque and specific tension**

49
50 293 Torque of the knee extensor muscle group was evaluated using a Biodex isokinetic
51
52 294 dynamometer (Shirely, NY). Measurements were done 72 hours after the muscle biopsy to
53
54
55 295 prevent acute effects on protein expression. Participants were seated with both the trunk-thigh
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1
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3 296 angle and the knee-thigh angle at 90°. Each participant was securely strapped to the test chair by
4
5
6 297 a crossover shoulder harnesses and a belt across the hip joint. The axis of the dynamometer was
7
8 298 aligned to the anatomical knee axis and the lever arm was attached 2-3 cm above the lateral
9
10 299 malleolus. Before measuring isometric torque, passive tension of the right knee extensor muscle
11
12 300 group was measured at 5, 30, 60, 90, 180, 270 degrees/sec as an index of spasticity. Isometric
13
14 301 torque was measured using current amplitude of 50 and 100 mA at a frequency of 30 Hz and
15
16 302 pulse duration 450 μ s. Isokinetic torque was measured at 60, 90 and 180 degrees/sec using the
17
18 303 same stimulation protocol.
19

20 304 **Serum and plasma analysis**

21
22 305 All metabolic profile analysis is presented in Table 2. Blood samples were collected after
23
24 306 an overnight fast. Total T was measured by liquid chromatography with isotope dilution mass
25
26 307 spectrometry detection after supported liquid extraction. Free T concentration was calculated
27
28 308 using sex hormone binding globulin and albumin concentrations (www.issam.ch/freetesto.htm)
29
30 309 [70]. Serum IGF-I concentration was measured by an immunoluminometric assay (Quest
31
32 310 Diagnostics, Madison, NJ). Fasting lipid profiles (HDL-C, LDL-C, total cholesterol, and
33
34 311 triglycerides) were determined as previously described [8, 20]. Inflammatory biomarkers CRP,
35
36 312 IL-6, TNF- α , and free fatty acids were determined by commercially available enzyme-linked
37
38 313 immunosorbent assay kits (ALPCO; Salem, NH).
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45 314 **Energy Expenditure**

46
47 315 After an overnight fast for 10-12 hours, participants were kept in a dark room for 20-30
48
49 316 minutes to attain a resting state during which BMR was measured as previously described [8].
50
51 317 Briefly, while in a supine position a canopy was placed over the subject's head. Each subject was
52
53 318 allowed 2-3 minutes before starting the test to ensure no signs of apnea or claustrophobic
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3 319 episodes. All subjects were instructed to stay awake during the entire test and to breathe
4
5
6 320 normally. The canopy was then attached to a vacuum to draw the expired gases to the flowmeter
7
8 321 of the metabolic unit (COSMED KB42). Prior to the test, the metabolic unit was calibrated
9
10 322 using the standard procedures identified by the manufacturer. Carbon dioxide and oxygen output
11
12 323 was used to calculate the respiratory exchange ratio and BMR (kcal/day) was calculated using
13
14 324 the average of the last 15 minutes of the test. This was used to measure the percentage of
15
16 325 substrate utilization (% fat vs. % carbohydrate) [8, 71].
17
18
19

20 326 To determine whether NMES-RT improves exercise performance, testing was performed
21
22 327 using a functional electrical stimulation bike (Restorative Therapies, RTI-300) against
23
24 328 progressive resistance protocol until fatigue. The protocol started with 3 minutes resting, 3
25
26 329 minute warm-up (35-37 RPM) using the servomotor and then a two minute incremental
27
28 330 progressive resistance protocol (1 Nm, 3 Nm, 5 Nm, etc.) until fatigue. After fatigue, a one
29
30 331 minute cool down period was allowed followed by 5 minutes of rest. Energy expenditure and
31
32 332 cardiovascular performance [VO_2 (l/min), blood pressure and heart rate] was collected at baseline
33
34 333 2 and post-interventions 1 and 2.
35
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39 334 Each participant met with a dietician at the start of the study and was asked to maintain a
40
41 335 5 day food dietary log monitoring their caloric intake for the duration of the study. Participants
42
43 336 were instructed to record all liquid and food consumption and no nutritional advice was given on
44
45 337 the size or the portion of the food. Dietary logs were analyzed on a weekly basis using a
46
47 338 nutritional software package (Nutrition Data System for Research version 2014) under the
48
49 339 supervision of a registered dietitian. After analysis was completed, the average caloric intake
50
51 340 (kcal) and percentage macronutrients (carbohydrates, fat and protein) were calculated. Each
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53 341 participant received monthly feedback via phone call with the registered dietician on how to
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1
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3 342 maintain appropriate dietary habits based on his BMR and percentage macronutrients (45%
4
5 343 carbohydrates, 30% fat and 25% protein). Every effort was made to balance the dietary habits
6
7
8 344 between the RT+TRT and the TRT group.
9

10 345 **Intravenous Glucose Tolerance Test (IVGTT)**

11
12 An IVGTT was used to determine insulin sensitivity and glucose effectiveness before
13 346 training and 48 hours after the last exercise bout. After an overnight fast, an intravenous line was
14 347 placed to facilitate infusion of glucose and blood sampling. Blood samples were taken every 2-3
15 348 minutes before and 30 minutes after glucose injection (0.3 gm/kg IV over 30 seconds), followed
16 349 by 5-10 minutes sampling ending at 180 minutes. Twenty minutes after the glucose injection a
17 350 bolus of insulin (0.02 U/kg) was injected to determine insulin sensitivity. Plasma glucose was
18 351 measured by the Autoanalyzer glucose oxidase method and plasma insulin concentrations were
19 352 determined by commercial radioimmunoassay (Table 2). The glucose disposal rate per unit of
20 353 secreted insulin per unit time and glucose mediated glucose disposal rate were calculated from a
21 354 least-squares fitting of the temporal pattern of glucose and insulin throughout the IVGTT using
22 355 the MINMOD program [72]. A representative analysis of IVGTT is presented in Figure 4. The
23 356 acute insulin response to IV glucose was calculated as the mean rise in plasma insulin above
24 357 baseline at 3, 4 and 5 minutes after IV glucose administration. KG, a measure of glucose
25 358 tolerance, was calculated as the least square slope of the natural log of absolute glucose
26 359 concentration between 5 and 20 minutes after the glucose bolus [73]. The homeostatic model of
27 360 assessment of insulin resistance (HOMA-IR) was calculated and insulin sensitivity was
28 361 determined using Matsuda and DeFronzo formula [73, 74].
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365 **III. Secondary Outcomes**

366 **Skeletal muscle biopsy**

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

375 **Mitochondrial Electron Transport Chain activities**

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

381 **Protein content**

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

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3 388 were rinsed and placed in a 1:2000 solution of a horseradish peroxidase-conjugated secondary
4
5 389 antibody (Cell Signaling) for 1 hour at room temperature. Membranes were then rinsed and
6
7
8 390 incubated with a horseradish peroxidase chemiluminescence detection reagent (GE Healthcare)
9
10 391 for 5 minutes. Proteins were visualized using an Amersham Imager 600 (GE Healthcare). Optical
11
12 392 densities were measured using iQuant software and all samples were normalized to the baseline
13
14 393 values for that participant.

17 394 **Histological Analysis**

19
20 395 Immediately after muscle biopsy, samples were mounted on tongue blades by using a
21
22 396 medium of OCT compound and tragacanth gum and stored at -70°C until analysis. Serial cross
23
24 397 sections (8-10 µM) were collected on glass slides and frozen at -20°C until analysis. Fiber type
25
26
27 398 and CSA will be determined by histochemical staining for myosin ATPase (preincubation at pH
28
29 399 4.3 or 9.4) as previously described [76]. Type I fibers will be identified by dark staining after
30
31 400 acid preincubation, type II fibers light staining, and type IIB intermediate. At pH 9.4 the staining
32
33 401 pattern was the opposite. Haematoxylin & Eosin (H&E) staining was performed according to
34
35 402 conventional histological procedures. Mitochondrial complex II and IV activity was estimated by
36
37 403 the activity of succinate dehydrogenase and cytochrome c oxidase activity, respectively, as
38
39 404 previously described [77, 78]. Stained muscle sections will be observed using an Olympus BX-
40
41 405 51 fluorescent microscope (Olympus, Tokyo, Japan) and analyzed using ImageJ software.

46 406 **Statistical Analyses**

47
48 407 Paired t-tests will be used to determine differences in body composition and metabolic
49
50 408 profile between baseline 1 and baseline 2. To determine the effect of interventions and
51
52 409 detraining, a repeated measures ANOVA will be used. Simple linear and multivariate regression
53
54 410 analyses will be used to examine the relationship between body composition and metabolic
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3 411 profile variables. For protein expression, we will use paired t-tests to examine the effects of each
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5 412 intervention.
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8 413 The effect size was calculated based on the effects of RT on body composition and
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10 414 metabolic profiles that were previously published [62]. The number of subjects necessary to find
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12 415 statistical differences in the major variables (muscle size, VAT and insulin concentration) of this
13
14 416 study was found to be 10 participants per group. One participant from the RT+TRT group
15
16 417 withdrew after developing side effects to TRT patches and the medical monitor personnel
17
18 418 recommended him to withdraw from the trial in week 8. However, we will perform intent to
19
20 419 treat analysis on his data, which means that despite his early withdrawal from the study his data
21
22 420 will be included in the final analysis. This will allow extrapolation of his post-intervention data
23
24 421 using the SPSS missing values option. We anticipate that we will collect sufficient data to
25
26 422 determine the effects of rehabilitation interventions on protein expression, mitochondrial
27
28 423 enzymatic and ETC activities in individuals with SCI. Statistical analysis will be performed
29
30 424 using SPSS version 23.0 (Chicago, IL) with a level of significance set at $p < 0.05$.
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35

36 425 **Ethics and Dissemination**

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38 426 Ethical approval has been obtained from the institutional review boards at the McGuire
39
40 427 VA Medical Center and Virginia Commonwealth University. All participants read and signed
41
42 428 approved consent forms prior to baseline assessment. Results of the study will be published in
43
44 429 peer-reviewed journal and presented at national and international conferences.
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48 430 **Data Monitoring**

49
50 431 The research staff oversaw and monitored the study to ensure data quality and participant
51
52 432 compliance. There were no adverse events. Only members of the research team will have access
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54 433 to data.
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434 Discussion

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

443 Ectopic adipose tissue accumulation, IMF and VAT, has been strongly associated with
444 altered metabolic profile after SCI [19, 20]. IMF has been determined to account for a 70%
445 reduction in glucose tolerance in individuals with complete SCI [19]. VAT is independently
446 associated with impairment in glucose tolerance, insulin resistance and dyslipidemia after SCI
447 [20]. Edwards et al noted significant positive association between VAT and insulin resistance
448 and a negative correlation between VAT:SAT ratio and HDL-C [13]. Increase in VAT is also
449 related to leptin and plasminogen activator inhibitor-1 concentrations [14]. It is possible that
450 increases in FM are associated with inflammatory biomarkers that trigger metabolic syndrome
451 after SCI [22]. Ectopic adipose tissue has been demonstrated to secrete pro-inflammatory
452 cytokines, including IL-6 and TNF- α . This stimulates hepatic production of CRP which is
453 suggestive of vascular inflammation [15-19].

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

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3 457 favorable body composition and metabolic adaptations. Twelve weeks of NMES-RT has shown
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5
6 458 to increase thigh muscle CSA by 35-40% as measured by MRI [59]. Moreover, there was a
7
8 459 reduction in %leg FM and a trend towards decrease in VAT CSA after 12 weeks of NMES-RT
9
10 460 [64]. The favorable adaptations in body composition were associated with decrease in plasma
11
12 461 insulin area under the curve and plasma triglycerides [59]. These findings were attributed to an
13
14 462 increase in plasma IGF-1. However, the effect of training appears to be limited to the trained
15
16 463 muscle and only modestly impacted whole body composition. It is unclear whether a RT
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18 464 program longer than 12 weeks may provide additional benefits to individuals with SCI.
19

20
21
22 465 The cellular changes underlying the alterations in skeletal muscle glucose utilization and
23
24 466 energy metabolism after SCI are unclear. Previous work indicated that expression of AMP-
25
26 467 activated protein kinase (AMPK), a key regulator of energy homeostasis for lipid and
27
28 468 carbohydrate utilization, was altered in persons with SCI compared to BMI matched able bodied
29
30 469 controls [79]. Another study revealed decreased expression of genes involved in glucose and
31
32 470 lipid metabolism [80]. Despite these abnormalities, one study reported that leg glucose uptake
33
34 471 during cycling was increased in individuals with SCI compared to able-bodied controls [61].
35
36 472 Another study showed similar glucose uptake of isolated muscle fibers from SCI and able bodied
37
38 473 individuals *in vitro* [81]. Benefits of other forms of functional electrical stimulation lower
39
40 474 extremity cycling (FES-LEC) have included improvements in body composition, carbohydrate-
41
42 475 and lipid metabolism and muscle fiber type composition [60-63, 82-84]. Similarly, we have
43
44 476 recently shown that 16 weeks of FES-LEC increased the protein abundance of GLUT-4, PGC-
45
46 477 1 α and AMPK by 3.8, 2.3 and 3.4 fold, respectively, in the vastus lateralis muscle in persons
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48 478 with motor complete SCI [85].
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3 479 TRT has been shown to increase skeletal muscle mass in hypogonadal men, men with
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6 480 chronic illness, and older men [53-55]. Androgen deficiency in men is associated with a loss of
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8 481 FFM and an increase in FM [53]. In epidemiologic studies, men with decreased free T had lower
9
10 482 appendicular skeletal muscle mass than those with normal T levels [86]. Previous work
11
12 483 documented that TRT increases muscle mass with a reciprocal decrease in total body FM [53,
13
14 484 54]. This reciprocal action has been suggested due to a switch from adipogenic toward myogenic
15
16 485 differentiation of mesenchymal stem cells [86]. In a randomized controlled double blinded
17
18 486 clinical trial, TRT was shown to improve insulin sensitivity, CRP and reduce VAT [54]. Sixty
19
20 487 percent of men with SCI have low T level and levels are associated with time since injury [49,
21
22 488 50]. TRT has also been reported to increase IGF-1 and several molecular mechanisms related to
23
24 489 the protective pathways have been recently elucidated [47, 48, 51, 87]. Therefore, enhancing the
25
26 490 decline in anabolic homeostasis by providing TRT may provide additional benefits as was
27
28 491 previously demonstrated by increasing lean mass and metabolic rate in individuals with SCI.
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33

34 492 It was very important to highlight that this protocol has 3 different phases including four
35
36 493 weeks of delayed entry, 16 weeks of intervention and 16 weeks of detraining. The delayed entry
37
38 494 period was included to allow each participant to serve as his own control. Moreover, we were
39
40 495 successful in retaining 12 participants (n=6/group) to complete the detraining phase. This
41
42 496 means that we had participants 6 participants that agreed to stick to our exercise program and the
43
44 497 use of TRT patches up to 9 months. This may reflect on the study protocol frequency that
45
46 498 ensured long-term adherence despite the length of the study. A very important point that is worth
47
48 499 highlighting is that our study protocol was designed to include 3 levels of outcome
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51 500 measurements including body composition, metabolic profile and cellular changes. This design
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3 501 is likely to provide mechanistic explanations to changes that occur at the body composition and
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5 502 metabolic levels
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8 503 **Limitations**
9

10 504 The current study was limited to those who were less than or equal to 50 years old.
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12 505 Because of advances in healthcare, many individuals with SCI have a near-normal life span
13
14 506 which may make the results of this study less generalizable. However, this age limit was
15
16 507 implemented because the effect of TRT on cardiovascular health has been controversial. There
17
18 508 are data showing that hypogonadism is a risk for cardiovascular disease [88]. Some replacement
19
20 509 studies show increased risk, but another study showed decreased mortality in men receiving
21
22 510 testosterone [89]. Another study in older men reported that injectable testosterone may be
23
24 511 associated with increased cardiovascular risk but topical testosterone was not [90]. Therefore, we
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26 512 set the inclusion criteria of less than or equal to 50 years old to reduce the likelihood of
27
28 513 developing cardiovascular complications.
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34 514 Currently NMES-RT is not readily available to the majority of SCI patients. Women
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36 515 were not included because administering TRT is not either appropriate or safe, because women
37
38 516 are at risk of virulization by testosterone. Thus, the trial was limited only to males with SCI.
39
40 517 Moreover, only 21% of individuals with motor complete SCI are women based on the Model
41
42 518 System Data.
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45

46 519 In summary, we anticipate that this trial will provide important insights into the body
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48 520 composition and metabolic benefits of 16 weeks of evoked RT+TRT or TRT. If beneficial, this
49
50 521 may be a feasible strategy for the rehabilitation of individuals with chronic SCI and increase the
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52 522 health of this and other clinical populations. Additionally, TRT alone may provide an alternative
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54 523 intervention for those who cannot benefit from training using surface NMES, because of lower
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3 524 motor neuron (LMN) denervation or intolerance to applications of electrical stimulation.
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6 525 Although the current study did not include any cauda equina participants, further research is
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8 526 warranted to examine the effects of TRT on muscle atrophy following LMN denervation. This is
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10 527 important because skeletal muscle following LMN denervation may respond differently than
11

12 528 innervated muscle. The study will also shed light on several molecular pathways that have been
13

14 529 suggested to influence both body composition and metabolic profile.
15

16
17 530 **Trial Status:** Enrollment into the study started in July 2012 and as of April 2016 all participants
18

19 531 have completed the study. Data collection and data analysis are expected to be completed in
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21 532 December 2016. The study is expected to be closed in June 2017.
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26
27 534 **Contributions:** ASG supervised all aspects of the trial including all interventions and
28

29 535 measurements and secured funding for the trial procedures. ASG, REK, LCO drafted the
30

31 536 manuscript. REK, JS, RK, and LCO contributed to data collection and analysis. RG, TL, TC,
32

33 537 DXC, CC, RA, EJJ and DRG are research physicians that contributed to patient monitoring and
34

35 538 study design. DXC and RA will provide guidance during data analysis and manuscript
36

37 539 preparation. All authors affirm that the manuscript is an honest, accurate, and transparent account
38

39 540 of the study being reported; that no important aspects of the study have been omitted; and that
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41 541 any discrepancies from the study as planned have been explained.
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48 543 **Competing interests:** The authors have no competing interests to declare
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52
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55 546 registration number NCT01652040.
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2
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30 559 works on different terms, provided the original work is properly cited and the use is non-
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32 560 commercial. See: <http://creativecommons.org/licenses/by-nc/3.0/>.
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570 **References**

- 571 1. Center, N.S.C.I.S., *Spinal cord injury facts and figures at a glance*. J Spinal Cord Med, 2013. **36**(1): p. 1-2.
- 572
- 573 2. DeVivo, M.J., B.K. Go, and A.B. Jackson, *Overview of the national spinal cord injury*
574 *statistical center database*. J Spinal Cord Med, 2002. **25**(4): p. 335-8.
- 575 3. Castro, M.J., et al., *Influence of complete spinal cord injury on skeletal muscle cross-*
576 *sectional area within the first 6 months of injury*. Eur J Appl Physiol Occup Physiol,
577 1999. **80**(4): p. 373-8.
- 578 4. Gorgey, A.S. and G.A. Dudley, *Skeletal muscle atrophy and increased intramuscular fat*
579 *after incomplete spinal cord injury*. Spinal Cord, 2007. **45**(4): p. 304-9.
- 580 5. Wielopolski, L., et al., *Measuring partial body potassium in the legs of patients with*
581 *spinal cord injury: a new approach*. J Appl Physiol (1985), 2009. **106**(1): p. 268-73.
- 582 6. Spungen, A.M., et al., *Soft tissue body composition differences in monozygotic twins*
583 *discordant for spinal cord injury*. J Appl Physiol (1985), 2000. **88**(4): p. 1310-5.
- 584 7. Spungen, A.M., et al., *Factors influencing body composition in persons with spinal cord*
585 *injury: a cross-sectional study*. J Appl Physiol (1985), 2003. **95**(6): p. 2398-407.
- 586 8. Gorgey, A.S., et al., *Relationship of spasticity to soft tissue body composition and the*
587 *metabolic profile in persons with chronic motor complete spinal cord injury*. J Spinal
588 Cord Med, 2010. **33**(1): p. 6-15.
- 589 9. Gater, D.R., Jr., *Obesity after spinal cord injury*. Phys Med Rehabil Clin N Am, 2007.
590 **18**(2): p. 333-51, vii.
- 591 10. Weaver, F.M., et al., *Prevalence of obesity and high blood pressure in veterans with*
592 *spinal cord injuries and disorders: a retrospective review*. Am J Phys Med Rehabil,
593 2007. **86**(1): p. 22-9.
- 594 11. Lavela, S.L., et al., *Diabetes mellitus in individuals with spinal cord injury or disorder*. J
595 Spinal Cord Med, 2006. **29**(4): p. 387-95.
- 596 12. Mojtahedi, M.C., et al., *The association between regional body composition and*
597 *metabolic outcomes in athletes with spinal cord injury*. Spinal Cord, 2008. **46**(3): p. 192-
598 7.
- 599 13. Edwards, L.A., J.M. Bugaresti, and A.C. Buchholz, *Visceral adipose tissue and the ratio*
600 *of visceral to subcutaneous adipose tissue are greater in adults with than in those without*
601 *spinal cord injury, despite matching waist circumferences*. Am J Clin Nutr, 2008. **87**(3):
602 p. 600-7.
- 603 14. Maruyama, Y., et al., *Serum leptin, abdominal obesity and the metabolic syndrome in*
604 *individuals with chronic spinal cord injury*. Spinal Cord, 2008. **46**(7): p. 494-9.
- 605 15. Manns, P.J., J.A. McCubbin, and D.P. Williams, *Fitness, inflammation, and the*
606 *metabolic syndrome in men with paraplegia*. Arch Phys Med Rehabil, 2005. **86**(6): p.
607 1176-81.
- 608 16. Matsuzawa, Y., *White adipose tissue and cardiovascular disease*. Best Pract Res Clin
609 Endocrinol Metab, 2005. **19**(4): p. 637-47.
- 610 17. Kern, P.A., et al., *Adipose tissue tumor necrosis factor and interleukin-6 expression in*
611 *human obesity and insulin resistance*. Am J Physiol Endocrinol Metab, 2001. **280**(5): p.
612 E745-51.
- 613 18. Blake, G.J. and P.M. Ridker, *Novel clinical markers of vascular wall inflammation*. Circ
614 Res, 2001. **89**(9): p. 763-71.

- 1
2
3 615 19. Elder, C.P., et al., *Intramuscular fat and glucose tolerance after spinal cord injury--a*
4 616 *cross-sectional study*. Spinal Cord, 2004. **42**(12): p. 711-6.
- 5 617 20. Gorgey, A.S., K.J. Mather, and D.R. Gater, *Central adiposity associations to*
6 618 *carbohydrate and lipid metabolism in individuals with complete motor spinal cord injury*.
7 619 Metabolism, 2011. **60**(6): p. 843-51.
- 8 620 21. Bauman, W.A. and A.M. Spungen, *Disorders of carbohydrate and lipid metabolism in*
9 621 *veterans with paraplegia or quadriplegia: a model of premature aging*. Metabolism,
10 622 1994. **43**(6): p. 749-56.
- 11 623 22. Grundy, S.M., et al., *Definition of metabolic syndrome: Report of the National Heart,*
12 624 *Lung, and Blood Institute/American Heart Association conference on scientific issues*
13 625 *related to definition*. Circulation, 2004. **109**(3): p. 433-8.
- 14 626 23. Nelson, M.D., et al., *Metabolic syndrome in adolescents with spinal cord dysfunction*. J
15 627 Spinal Cord Med, 2007. **30 Suppl 1**: p. S127-39.
- 16 628 24. Bauman, W.A. and A.M. Spungen, *Coronary heart disease in individuals with spinal*
17 629 *cord injury: assessment of risk factors*. Spinal Cord, 2008. **46**(7): p. 466-76.
- 18 630 25. Lee, C.S., et al., *Evaluating the prevalence of silent coronary artery disease in*
19 631 *asymptomatic patients with spinal cord injury*. Int Heart J, 2006. **47**(3): p. 325-30.
- 20 632 26. Bauman, W.A., et al., *Depressed serum high density lipoprotein cholesterol levels in*
21 633 *veterans with spinal cord injury*. Paraplegia, 1992. **30**(10): p. 697-703.
- 22 634 27. Wilt, T.J., et al., *Carbohydrate and lipid disorders and relevant considerations in persons*
23 635 *with spinal cord injury*. Evid Rep Technol Assess (Full Rep), 2008(163): p. 1-95.
- 24 636 28. Itoh, K., et al., *Mitochondrial dynamics in neurodegeneration*. Trends Cell Biol, 2013.
25 637 **23**(2): p. 64-71.
- 26 638 29. Chan, D.C., *Mitochondria: dynamic organelles in disease, aging, and development*. Cell,
27 639 2006. **125**(7): p. 1241-52.
- 28 640 30. Zhao, J., et al., *Mitochondrial dynamics regulates migration and invasion of breast*
29 641 *cancer cells*. Oncogene, 2013. **32**(40): p. 4814-24.
- 30 642 31. Phielix, E. and M. Mensink, *Type 2 diabetes mellitus and skeletal muscle metabolic*
31 643 *function*. Physiol Behav, 2008. **94**(2): p. 252-8.
- 32 644 32. Ritov, V.B., et al., *Deficiency of electron transport chain in human skeletal muscle*
33 645 *mitochondria in type 2 diabetes mellitus and obesity*. Am J Physiol Endocrinol Metab,
34 646 2010. **298**(1): p. E49-58.
- 35 647 33. Erickson, M.L., et al., *Near-infrared assessments of skeletal muscle oxidative capacity in*
36 648 *persons with spinal cord injury*. Eur J Appl Physiol, 2013. **113**(9): p. 2275-83.
- 37 649 34. Martin, T.P., et al., *Influence of electrical stimulation on the morphological and*
38 650 *metabolic properties of paralyzed muscle*. J Appl Physiol (1985), 1992. **72**(4): p. 1401-6.
- 39 651 35. Grimby, G., et al., *Muscle fiber composition in patients with traumatic cord lesion*. Scand
40 652 J Rehabil Med, 1976. **8**(1): p. 37-42.
- 41 653 36. Goodpaster, B.H., *Mitochondrial deficiency is associated with insulin resistance*.
42 654 Diabetes, 2013. **62**(4): p. 1032-5.
- 43 655 37. Holloszy, J.O., *"Deficiency" of mitochondria in muscle does not cause insulin resistance*.
44 656 Diabetes, 2013. **62**(4): p. 1036-40.
- 45 657 38. Scarpulla, R.C., *Metabolic control of mitochondrial biogenesis through the PGC-1 family*
46 658 *regulatory network*. Biochim Biophys Acta, 2011. **1813**(7): p. 1269-78.
- 47 659 39. Arany, Z., *PGC-1 coactivators and skeletal muscle adaptations in health and disease*.
48 660 Curr Opin Genet Dev, 2008. **18**(5): p. 426-34.

- 1
2
3 661 40. Summermatter, S., et al., *Peroxisome proliferator-activated receptor {gamma} coactivator 1{alpha} (PGC-1{alpha}) promotes skeletal muscle lipid refueling in vivo by*
4 662 *activating de novo lipogenesis and the pentose phosphate pathway.* J Biol Chem, 2010.
5 663 **285**(43): p. 32793-800.
6 664
7
8 665 41. Pagel-Langenickel, I., et al., *PGC-1alpha integrates insulin signaling, mitochondrial*
9 666 *regulation, and bioenergetic function in skeletal muscle.* J Biol Chem, 2008. **283**(33): p.
10 667 22464-72.
11
12 668 42. Adhihetty, P.J., et al., *Effect of denervation on mitochondrially mediated apoptosis in*
13 669 *skeletal muscle.* J Appl Physiol (1985), 2007. **102**(3): p. 1143-51.
14 670 43. Kramer, D.K., et al. *Human skeletal muscle fibre type variations correlate with*
15 671 *PPAR alpha, PPAR delta and PGC-1 alpha mRNA.* Acta Physiol (Oxf),
16 672 2006. **188**(3-4):p.207-16.
17
18 673 44. O'Brien, L.C.Gorgey, A.S., *Skeletal muscle mitochondrial health and spinal cord injury.*
19 674 World J Orthop., 2016. **18**;7(10):p.628-637.
20 675 45. Tsitouras, P.D., et al., *Serum testosterone and growth hormone/insulin-like growth*
21 676 *factor-I in adults with spinal cord injury.* Horm Metab Res, 1995. **27**(6): p. 287-92.
22 677 46. Bauman, W.A., et al., *Blunted growth hormone response to intravenous arginine in*
23 678 *subjects with a spinal cord injury.* Horm Metab Res, 1994. **26**(3): p. 152-6.
24
25 679 47. Bhasin, S., *Regulation of body composition by androgens.* J Endocrinol Invest, 2003.
26 680 **26**(9): p. 814-22.
27 681 48. Bhasin, S. and J.G. Buckwalter, *Testosterone supplementation in older men: a rational*
28 682 *idea whose time has not yet come.* J Androl, 2001. **22**(5): p. 718-31.
29 683 49. Clark, M.J., et al., *Testosterone levels among men with spinal cord injury: relationship*
30 684 *between time since injury and laboratory values.* Am J Phys Med Rehabil, 2008. **87**(9): p.
31 685 758-67.
32
33 686 50. Kostovski, E., et al., *Decreased levels of testosterone and gonadotrophins in men with*
34 687 *long-standing tetraplegia.* Spinal Cord, 2008. **46**(8): p. 559-64.
35 688 51. Zhao, W., et al., *Testosterone protects against dexamethasone-induced muscle atrophy,*
36 689 *protein degradation and MAFbx upregulation.* J Steroid Biochem Mol Biol, 2008. **110**(1-
37 690 2): p. 125-9.
38
39 691 52. Gregory, C.M., et al., *Effects of testosterone replacement therapy on skeletal muscle after*
40 692 *spinal cord injury.* Spinal Cord, 2003. **41**(1): p. 23-8.
41 693 53. Isidori, A.M., et al., *Effects of testosterone on body composition, bone metabolism and*
42 694 *serum lipid profile in middle-aged men: a meta-analysis.* Clin Endocrinol (Oxf), 2005.
43 695 **63**(3): p. 280-93.
44
45 696 54. Aversa, A., et al., *Effects of testosterone undecanoate on cardiovascular risk factors and*
46 697 *atherosclerosis in middle-aged men with late-onset hypogonadism and metabolic*
47 698 *syndrome: results from a 24-month, randomized, double-blind, placebo-controlled study.*
48 699 J Sex Med, 2010. **7**(10): p. 3495-503.
49 700 55. Sinha-Hikim, I., et al., *Effects of testosterone supplementation on skeletal muscle fiber*
50 701 *hypertrophy and satellite cells in community-dwelling older men.* J Clin Endocrinol
51 702 Metab, 2006. **91**(8): p. 3024-33.
52
53 703 56. Ryan, A.S., et al., *Skeletal muscle hypertrophy and muscle myostatin reduction after*
54 704 *resistive training in stroke survivors.* Stroke, 2011. **42**(2): p. 416-20.

- 1
2
3 705 57. Ibanez, J., et al., *Twice-weekly progressive resistance training decreases abdominal fat and improves insulin sensitivity in older men with type 2 diabetes*. *Diabetes Care*, 2005.
4 706 **28**(3): p. 662-7.
5 707
6 708 58. Chromiak, J.A., et al., *Effect of a 10-week strength training program and recovery drink*
7 709 *on body composition, muscular strength and endurance, and anaerobic power and*
8 710 *capacity*. *Nutrition*, 2004. **20**(5): p. 420-7.
9 711 59. Mahoney, E.T., et al., *Changes in skeletal muscle size and glucose tolerance with*
10 712 *electrically stimulated resistance training in subjects with chronic spinal cord injury*.
11 713 *Arch Phys Med Rehabil*, 2005. **86**(7): p. 1502-4.
12 714 60. Mohr, T., et al., *Insulin action and long-term electrically induced training in individuals*
13 715 *with spinal cord injuries*. *Med Sci Sports Exerc*, 2001. **33**(8): p. 1247-52.
14 716 61. Kjaer, M., et al., *Fatty acid kinetics and carbohydrate metabolism during electrical*
15 717 *exercise in spinal cord-injured humans*. *Am J Physiol Regul Integr Comp Physiol*, 2001.
16 718 **281**(5): p. R1492-8.
17 719 62. Crameri, R.M., et al., *Effects of electrical stimulation-induced leg training on skeletal*
18 720 *muscle adaptability in spinal cord injury*. *Scand J Med Sci Sports*, 2002. **12**(5): p. 316-
19 721 22.
20 722 63. Gerrits, H.L., et al., *Peripheral vascular changes after electrically stimulated cycle*
21 723 *training in people with spinal cord injury*. *Arch Phys Med Rehabil*, 2001. **82**(6): p. 832-9.
22 724 64. Gorgey, A.S., et al., *Effects of resistance training on adiposity and metabolism after*
23 725 *spinal cord injury*. *Med Sci Sports Exerc*, 2012. **44**(1): p. 165-74.
24 726 65. Kirshblum, S.C., et al., *International standards for neurological classification of spinal*
25 727 *cord injury (revised 2011)*. *J Spinal Cord Med*, 2011. **34**(6): p. 535-46.
26 728 66. Gorgey, A.S., et al., *Effects of neuromuscular electrical stimulation parameters on*
27 729 *specific tension*. *Eur J Appl Physiol*, 2006. **97**(6): p. 737-44.
28 730 67. Gorgey, A.S., et al., *Effects of electrical stimulation parameters on fatigue in skeletal*
29 731 *muscle*. *J Orthop Sports Phys Ther*, 2009. **39**(9): p. 684-92.
30 732 68. Gorgey, A.S., et al., *Influence of motor complete spinal cord injury on visceral and*
31 733 *subcutaneous adipose tissue measured by multi-axial magnetic resonance imaging*. *J*
32 734 *Spinal Cord Med*, 2011. **34**(1): p. 99-109.
33 735 69. Gorgey, A.S. and C. Shepherd, *Skeletal muscle hypertrophy and decreased intramuscular*
34 736 *fat after unilateral resistance training in spinal cord injury: case report*. *J Spinal Cord*
35 737 *Med*, 2010. **33**(1): p. 90-5.
36 738 70. Vermeulen, A., L. Verdonck, and J.M. Kaufman, *A critical evaluation of simple methods*
37 739 *for the estimation of free testosterone in serum*. *J Clin Endocrinol Metab*, 1999. **84**(10): p.
38 740 3666-72.
39 741 71. Collins, E.G., et al., *Energy cost of physical activities in persons with spinal cord injury*.
40 742 *Med Sci Sports Exerc*, 2010. **42**(4): p. 691-700.
41 743 72. Bergman, R.N., *Lilly lecture 1989. Toward physiological understanding of glucose*
42 744 *tolerance. Minimal-model approach*. *Diabetes*, 1989. **38**(12): p. 1512-27.
43 745 73. Matthews, D.R., et al., *Homeostasis model assessment: insulin resistance and beta-cell*
44 746 *function from fasting plasma glucose and insulin concentrations in man*. *Diabetologia*,
45 747 1985. **28**(7): p. 412-9.
46 748 74. Matsuda, M. and R.A. DeFronzo, *Insulin sensitivity indices obtained from oral glucose*
47 749 *tolerance testing: comparison with the euglycemic insulin clamp*. *Diabetes Care*, 1999.
48 750 **22**(9): p. 1462-70.

- 1
2
3 751 75. Brass, E.P., et al., *Decreased NADH dehydrogenase and ubiquinol-cytochrome c*
4 752 *oxidoreductase in peripheral arterial disease*. Am J Physiol Heart Circ Physiol, 2001.
5 753 **280**(2): p. H603-9.
- 6
7 754 76. Brooke, M.H. and K.K. Kaiser, *Three "myosin adenosine triphosphatase" systems: the*
8 755 *nature of their pH lability and sulfhydryl dependence*. J Histochem Cytochem, 1970.
9 756 **18**(9): p. 670-2.
- 10
11 757 77. Nachlas, M.M., et al., *Cytochemical demonstration of succinic dehydrogenase by the use*
12 758 *of a new p-nitrophenyl substituted ditetrazole*. J Histochem Cytochem, 1957. **5**(4): p.
13 759 420-36.
- 14 760 78. Wong-Riley, M., *Changes in the visual system of monocularly sutured or enucleated cats*
15 761 *demonstrable with cytochrome oxidase histochemistry*. Brain Res, 1979. **171**(1): p. 11-28.
- 16
17 762 79. Kostovski, E., et al., *Altered content of AMP-activated protein kinase isoforms in skeletal*
18 763 *muscle from spinal cord injured subjects*. Am J Physiol Endocrinol Metab, 2013. **305**(9):
19 764 p. E1071-80.
- 20 765 80. Long, Y.C., et al., *Differential expression of metabolic genes essential for glucose and*
21 766 *lipid metabolism in skeletal muscle from spinal cord injured subjects*. J Appl Physiol
22 767 (1985), 2011. **110**(5): p. 1204-10.
- 23
24 768 81. Aksnes, A.K., et al., *Intact glucose transport in morphologically altered denervated*
25 769 *skeletal muscle from quadriplegic patients*. Am J Physiol, 1996. **271**(3 Pt 1): p. E593-
26 770 600.
- 27 771 82. Hjeltnes, N., et al., *Improved body composition after 8 wk of electrically stimulated leg*
28 772 *cycling in tetraplegic patients*. Am J Physiol, 1997. **273**(3 Pt 2): p. R1072-9.
- 29 773 83. Hjeltnes, N., et al., *Exercise-induced overexpression of key regulatory proteins involved*
30 774 *in glucose uptake and metabolism in tetraplegic persons: molecular mechanism for*
31 775 *improved glucose homeostasis*. FASEB J, 1998. **12**(15): p. 1701-12.
- 32
33 776 84. Mohr, T., et al., *Long-term adaptation to electrically induced cycle training in severe*
34 777 *spinal cord injured individuals*. Spinal Cord, 1997. **35**(1): p. 1-16.
- 35 778 85. Gorgey, A.S., et al., *Abundance in proteins expressed after functional electrical*
36 779 *stimulation cycling or arm cycling ergometry training in persons with chronic spinal*
37 780 *cord injury*. J Spinal Cord Med, 2016: p. 1-10.
- 38
39 781 86. Herbst, K.L. and S. Bhasin, *Testosterone action on skeletal muscle*. Curr Opin Clin Nutr
40 782 Metab Care, 2004. **7**(3): p. 271-7.
- 41 783 87. Wu, Y., et al., *Identification of androgen response elements in the insulin-like growth*
42 784 *factor I upstream promoter*. Endocrinology, 2007. **148**(6): p. 2984-93.
- 43
44 785 88. Araujo, A.B., et al., *Clinical review: Endogenous testosterone and mortality in men: a*
45 786 *systematic review and meta-analysis*. J Clin Endocrinol Metab, 2011. **96**(10): p. 3007-19.
- 46 787 89. Shores, M.M., et al., *Testosterone treatment and mortality in men with low testosterone*
47 788 *levels*. J Clin Endocrinol Metab, 2012. **97**(6): p. 2050-8.
- 48
49 789 90. Layton, J.B., et al., *Comparative Safety of Testosterone Dosage Forms*. JAMA Intern
50 790 Med, 2015. **175**(7): p. 1187-96.

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794 **Table 1.** Randomization of individuals with motor complete SCI into either RT+TRT (n=13) or
 795 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

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

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803 **Table 2.** Metabolic health variables measured at baseline 1, baseline 2, post-intervention 1 and
 804 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, TNF α)	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

805 *, Only at baseline 2 and post-intervention 1 to calculate free testosterone. ELISA, enzyme-
 806 linked immunosorbent assay; HBA1C, hemoglobin A1c; SHBG, sex hormone binding globulin;
 807 SST, serum separator tube; IGF-1, insulin-like growth factor 1; IGF-BP, insulin-like growth
 808 factor binding protein; CRP, C-reactive protein; IL-6, interleukin 6; TNF α , tumor necrosis factor
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3 820 **Figure Legends**
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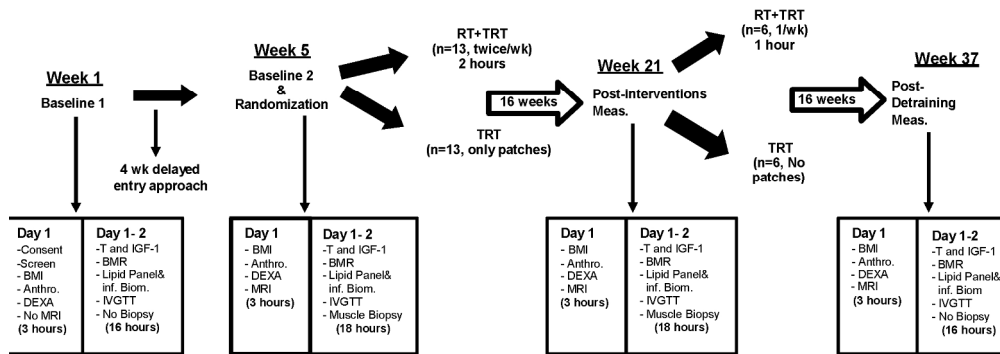
6 821 **Figure 1.** Timeline and main procedures of the TEREX-SCI trial for both the RT+TRT and TRT
7 822 groups during baseline 1, baseline 2, post-intervention 1 and post-intervention 26 for persons
8 823 with motor complete SCI.
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12 824 **Figure 2.** A person with T4 motor complete SCI undergoing both electrically evoked RT (left
13 825 panel) and TRT using transdermal patches (right panel) as a part of a 16 week intervention.
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17 826 **Figure 3.** Schematic diagram showing the process of recruitment over the 3 year period of the
18 827 TEREX trial.
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21 828 **Figure 4.** A representative figure of analysis for IVGTT in a person with SCI after infusion of
22 829 dextrose followed by insulin 20 minutes later. The dextrose concentration is calculated by
23 830 multiplying 0.3g x body weight (kg) in 50% solution. Insulin concentration is determined by
24 831 multiplying 0.02 units x body weight (kg). Blue line represents fasting glucose concentration,
25 832 resting, following infusion, and over 120 minutes. Red line represents the line of best fit of
26 833 glucose concentration. Green line represents fasting insulin level, spike following 20 minutes of
27 834 dextrose infusion and over 120 minutes. Black line represents the line of best fit for insulin
28 835 concentration.
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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)

Peer review only

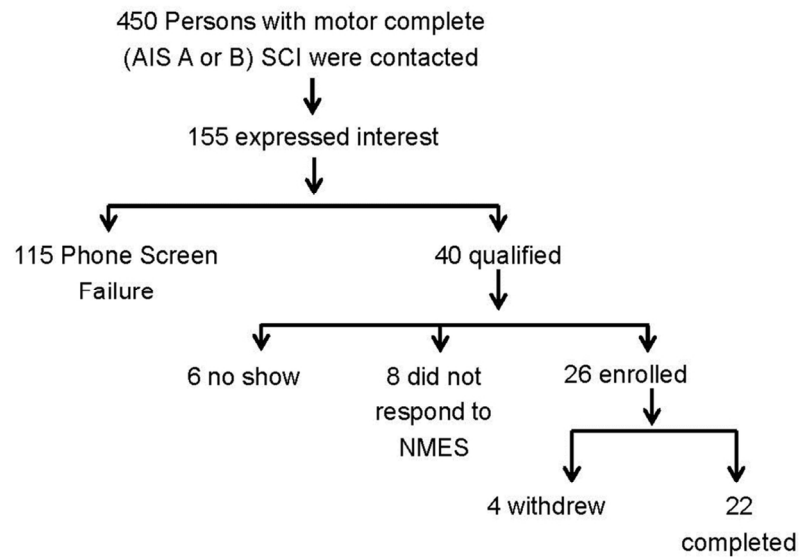
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A person with T4 motor complete SCI undergoing both electrically evoked RT (left panel) and TRT using transdermal patches (right panel) as a part of a 16 week intervention.

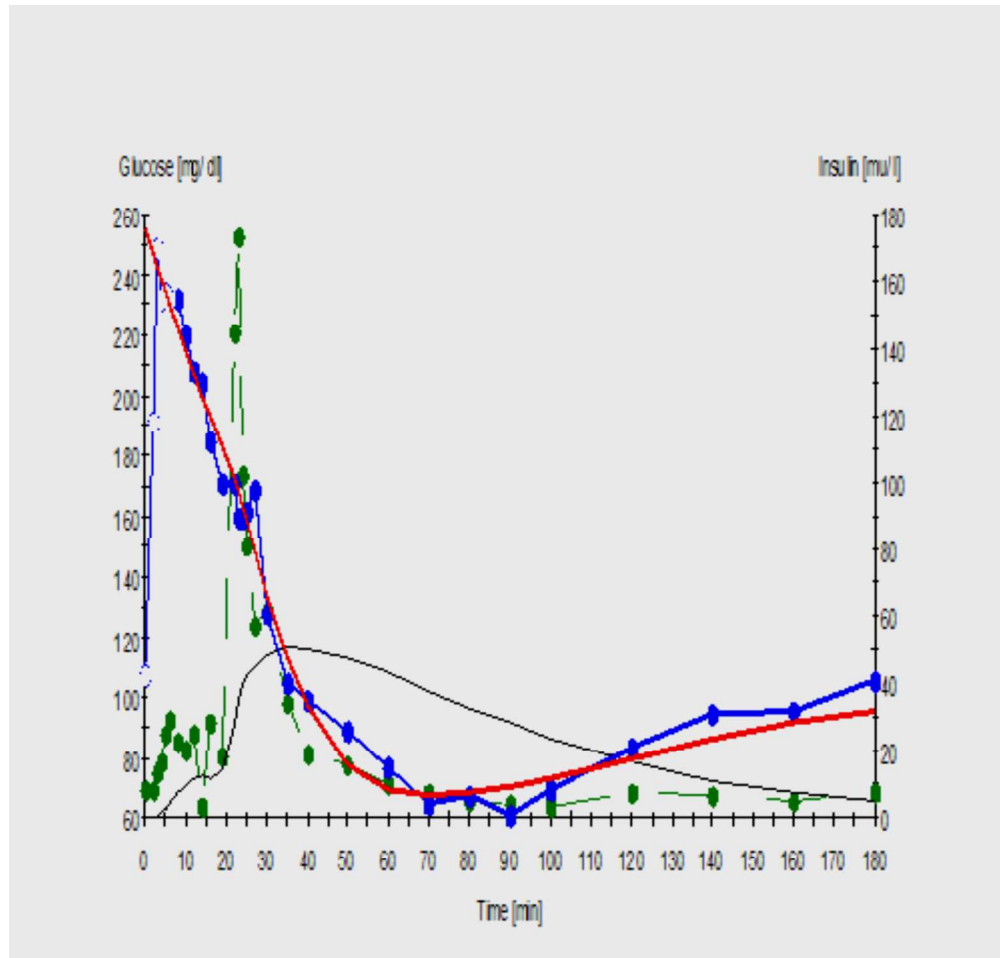
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Recruitment



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 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

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3	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____
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6	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	____9-10____
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8 **Methods: Assignment of interventions (for controlled trials)**

9 Allocation:

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12	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____
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18	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____
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22	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____9____
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25	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	____11____
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28		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____N/A____
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32 **Methods: Data collection, management, and analysis**

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34	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____
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39		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____
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3	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 ___
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7	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 ___
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10		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	___ N/A ___
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12		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 ___
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16	Methods: Monitoring			
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18	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 ___
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23		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 ___
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26	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 ___
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29	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 ___
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33	Ethics and dissemination			
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35	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	___ 3, 8-9, 19 ___
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38	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 ___
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3	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	___ 9 ___
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6		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	___ N/A ___
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9	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 ___
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12	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	___ 24 ___
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15	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 ___
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18	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 ___
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21	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 ___
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26		31b	Authorship eligibility guidelines and any intended use of professional writers	___ 24 ___
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28		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	___ N/A ___
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30	Appendices			
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32	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	___ attached ___
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35	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 ___
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*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.