Effects of Evoked Resistance Training and Testosterone after Spinal Cord Injury

Abstract

Individuals with spinal cord injury (SCI) are at a lifelong risk of increasing obesity and several chronic metabolic disorders such as glucose intolerance, insulin resistance and dyslipidemia secondary to deterioration in body composition. Within few weeks of injury, there are significant decrease in whole body fat-free mass (FFM), particularly lower extremity skeletal muscle mass and subsequent increase in fat mass (FM). Resistance training (RT) is an important type of exercise that has been shown to induce positive physiological adaptations such as increasing lean mass and reducing metabolic disorders in other clinical populations.

In a pilot work, we provided evidence that evoked RT using surface neuromuscular electrical stimulation (NMES) for knee extensor muscle group resulted in significant increase skeletal muscle cross-sectional area (CSA), reduction in % leg FM and a trend towards decrease in visceral adipose tissue (VAT) CSA. The favorable adaptations in body composition were associated with decrease in plasma insulin area under the curve and plasma triglycerides. We attributed the adaptations in body composition and metabolic profile to an associated increase in plasma insulin-like growth factor (IGF-1). We concluded that twelve weeks of evoked RT targeted towards evoking skeletal muscle hypertrophy could result in significant body composition and metabolic adaptations in individuals with SCI.

It is unclear if a longer RT program greater than 12 weeks would provide additional benefits to veterans with SCI. It is also unknown whether enhancing the decline anabolic homeostasis by providing testosterone (T) replacement therapy (TRT) would reverse body composition and metabolic profile changes in veterans with SCI. The major research goal of this proposal is to investigate the effects of 16 weeks of evoked RT+TRT vs. TRT on body composition (muscle CSA, VAT, %FM) and the metabolic profiles (glucose and lipid metabolism) in individuals with motor complete SCI. To address this goal, surface NMES accompanied with ankle weights will be conducted twice weekly to exercise the knee extensor skeletal muscle groups from sitting position. After 4 weeks of delayed entry approach, participants (n =24) will be randomly assigned into RT+TRT (n =12) or TRT (n =12) groups. The TRT will be provided via transdermal T patches that will be alternated on both shoulders over the course of the study. We also propose to study the effects of detraining on body composition and metabolic profiles.

Specific aims and objectives

Specific aim 1 will demonstrate the effects of NMES RT and/or Testosterone patches (Tp) on the CSA of thighs and legs skeletal muscle groups, percentage FFM, and the CSA of VAT, intramuscular fat and percentage FM after 16 weeks of training + Tp and 16 weeks of detraining.

Specific aim 2 will determine the changes in metabolic milieu (resting energy expenditure, glucose homeostasis, lipid profile, free fatty acids, serum total and free testosterone and IGF-1), and cytokines (c-reactive protein, tumor necrosis factor alpha and IL-6 as inflammatory biomarkers) after 16 weeks of training+Tp and detraining.

Specific aim 3 will determine if 16 weeks of evoked RT and Tp will increase GLUT-4 concentration, muscle IGF-1 and peroxisome-proliferator-activated receptor-gamma co-activator 1 (PGC-1) expressions, altered fiber type distribution and enhance the mitochondrial enzymatic activities (electron transport chain) compared to Tp only.

Background and Significance

Our nation has recognized the importance of continuously developing rehabilitation protocols for individuals with chronic disabilities especially for those with spinal cord injury (SCI). Adopting new therapeutic strategies should be considered a national goal to minimize the SCI-related secondary chronic disorders and expansion in the cost-related increase in life expectancies post-injury. According to the National SCI Statistical Center, there are approximately 11,000-12,000 new cases of SCI annually.[1,2] There is an estimated 14% growth in the prevalence of SCI since 1988. The prevalence of individuals with SCI has been estimated to be 250,000-400,000. Vehicle accidents account for 47.5% of SCI reported cases. Falls and violence are secondary common causes of SCI, accounting for 22.9 and 13.8% respectively.[1] There are an estimated 46,000 veterans in the United States with SCI (currently 30,252 individuals listed in the national VA medical center (VAMC) Spinal Cord Dysfunction database), and recent literature would suggest that more than 60% are overweight or obese using body mass index (BMI) standards, and more than 50% are glucose intolerant, while one out of five is frankly diabetic.[2-7] *In the opening ceremony of the 2010 Academy of SCI Professionals meeting in Las Vegas, Dr. Margaret Hammond, Head of the Veteran's Administration SCI Program, announced that fighting against obesity and associated co-morbidities in people with SCI is one of the top priorities of her office*. The national aggregate direct costs of SCI in the United States have been continuously increasing in the last two decades accompanied with a decline in mortality over the first year after SCI. The average initial charge/ case could range from $185,000 to $295,000 with annual charges thereafter that range from $17,000 to 33,000.[8,9] It has been estimated that the annual total cost that resulted from SCI is about 7.7 billion dollars.[8] These figures reveal the substantial economic burden that would be enough to overwhelm the available resources. Physical paralysis and related health secondary complications such as cardiovascular disease, respiratory infection, and type II diabetes mellitus (DM) and dyslipidemia may contribute into the high costs of care after SCI.

Body composition after SCI

Deterioration in body composition following SCI has gained the attention of a handful of investigators.[3,4 10-18] A few months post-injury, there is a rapid onset of skeletal muscle atrophy[19,20,21], increase of fat mass (FM)[10,13,16], waist circumference[17,18] and decrease of fat free mass (FFM).[10,13] Healthy BMI fails to reflect the exact percentage of FM after SCI and there is a significant gain in FM post-injury.[3,4,22] The deterioration in body composition and the associated muscle atrophy has been attributed to number of factors including reduction in physical activity, unloading, disuse and reduction in anabolic hormone secretion.[3,4,23,24] Individuals with SCI suffer dramatic muscle atrophy that begins within a few weeks of injury and it continues at least until the end of the first year.[19,20] Skeletal muscle cross-sectional area (CSA) could be as low as 50% compared to healthy able-bodied (AB) controls.[19] Spungen et al. had reported that monozygotic twins with acquired paraplegia had significantly more total body FM and percent fat per unit BMI than their AB twins.[10] Those with SCI showed an increase in FM (7%) compared with their AB co-twins. Spungen et al. has demonstrated that 133 men with SCI were 13.1% fatter per unit of BMI compared with age-, height-, and ethnicity-matched AB controls.[13] Individuals with SCI have greater trunk FM, visceral adipose tissue (VAT) CSA (58%) and VAT: subcutaneous adipose tissue (SAT) ratio (48%) compared to age and waist circumference matched AB population.[25-27] *The body composition changes may be further exacerbated by associated reductions in the anabolic hormones, testosterone (T), and growth hormone (GH) and the GH secondary messenger insulin like growth factor-1 (IGF-1).*[24, 28, 29] Growth hormone release is blunted and chronically depressed in SCI, as evidenced by reduced levels of IGF-1, a

convenient indicator of chronic GH secretion.[28, 29] In the rat and human models, reduction in IGF-1 has been associated with skeletal muscle atrophy and increase in the FM.[30, 31]

Body composition and metabolic profile after SCI

Approximately 2/3 of individuals with SCI are either overweight or obese and the prevalence of obesity after SCI may exceed its occurrence in the healthy community.[3, 4] After injury, the loss of metabolically active muscle mass results in reduction of the resting energy expenditure (EE).[32, 33] The resting EE accounts for ~84% of the total daily energy expenditure and the decline in EE after SCI thus disturbs energy balance. Bauman et al (2004) has demonstrated significantly reduced muscle mass and viscera in SCI vs. monozygotic AB twins using whole body potassium counts (2534 ± 911 vs. 3515 ± 916); resting EE were similarly reduced in SCI vs. AB twins (1634 ± 290 vs. 1735 ± 295 kcal/d). [34]

Kocina *et al.* demonstrated that the prevalence of cardiovascular diseases and type II DM is higher in the SCI compared to healthy AB controls.[35] The mortality rate for cardiovascular disease is 228% higher in the sedentary SCI population and occurs at younger age after SCI.[35] Duckworth et al. demonstrated that approximately one half of 45 patients with chronic SCI were susceptible to problems related to glucose intolerance, insulin resistance and type II DM.[36] Bauman and Spungen documented that 50% of those with paraplegia and 82% with quadriplegia were diabetic.[6,37] Additionally, serum lipid levels in individuals with SCI showed a decrease in high-density lipoprotein (HDL-C) cholesterol that negatively correlated with an increase in serum triglycerides (TG).[38, 39] Nash et al. reported that 76% of individuals with paraplegia had HDL-C less than 40 mg/dl and 34% had the Adult Treatment Panel III- defined metabolic syndrome.[40,41] The above complications have been strongly correlated to a reduction in physical activity and deterioration in body composition (increase body adiposity and reduction in FFM) that predispose individuals with SCI to a spectrum of health-related secondary complications. An association was noted between whole body insulin-mediated glucose uptake and skeletal muscle mass in tetraplegics, suggesting loss of muscle mass as the primary reason for insulin insensitivity.[42] Another study reported that 55% of individuals with SCI were at risk of developing metabolic syndrome.[43-44] It is also possible that increases in FM are associated with inflammatory biomarkers that trigger metabolic syndrome after SCI.[43] Adipose tissue has been demonstrated to secrete large amounts of proinflammatory cytokines, including interleukin-6 (IL-6) and tumor-necrosis factor-α (TNF-α).This stimulates hepatic production of C - reactive protein (CRP) which is tied to vascular inflammation.[45-48]

Ectopic adipose tissue accumulation, intramuscular fat (IMF) and VAT, has been strongly associated with altered metabolic profile after SCI.[49,50] IMF has been determined to account for a 70% reduction in glucose tolerance in individuals with complete SCI.[49] We have recently shown that VAT is independently associated with impairment in glucose tolerance, insulin resistance and dyslipidemia after SCI.[50] Edwards et al noted significant association between VAT, plasma insulin and insulin resistance index; while a negative correlation was noted between VAT: SAT ratio and HDL-C. Increase in VAT is also related to leptin and plasminogen activator inhibitor-1.[26,27]

Mitochondrial adaptations after SCI

Mitochondria are the site of oxygen consumption and energy production of all tissues including skeletal muscle. Oxidative glucose and lipid metabolism are dependent on mitochondria to generate energy in the cells. Mitochondrial biogenesis dysfunction leads to decreased B- oxidation and insulin resistance. It is well established that fewer and small sized

mitochondria are found in skeletal muscle of insulin resistant, obese and type 2 DM subjects.[51] This is associated with reduction in several of the key enzymes including cytochrome c oxidase, succinate dehydrogenase (SDH), pyruvate dehydrogenase and carnitine palmitoyltransferase I which further overwhelms the metabolic processes.[52] The molecular mechanism of mitochondrial biogenesis is driven in part through peroxisome proliferator-activated receptor (PPAR) coactivator 1 alpha (PGC-1 α).[53, 54] PGC-1α expression is known to influence fiber type phenotype and enhances the shift from fast to slow myosin heavy chain. The expression is increased with increasing ATP cellular demands during exercise and GLUT-4 expression resulting in insulin sensitizing effects.[54] It is also decreased in obese, diabetic individuals, following denervation and 42 days after SCI; resulting in reduction in the size and the number of mitochondria.[55, 56] Reduction in the size or the number of mitochondria is also accompanied by decreases in mitochondrial electron transport chain (ETC) activity. This may imply that mitochondrial activity can play a significant role in altered metabolic profile. Individuals with SCI occupy the lowest end of the physical activity spectrum, with a VO_2 peak as low as 0.9 l/min. In a comparison between elite rowers and individuals with complete SCI, more than 70% demonstrated lower VO_2 peak following chronic SCI.[57] This lower VO_2 peak was attributed to smaller muscle fibers, transformation to type II fibers, and/or a decrease in aerobic-oxidative enzyme activity.[58, 59,60] It would be safe to suggest that reduction in VO_2 peak is due to reduction in the size, number or the activity of mitochondria in chronic SCI. Martin et al. reported a 48-67% lower SDH activity per unit fiber volume in tibialis anterior muscle fibers of patients 2-11 years after injury compared with AB controls.[61]

The impact of Resistance Training

Exercise is commonly prescribed as an effective and therapeutic modality to reduce obesity and its associated metabolic consequences in the healthy community. The Centers for Disease Control and the American College of Sports Medicine recommendations have indicated that daily exercise for 30 minutes should be sufficient to prevent the occurrence of health and obesity related secondary complications.[62] Although exercise and active lifestyle have been recommended as advantageous tools to prevent secondary complications after SCI, several barriers have been perceived that interfere with maximizing the benefits to any exercise protocol. After SCI, exercise opportunities are either not possible or limited to small muscle mass above the level of lesion, and do not exercise large muscle groups in the lower extremities.[63] This renders exercise as an ineffective method to attenuate the deterioration in body composition and metabolic profile after SCI. During upper extremity exercise in persons with spinal cord injury, left ventricular end diastolic volume remains largely constant, resulting from unchanged or decreased venous return, ultimately contributing to fairly limited changes in cardiac stroke volume. Inability to shunt blood from the viscera and non-exercising trunk and lower extremity musculature to the working muscles is due to impaired sympathetic responses in SCI; venoconstriction is also impaired, and a large amount of blood remains pooled in the lower extremities and inferior vena cava. Further, the inability to voluntarily contract lower extremity musculature eliminates activity of the venous pump system. Circulatory hypokinesis has been reported by several investigators in which upper extremity exercise often causes hypotension in tetraplegia, as the increased metabolic demands of exertion are not matched by appropriate hemodynamic responses.[63]

Resistance training (RT) has been identified as a very important rehabilitation tool that helps to increase lean mass and decrease fat mass.[64,65] It helps in increasing activity, altering body composition and attenuating the process of sarcopenia in the elderly and muscle atrophy after SCI.[66-73] In healthy adults after 12 weeks of RT, skeletal muscle CSA has increased by 7

to 10%.[67] In elderly individuals, RT for 12 weeks increased specific tension by more than 30%.[66] Moreover, RT has been shown to influence body composition and reduce VAT, suggesting that the benefits of RT could be extended to decrease independent risk factors of developing insulin resistance.[66] After SCI, circuit RT has been shown to improve cardio-respiratory fitness, muscle strength and increase caloric expenditure after exercising upper extremities in a group with chronic paraplegia.[63] RT has also been used to exercise both the paralyzed and non-paralyzed muscles of the lower extremities.[70-72] Neuromuscular electrical stimulation (NMES) was used in individuals with chronic SCI to evoke exercise-induced RT using regular ankle weights.[68,71] The training procedure took an advantage of exercising the large knee extensor muscle groups. The findings were promising by evoking skeletal muscle hypertrophy by more than 40% and improving glucose tolerance after years of injury.[68] However, It is worth noting that training with NMES may not be possible for a large segment of the SCI. For instance, those with SCI below L2 who commonly experience peripheral denervation or those with osteoporosis for the fear of lower extremity bony fractures. Therefore, providing an alternative approach may be highly desirable especially for this segment of SCI.

Testosterone (T) is an anabolic and androgenic hormone that is associated with growth. T replacement therapy (TRT) has been shown to increase skeletal muscle mass in hypogonadal men, men with chronic illness, and older men.[73] It has been postulated that testosterone administration increases the number of satellite cells responsible for muscle hypertrophy which stimulates muscle protein synthesis and inhibits protein degradation.[74] Androgen deficiency in men is associated with a loss of FFM and an increase in FM. In epidemiologic studies, men with decreased free T-index had lower appendicular skeletal muscle mass than those with normal T levels. Previous work documented that TRT increases muscle mass with a reciprocal decrease in total body FM. This reciprocal action has been suggested due to a switch in the differentiation of mesenchymal stem cells into muscle cells over adipocytes.[75] In a randomized controlled double blinded clinical trial, TRT has been shown to improve insulin sensitivity, CRP and reduce VAT.[76] Sixty percent of men with SCI have low T level and especially within the first 6 months of SCI.[77] A recent study demonstrated a decreased level of T in men with tetraplegia after blood sampling for 24 h period compared to AB-controls by more than 37%.[78] *In rats with complete SCI, TRT has been shown to attenuate the decrease in muscle size to only 30% compared to 50% in the sham group and attenuated the decline in the oxidative and glycolytic enzymatic activities.*[78] TRT has also been reported to increase IGF-1 in men and several molecular mechanisms related to the protective pathways have been recently elucidated.[80,81]

The **Significance of this research** to the VA population lies in the fact that currently more than 30,000 individuals are listed in the national VAMC spinal cord dysfunction registry, and recent literature suggests that more than 50% of them have insulin resistance resulting in glucose intolerance or type II DM, dyslipidemia and cardiovascular diseases. While it is generally accepted that using a wheelchair is reasonable, the health consequences of losing more than 25% of muscle mass is appreciated after SCI. As a result the National Center for Medical Rehabilitation Research of the National Institutes of Health has made research designed to limit secondary complications of the people with disabilities its primary goal (http:// www. nichd. nih. gov /about/org/ncmrr/). Advances in therapeutic interventions (stem cell, gene therapy) may alleviate problems associated with SCI. However, there will likely be many individuals with long-standing complete SCI that cannot benefit from these interventions and deserve to live in good health. This special population already has significant functional deficits including reduced mobility, myocardial atrophy, and restrictive lung disease and bowel and bladder dysfunction. Thus, a simple and inexpensive means to slow the development of these health related

secondary complications is worth investigating. Developing a rehabilitation strategy that could reduce complications after SCI and to enhance the standard of care for veterans who have suffered SCI is clearly needed. While there is evidence that loading the paralyzed skeletal muscles results in significant muscle hypertrophy, improvement of body composition and metabolic profile, depression of T after SCI may limit the effects of RT on these variables. Therefore, a complimentary approach of RT and TRT may work as an effective rehabilitation strategy to counterbalance the growing rate of obesity, type II DM and cardiovascular disease among veterans with SCI. Moreover, TRT may provide an effective intervention for those who cannot effectively benefit from applications of NMES.

Subjects: Twenty four chronic (1year or more post-injury) individuals with motor complete SCI will be recruited from the Hunter Holmes McGuire VA Spinal Cord Dysfunction registry (n=1,825) over 5 years to participate in the study.

Inclusion Criteria : All participants will be between 18-50 years old, male, with BMI $\leq$ 30 Kg/m^2, traumatic motor complete C5-L2 level of injury, American Spinal Injury Classification (A and B; i.e. motor deficit below the level of injury). Different studies have shown that the BMI criteria used in healthy AB controls underestimates the %FM in individuals with SCI.[3,4,12,22] Therefore, a BMI $\leq$ 30 may have about 30-40% FM which would be sufficient to determine the effects of the proposed interventions on %FM.

Exclusion Criteria: Participants with pre-existing medical conditions will be excluded (cardiovascular disease, uncontrolled type II DM and those on insulin, pressures sores stage 2 or greater, and those with supra-physiological T level), hematocrit above 50% and urinary tract infection or symptoms. All participants will undergo body composition assessments (specific aim 1), metabolic studies (specific aim 2) and muscle biopsies (specific aim 3). Approved flyers and advertisements will also be posted at the VAMC, VCU Hospitals and clinic sites.

After informed consent, each subject will undergo a complete physical examination by a physiatrist board certified in SCI medicine (Drs. Castillo or Lavis), including neurological assessment, EKG and ASIA examination. The study visit will include estimation of body composition anthropometry, and dual x-ray absorptiometry (DXA, baseline 1 and 2). Additionally, MRI scans will be obtained for trunk VAT, lower extremities skeletal muscles and IMF CSAs (baseline 2). Participants will then be escorted to the VCU GCRC for dinner, and will remain in the GCRC overnight. At 6 am, the subject will be gently awakened for determination of basal metabolic rate (BMR). An am IV line will then be placed, serum total T, free T, sex hormone binding globulin(SHBG) and IGF-1 concentrations will be measured at 6.30, 7.00 and 7.30 am. The free T and SHBG will only be measured at baseline 2 and post-intervention 1 because of financial restriction. Resting blood pressure and fasting metabolic labs will be obtained including HbA1c, as well as lipid panels, CRP, IL-6, TNF-alpha, and free fatty acids (FFA). This will be followed by a 3-hour IVGTT which will begin at 8 am and terminate at 11 am. After 11.00 am the vastus lateralis muscle will be biopsied to measure GLUT-4, IGF-1and PGC-1 proteins, myosin heavy chain expression and to determine mitochondrial ETC and enzymatic activities. During the 3h-IVGTT, a dietitian will meet with each participant individually to ensure that they will follow a standard diet pattern during the 16 week-interventions (45% carbohydrate, 35% fat and 25% protein) to avoid any confounding effects on our measurements. All participants will be asked to maintain a **5 day food record** monitoring their energy intake during the course of the study. The diaries will be evaluated weekly by the dietitian to provide monthly feedback. All

participants will meet with the dietitian five times during the course of the first 20 weeks (baseline 1, baseline 2, 4, 8 and 12 weeks) to ensure appropriate adherence to the diet pattern throughout the study, no meetings will be conducted during the detraining period.

A delayed entry approach will be adopted for 4 weeks so each participant will serve as his own control, to control for body weight and dietary habits **(baseline 1)**. Following the delayed entry period, the twenty four participants will be randomly assigned to a RT+Tp group (n = 12) or a Tp group (n = 12). Testosterone patches (Tp; 4-6 mg/d) will be re-placed daily on alternating shoulders at bedtime for 16 weeks. Randomization will be done at the end of the two-day assessment period using a computer program **(baseline 2).** The participants will be age, level of injury, time since injury (TSI) and WC matched between both groups. The RT+Tp group will undergo 16 weeks of supervised unilateral progressive RT using surface NMES and ankle weights. Following training **(post-intervention measurements)**, the two day assessment period will be repeated using the same sequence. This will be followed by detraining phase for 16 weeks in which six participants of the RT+Tp group will be asked to train once weekly and the other six will receive no intervention and only six participants of the Tp group will receive no intervention. The purpose of the detraining phase is to determine if there is a carryover effects to the interventions or once weekly can result in a maintenance effect. Following detraining, the two day assessment period will be repeated *without skeletal muscle biopsy* to minimize subjects' burden (**post-detraining measurements**). The study timeline is highlighted in figure 1.

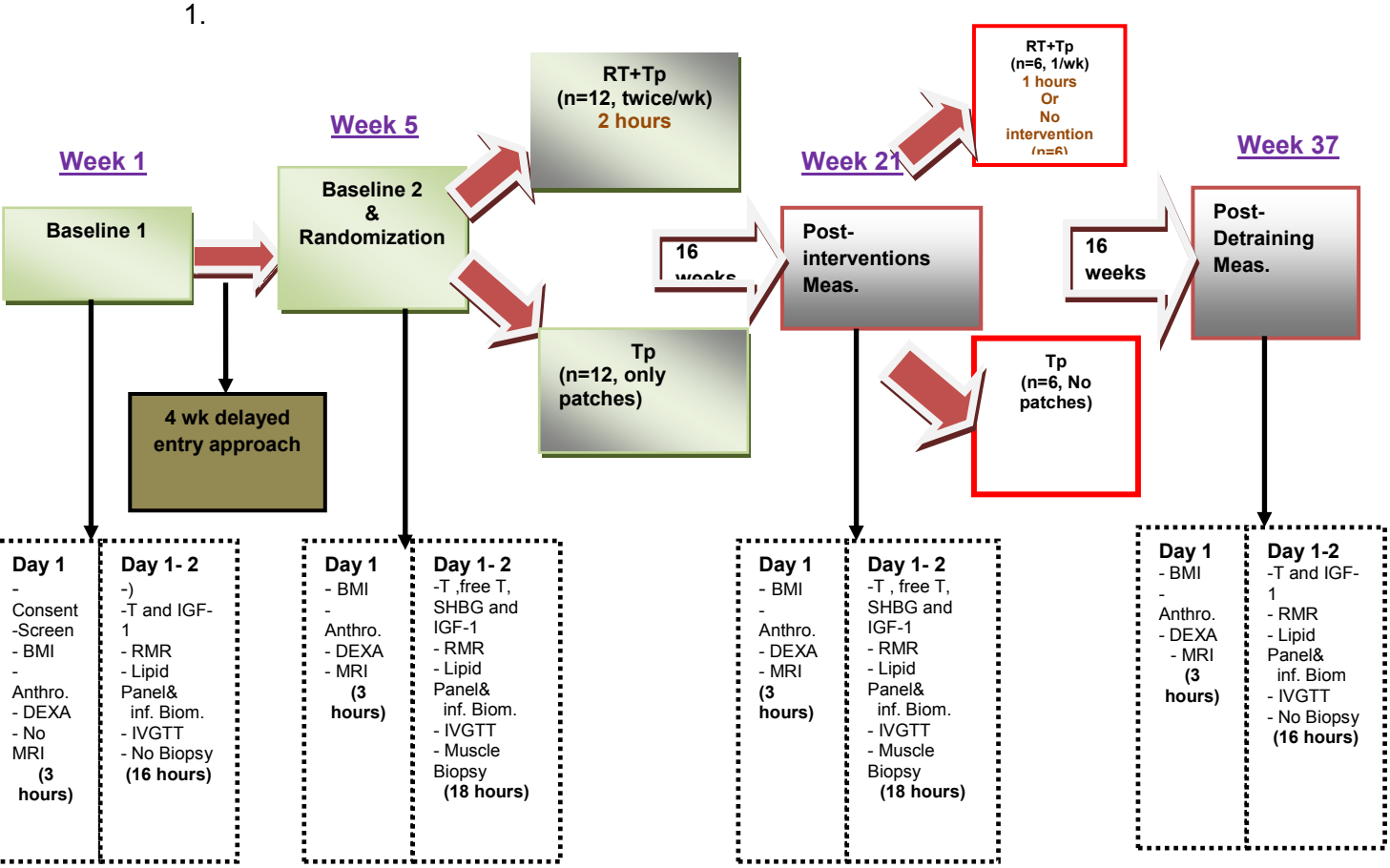

Fig 1. Timeline of the study.

Interventions

1. Delayed entry approach. A 4 week initial period will be included to obtain baseline measurements (baseline 1 within the first week), stabilize body weight and educate participants on how to monitor their dietary intake. During this period, participants ***will not*** be asked to make changes in their dietary intake. This initial period of delayed entry will allow each participant to serve as his own control and no interventions will be provided. All the measurements will be carried as proposed except of MRI and muscle biopsy to reduce subjects' burden and because of insufficient funding for MRI scans.

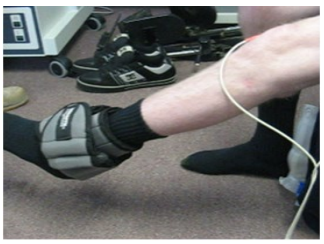

Fig 2. NMES RT & ankle weights

2.Resistance training. The first week of the RT (16 weeks) will be conducted with no ankle weights to ensure that the knee extensor muscles can extend the weight of the lower leg against gravity.[73] Once full knee extension is achieved in a sitting position, an increment of 2 lbs will be used on a weekly basis with the criteria that full knee extension should be achieved before further increase in load.[67,84,85] All training procedures will be conducted with the participants sitting in their wheelchairs with enough space to clear their foot off the ground. Knee extension will be performed with the ankle weights attached to the shin. Surface NMES will be applied to the knee extensor muscles via surface electrodes to induce concentric-eccentric actions as shown in figure 11. Two 8 X10 cm^2 (Uni-Patch, 1313 West Grant Boulevard, Wabasha, MI, USA) adhesive carbon electrodes will be placed on the skin over the knee extensor muscle group. One electrode will be placed 2–3 cm above the superior aspect of the patella over m. vastus medialis, and the other lateral to and 30 cm above the patella over m. vastus lateralis. Current from the stimulator will be manually increased in 5-second intervals to evoke full knee extension against gravity followed by the lengthening action during relaxation. The current (mA) that causes full knee extension will be recorded after each stimulation bout (Please see the attached form). Training will be performed *twice weekly for 16 weeks* under full supervision from the PI. Each training session will consist of 4 sets of 10 repetitions of NMES-induced knee extensions and it will last for 30-40 minutes. A unilateral training will be conducted (right then left knee extensor muscle groups) to ensure monitoring and to avoid autonomic dysreflexia especially for those above T4 SCI. A 5 second/5 second work/rest ratio will be used with a 3-minute rest between sets, 30 Hz, 450μs pulses and a current sufficient to evoke full knee extension.[68, 86-88]

3.Testosterone replacement patches (Tp). Testosterone for both groups will be administered by a shoulder patch (Testoderm, Alza Corp., Palo Alto, CA) that will deliver between 4-6 mg/day.[73,88] Each subject will be asked to use a patch that delivers 4 mg/day initially, to wear it at all times and to change the patch once a day before bedtime. The patch will be worn daily on the right or the left shoulder and the application sites will be reversed on the following day to the opposite shoulder over the course of the 16 weeks. The participant will be instructed on how to place the patches on a clean dry area of skin. The serum testosterone concentration will be measured and reviewed in a blinded fashion weekly for the first month and then every 4 weeks.

The dose will be decreased to 2 mg/day if the serum T concentration is more than 1000 ng/dL (34.7 nmol/L) and that the participant will be reeducated in the patch technique if the concentration was less than 250 ng/dL (8.7nmol/L) above the pretreatment concentration. Free T will be calculated using total T, sex hormone binding globulin (SHBG) and albumin equation.[89] Because the patches only can be dispensed into 4 or 2 mg, monitoring the T level will allow to make the decision either to taper the dose down to 2 mg or add 2 mg patch in addition to the 4 mg (i.e. asking the participant to wear 2 patches together). If the serum T level did not attain the physiological level (based on the lab results), the dose will be increased to 6 mg (using 2 patches of 4 and 2 mg) by the end of the first month and to 8 mg (2 patches of 4 mg) by the end of the 2nd month of the study. Following baseline 2 measurements, each participant will receive a box of 30 patches (4 mg) that should cover the first 4 weeks (28 days) of the study with additional 2 days of patches incase the participant is unable to report on 28th day of the study. This will continue throughout the 16 weeks of the study. All participants will be asked to return the boxes back with the empty sleeves of the patches to account for compliance. The PI will be used the research data log to account for the numbers of patches that were dispensed and used during the course of the study.

4. Detraining after 16 week intervention. Many research investigations have focused primarily on the training aspect and overlooked the physiological adaptations following training. It is unknown to what extent that training evoked skeletal muscle hypertrophy can be maintained in these individuals with SCI despite a marked reduction in the total amount of training. We propose to evaluate body composition and metabolic profile after 16 weeks of detraining.[91,92] Six participants of the RT+Tp group will continue training once weekly in addition to the TRT to determine if we could attenuate the effects of detraining on the examined variables.[92] The other six participants of the RT+Tp will receive no intervention but they will be asked to return for testing. Six participants of the Tp group will receive *no intervention* and will be reexamined after 16 weeks. Both groups will be asked to turn in a weekly dietary recall forms without meeting with the dietitian.

5. Home based NMES Training (Pilot work). Five participants from the RT+Tp group will continue unilateral training of one knee extensor muscle group to perform dynamic leg extension for additional 8 weeks. After 16 weeks of the last training bout, 5 participants on the basis of first-come, first served will be invited to join this program. Training will be conducted at home and will be monitored via Telehealth communication (a service offered by the Department of Veterans Health Administration). Previous trials were successful in conducting home based training and monitored their participants via telephone or internet communications. Participants will be provided a battery operated NMES unit and ankle weights that do not exceed 20 lbs. Training will be conducted twice weekly (3 sets x 10 reps) for 8 weeks and one leg will be trained and the other leg will serve as a control. Pre-set parameters will be adjusted by the PI and will allow the participants enough time to practice using the NMES unit. Before and at the end of the 8 week training, participants will conduct MRI of both knee extensor muscle groups (see below).

Measurements

Specific aim 1. We will determine the efficacy of evoked RT and/or Tp on the size of thigh skeletal muscle groups and percentage fat-free mass, and the CSA of visceral, intramuscular fat and percentage fat mass after 16 weeks of training and 16 weeks of detraining. Anthropometrics, DXA, MRI will be captured pre-intervention, 16 week post-training and 16

weeks following detraining. The ***rationale*** is that elevating level of T and free T to the supra-physiological level will render the RT effective and optimize the outcomes on body composition.

1.1 Body mass index (BMI) and waist circumference (WC): Each participant will be asked to void his bladder and then will propel onto a wheelchair weighing scale. After weighing the participant and his wheelchair (kg-1), he will be helped to transfer to an adjustable mat and his/her wheelchair will be weighted empty (Kg-2). The weight of each participant will be determined by subtracting (**2**) from **(1)** (kg). The height of each participant will be determined at the right side in the supine position. After transferring to the mat, the PI will help each participant to properly align. Two smooth wooden boards will be placed at the participant's head and heels and the distance between them correspond to the height in nearest cm. Every effort will be taken to maintain the knees in an extended position.[12,16] The BMI (Kg/m^2) will be calculated as weight (Kg)/ $height^2$ (m^2). Measurement of WC will be determined in duplicate by identifying the narrowest region of the trunk from sitting and lying positions. A black marker will be used to identify the anatomical site in a sitting position. After normal expiration, a tape measure will be used around the participant's trunk to measure WC. If the value differs by > 1 cm, a third measurement will be taken.[12, 16, 17, 18]

1.2 Dual energy x-ray absorptiometry (DXA): DXA will be used to study body composition in SCI individuals, specifically regional and total FM and FFM. Total body and regional (lumbar spine, proximal femur, and forearm) DXA scans will be performed using a Lunar Prodigy Advance (Lunar Inc., Madison, WI) bone densitometer at the VAMC hospital. We perform testing after lower extremity elevation for at least 30 minutes to minimize fluid shift. All scans will be performed and analyzed by a trained, certified DXA operator using the Lunar software version 10.5. The subject will be assisted to lie on a padded table and both legs will be strapped proximal to the knees and the ankles. The arms and legs will be positioned to ensure proper alignment and to lie still for 10 minutes during the period of the scan. After scanning, total and regional (% FM and FFM) will be determined using total and regional DXA software; the coefficient of variability of two repeated scans is less than 3%.[16, 83, 84]

1.3 Magnetic resonance imaging (MRI): MRI will be performed at the VAMC Hospital using a 1.5 Tesla magnet (GE). A well trained technician will be responsible for reviewing a checklist to ensure that no contra-indications or precautions to conduct the scans. Outside the scanner chamber, the MRI table will be lowered to the level of the participant's wheelchair to easily transfer him. On the scanning table, the subject will allow a few minutes to relax and to avoid the occurrence of muscle spasms that could affect the quality of the scan. Both lower limbs will be strapped together using a soft thera-band to avoid any movement inside the magnet. The participant's head slides in first and the arms are placed across the chest due to range of motion limitations preventing overhead placements. All participants will be instructed to lie still inside the magnet and they will be provided with ear plugs to protect their ears against the magnet noise. The technician will roll the table and dock it to the scanner and then adjust the table at the magnet bore to ensure scanning of the correct region of interest. All participants will have access to a safety belt and a microphone that may be used in case of emergency. The duration of the whole scan (leg, thigh, and visceral fat) including the preparation time should not exceed 30 minutes.[19,20, 49, 50, 68,83, 85]

1.3.1 Skeletal muscle CSA. The skeletal muscle CSAs will be determined before (baseline 2), after interventions and after 16 week-detraining period. Images of both thighs and legs will be collected using the following scanning parameters (repetition time, 500; echo time, 14; field of

view, 20cm; matrix, 256×256). Transaxial images, 10 mm thick and 10 mm apart, will be taken from the hip joint to the knee joint (thigh) and from knee to the ankle (leg) using the whole body coil. The location of the scan will be specifically identified by placing a mark 6 inches proximal to (thigh) or distal to (leg) the patella and matched with the follow up scans.[19,20, 49, 68,83, 85]

1.3.2 Visceral fat. T1-weighted imaging will be performed using a fast spin-echo sequence with the following parameters: (axial in-phase /out-phase with a repetition time of 140 ms and echo time of 4.2 and 1.8 ms for the in-phase and the out-phase, respectively; a 46 cm field of view, matrix size of 256 x 256 or 320x320, one NEX and acquisition time of 4-5 minutes). Transverse slices (0.8 cm thickness) are acquired every 0.4 cm gap from the xyphoid process to the femoral heads. Images will be acquired in series of two stacks with L4-L5 used as a separating point. After acquisition of a localizer sequence, the intervertebral space between the fourth and fifth lumbar vertebrae will be identified by locating the umbilicus. To ensure a short breath holding duration, two sets of 9-12 slices will be captured. The first set will extend superiorly from L4-L5 to the xyphoid process and the second distally from L4-L5 to the femoral heads. Participants will be asked to take a deep breath in and hold their breath for 10-15 seconds to reduce the respiratory-motion artifact associated with MRI for the abdominal region.[50,83]

1.3.3 Processing and analysis. Images will be downloaded to a CD and analysis will be performed using commercial available software (X-vessel). Briefly, the thigh and leg images will be automatically segmented into fat (high intensity), skeletal muscle (mid intensity) and background/bone (low intensity). This first pass segmentation will be used to correct for intensity variations across the original image caused by radio frequency heterogeneity. The corrected original image is then resegmented into the three intensity components using a fuzzy c-mean clustering algorithm. Manual selection of a pixel of skeletal muscle highlights all skeletal muscle pixels and provides the total number of skeletal muscle pixels exclusive of fat or low intensity pixels. **VAT measuring** will start by determining the region of interest and then exclude other bony or muscular areas within this region. This will be done by tracing around the anatomical borders of VAT using the cursor. The high signal intensity and the bright color of the fat pixels will ensure proper tracing (Fig 3). The number of pixels in the highlighted region will be multiplied by the field of view/ matrix size to measure VAT and SAT CSA (cm^2).[19,20, 49, 50, 68,83, 85]

1.3.4 Pilot work for future grants to measure muscle damage and trabecualar bone. Six to eight participants per group will undergo these additional measurements. To measure muscle damage, T2-dual echo MRI at 30 and 60 ms acquisition will be performed before training and 48 hours after the end of week 1 and 48 hours after the end of week 16. **For trabecualar bone,** A 3D fast gradient-echo sequence will be used to obtain the high resolution images (10 cm FOV), with a spatial reconstructed resolution of 195·195·1000 lm. Abilateral phased array coil (USA Instruments) will be used to collect 30 contiguous 1 mm slices in the axial plane, starting with the distal end of the femur, and another block of 30 starting with the proximal end of the tibia. A 3-D low-pass filter will be applied to correct for heterogeneous signal across the phased array surface coil. Regions of interests will be manually identified and a set of parallel lines rotated by 5 through the slice to determine mean intercept length. Parameters measured will be apparent trabecular bone volume to total volume (app.BV/TV) and apparent trabecular thickness (app.Tb.Th, mm), from which apparent trabecular number (app.Tb.N, mm) and separation (app.Tb.Sp, mm) will be derived.

1.4 Skeletal muscle torque and specific tension (torque / muscle CSA) of the knee extensor muscle groups (left and right) will be evaluated prior, 16 weeks after training and then after

detraining using Biodex isokinetic dynamometer (Shirely, NY). Measurements *will be done 72h after the muscle biopsy* to prevent acute effects. Participants will be in a seated posture with the trunk-thigh angle and the knee flexed at 90º (where 0 corresponds to the full knee extension). Each participant will be securely strapped to the test chair by crossover shoulder harnesses and a belt across the hip joint. The axis of the dynamometer will be aligned to the anatomical knee axis and the lever arm will be attached 2-3 cm above the lateral malleolus. Before measuring isometric torque, passive tension of the right knee extensor muscle group will be measured at 5, 30, 60, 90,180, 270 degrees/sec as an index of spasticity. Isometric torque will be measured after adjusting the amplitude of the current to 50, 100 and 150 mA (frequency 30 Hz and pulse duration 450 μs). Isokinetic torque will be measured at 60, 90 and 180 degrees/sec using the same electrical stimulation protocol (Please see the attached form).[87,88]

Specific Aim #2: We will determine the changes in metabolic milieu (basal energy expenditure, glucose homeostasis, lipid profile, FFA, serum total and free T, and insulin-like growth-factor), and cytokines (c-reactive protein, tumor necrosis factor alpha and IL-6 as inflammatory biomarkers) after the delayed entry approach, as result of training and detraining. The ***rationale*** is the preliminary data suggest that maintenance of lean mass is associated with increase in basal energy expenditure, improvement in lipid profile and insulin sensitivity. We hypothesize that adding RT to TRT may result in improvement of carbohydrate and lipid profiles and associated decrease in FFA and inflammatory biomarkers.

2.1 Serum total, free testosterone and IGF-1 The plasma T, free T, SHBG and IGF-1 will be measured in the morning at 6.30, 7.00and 7.30 am (2 ml/ sample).[89, 93] The analysis of total T will be performed by radioimmunoassay after sample extraction and column chromatography. The interassay coefficient of variation (CV) is 12.5% or less for all quality control samples analyzed with the study samples. Free T concentrations will be calculated by measuring SHBG and albumin (www.issam.ch/freetesto.htm).[90] Plasma IGF-I concentrations will be measured by immunoluminometric assay (Quest Diagnostics, Madison, NJ) and RIA (Diagnostics Systems Laboratories Inc., Webster, TX), respectively. Intra-assay precision of IGF-1 is 4.6% at 50 ng/ml and 3.6% at 168 ng/ml.

2.2 Basal metabolic rate and respiratory exchange ratio by indirect calorimetry
After overnight fast, participants will then be kept in a dark room for 20-30 minutes to attain a resting state during which basal metabolic rate will be measured by using a disposable mask placed on the face. The gasses (VCO_2 and VO_2) collected will help to measure the respiratory exchange ratio. This will help to determine the changes in the percentage of substrate utilization (% fat vs. % carbohydrate) after interventions.[16, 95] Participants will also be asked to ride an FES bike for 20 – 30 minutes to determine energy expenditure for both groups at the start of the study and again at the end of 16 weeks of training. Also energy expenditure in conjunction with heart rate will be determined during weight lifting procedure and every 4 weeks for RT + Tp group only.

2.4 Blood lipids: Each subject will have fasting lipid profiles (HDL-C, LDL-C, total cholesterol, and TG) assessed, with total cholesterol: HDL-C ratios utilized as the criterion variable. Concurrent with the IVGTT and following a 12-hour fast, 10 ml of blood will be collected from the indwelling venous catheter and lipids determined by standard analyses procedures employed by the VCU GCRC.[16,50]

2.5 Inflammatory biomarkers: Before starting the IVGTT and following a 12-hour fast, 10 ml of blood will be collected from the indwelling venous catheter and CRP, IL-6, TNF-α , and FFA will be determined by standard analyses procedures employed by the VCU GCRC using commercially available assay kits.[45,70]

2.6 Intravenous Glucose Tolerance Test (IVGTT) An IVGTT will be used to determine insulin sensitivity and glucose effectiveness. Each subject will undergo an IVGTT *before (baseline 1 and 2)*, 48 hours after the cessation of the last exercise bout and 16 weeks after detraining. After a 10 to 12-hour fast, an indwelling catheter with an intravenous saline drip (0.9% NaCl) will be placed in an antecubital vein, and another intravenous line will be placed in a contralateral hand vein to facilitate infusion of glucose and blood sampling during the IVGTT. Glucose samples will be taken at –6, -4, -2, 0, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes after the rapid glucose injection (0.3 gm/kg IV over 30 seconds at time zero). In addition, 20 minutes after the glucose injection a bolus of insulin (0.02 U/kg) will be injected to determine insulin sensitivity. Blood pressure and heart rate will be assessed at minutes 22, 23, and 24 of the protocol. Duplicate blood samples will be taken, the plasma will be separated from the cells, placed in tubes containing 10% EDTA, and frozen at -70 degrees Celsius until analysis. Plasma glucose will be measured by the Autoanalyzer glucose oxidase method and plasma insulin concentrations will be determined by commercial radioimmunoassay using single-antibody kits at the VCU GCRC core laboratory. The S_I (glucose disposal rate per unit of secreted insulin per unit time) and S_G (glucose mediated glucose disposal rate) will be calculated from a least-squares fitting of the temporal pattern of glucose and insulin throughout the IVGTT using the MINMOD program.[96] The coefficient of variation is approximately 15%. The acute insulin response to IV glucose is calculated as the mean rise in plasma insulin above baseline at 3, 4 and 5 minutes after IV glucose administration. KG, a measure of glucose tolerance, is calculated as the least square slope of the natural log of absolute glucose concentration between 5 and 20 minutes after the glucose bolus.[97] The homeostatic model of assessment of insulin resistance (HOMA-IR) will be calculated and insulin sensitivity will be determined using Matsuda and Defronzo formula. Insulin sensitivity = 10.000/ *sq. root* (fasting plasma glucose X fasting plasma insulin) X (mean OGTT glucose concentration x mean OGTT insulin concentration).[97,98]

Specific aim 3. We will determine if 16 weeks of evoked RT+Tp will increase protein levels in vastus latereralis biopsy samples of GLUT-4, IGF-1 and PGC-1 alpha proteins. We will also test whether RT+Tp altered muscle phenotype by measuring MHC isoform expression or enhanced mitochondrial enzymatic activities and ETC compared to Tp only. Skeletal muscle biopsy of the right vastus lateralis muscle *will be taken prior to and 48 hours after 16 weeks* of interventions. We hypothesize that the RT+Tp will stimulate proteins expression and mitochondrial enzymatic and ETC activities compared to the Tp group.[99] A recent study showed that the *random recruitment pattern of NMES[100] can result in atypical protein pattern adaptations commonly seen after RT and endurance type training*.[101]

3.1 Skeletal muscle biopsy Three biopsy samples of vastus lateralis muscle (100 mg wet wt) will be obtained by a 5-mm Bergstrom needle (Dr. Savas) and applying suction, before and 48 hours after 16 weeks of the interventions. Area overlying the muscles will be prepped and draped in sterile fashion, 3cc 2% lidocaine will be locally administered, and a 5 mm skin incision will be made with a #10 scalpel. The biopsy will be obtained through three different incisions, after which the sites will be closed with Steristrips and an overlying sterile adhesive dressing, with a pressure dressing administered for at least 10 minutes. The biopsy samples will be quick

frozen in liquid nitrogen and stored at -70C until further analysis. One sample will split into two halves and will be used for measuring activities of mitochondrial enzymes and ETC complexes (I- IV). The second sample will be used for measuring GLUT-4 and IGF-1 proteins. The third sample will be split for PGC-1α and for histological assessments of MHC fiber types.

3.2 Mitochondrial ETC activities. The assays will be performed in fresh cholate-treated skeletal muscle homogenates. ETC complex activities will be measured spectrophotometrically as specific donor-acceptor oxidoreductase activities in 0.1Mphosphate buffer (HP 8453 and Lambda 35 UV/VIS). Both donors and acceptors span specific regions of the ETC. Rotenone-sensitive NADH cytochrome c reductase will measure complexes I and III. NADH coenzyme Q reductase will be measured as the rotenone-sensitive oxidation of NADH with decylubiquinone as acceptor, and assesses complex I. NADH ferricyanide reductase measures NADH dehydrogenase in **complex I**. Antimycin-sensitive succinate-cytochrome c reductase assesses **complexes II and III**. Complex II activity will be measured as the thenoyltrifluoroacetone sensitive reduction of 2,6 dichlorophenolindophenol with succinate as substrate, whereas total complex II will be measured in the same manner but with duroquinone added as a source of exogenous coenzyme Q. Succinate dehydrogenase measures the first two subunits of complex II. Decylubiquinol-cytochrome c oxidoreductase will be measured as the antimycin-sensitive reductase to assess complex III. Cytochrome c oxidase will be measured as the oxidation of reduced cytochrome c and expressed as the first order rate constant. To measure activity of mitochondrial ubiquinol:O_2 oxidoreductase (**complex III/IV**), a pyridine nucleotide HPLC-based enzymatic assay will be developed that utilized the enzyme diaphorase to regenerate ubiquinol by oxidizing an NADH. [51,52] Flurometric measurements will be conducted to measure the primary oxidative and glycolytic enzymes' activities including citrate syntahse, SDH, lactate dehydrogenase, hexokinase, and carnitine palmitoyltransferase I enzymes.[58, 59,91]

3.3 Protein content. Muscle biopsy samples will be homogenized on ice using the appropriate chemical reagents (50 mM HEPES, 10 mM EDTA, 100 mM NaF, 50 mM Na pyrophosphate, 10 mM Na Orthovanadate and protease inhibitor cocktail). Western blot analysis will then be performed to determine the protein concentrations of **GLUT-4**, **IGF-1** and **PGC-1 α**.[55, 56, 69] Briefly, proteins will be resolved by SDS-PAGE then transferred to two supported nitrocellulose membranes by wet electromembrane transfer at 110 V for 2 hours, and then blocked in 6% bovine serum albumin (BSA)/Tween-Tris-buffered saline (TTBS) for 1 hour. Membranes will be incubated separately with the appropriate primary antibody overnight. The membranes will be washed separately (three times for 20 minutes) in 6% BSA/TTBS and incubated with appropriate secondary antibody in 4 mL 3% BSA/TTBS at 4°C for 1 hour. The membranes will be washed (four times for 45 minutes) in 6% BSA/TTBS. Proteins will be visualized using an enhanced chemiluminescence detection system (GE Lifesciences) according to the manufacturer's instructions. Western blots will be quantified by scanning with A GS800 densitometer. Optical densities of the Western blots will be measured using image-analysis software (Molecular Analyst; Bio-Rad).

3.4. Immunohistochemistry and myosin heavy chain (MHC) Immediately after muscle biopsy, half the third sample will be mounted on tongue blades by using a medium of OCT compound and tragacanth gum then stored in -70°C until analyzed. Fiber type percent and CSA will be determined by using immunohistochemical methods. Using a cryostat, (Leica CM3050) 16 micron cryo-sections will be collected on glass slidesand frozen at -20°C until assayed. Slides will be removed from -20°C, allowed to dry at room temperature, and then gently fixed in cold 4% PFA (in 1x PBS) for 10 minutes. Sections were then permeablized for 10 minutes and

protein blocked. Sections will be incubated with slow skeletal MHC antibody (abcam®ab1103) at a dilution of 1:100 for 60 minutes at room temperature and then rinsed (3x for 2min in 1xPBS+0.1%tween) and treated with an Expose Mouse and Rabbit Specific AP (red) Detection Kit (abcam). Sections will once again be rinsed (3x for 5min in 1xPBS+0.1%tween) and prepared for the next antibody incubation by re-permablizing and protein blocking. Sections will be then incubated with myosin for human fast fibers (2A+/+, 2X+/-) antibody at a dilution 1:100 overnight at room temperature. Then it will be again rinsed (3x for 2min in 1xPBS+0.1%tween) and treated with an Expose Mouse and Rabbit Specific HRP/DAB Detection IHC Kit (abcam). Sections were then rinsed, mounted, and cover slipped using cytoseal™ XYL (Richard-Allan Scientific®). Stained muscle sections were observed using a Nikon Eclipse E600 at 100X and images collected using a Nikon Digital Camera DXM1200 and Nikon ACT-1 software. Images were analyzed for fiber type discrimination by color, slow fibers were stained dark pink, fast 2A fibers were stained dark brown and fast 2X fibers were stained light brown.

4.1 Statistical Analyses. A delayed entry approach for 4 weeks will allow each participant to serve as his own control. After the initial 4 weeks, the PI will have each number from 1-24 randomly assigned to one of the two groups using a computer program (random number generator). *The order of the enrollment will determine the participant's number based on age, level of injury, time since injury and %FM*. Paired t-tests will be used to determine differences in body composition and metabolic profile between baseline 1 and baseline 2 (see Figure 10). We will first examine the effects of the RT+Tp vs. Tp(pre-training vs. post-training) on body composition and the metabolic profile variables. 1) **To determine the effects of interventions (n =12 /group)**, Repeated Measures ANOVA with one between subjects factor (Group, RT +Tp vs. Tp Only), one within subjects factor (Time) and the interaction between the two factors. A simple linear and multivariate regression analyses will be used to examine the relationship between body composition and metabolic profile variables. Inflammatory biomarkers (CRP, TNF and IL-6) and HOMA-IR will be log-transformed before analysis. For the protein expression, we will use paired t-tests to examine the effects of each intervention. 2) **To determine the effects of detraining** , Repeated Measures ANOVA will be used to examine the effects on body composition and metabolic variables. The sample size was calculated based on the mean and standard deviation (SD) values derived from our preliminary work. Power was set at 0.8 with a level of significance = 0.05. The effect size is calculated based on the effects of RT on body composition and the metabolic profiles (Table 1). The number of subjects necessary to find statistical differences in the major variables of this study was found to be 10 participants /group. The results will be expressed as mean ± SD and all statistical analysis will be performed using SPSS version 17.0 (Chicago, IL).

ADVERSE EVENTS: All subjects will be closely monitored for any unexpected event. An unexpected event is any unfavorable and unintended sign, symptom, or disease temporally associated with subjects participation in the study , whether or not considered related to the study. All adverse events will be assessed in terms of seriousness, severity, and relationship to participation in the study. All serious adverse events will be promptly reported in the IRB. Subjects will be monitored for any changes in baseline health by the study staff. Their SCI provider will be notified of new changes. An adverse event is any experience that has taken place during the course of a research project, which, in the opinion of the investigators, was harmful to a subject participating in the research, increased the risks of harm in the research, or had an unfavorable impact on the risk/benefit ratio. Adverse events will be monitored throughout the study via review of medical charts and verbal concerns voiced by the participant or an associated friend or family member.

ETHICAL CONDUCT OF THE STUDY: The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, will be carried out by all study staff in keeping with Good Clinical Practice Guidelines and under the guiding principles in the Declaration of Helsinki. Any deviations fron the protocol will be fully explained and documented by the investigator and promptly reported to the IRB.

CONFIDENTIALITY: All records identifying the subject will be kept confidential. Only the subject number will be recorded on study documents. The PI, Ashraf S. Gorgey, will maintain a list to enable the subjects' records to be identified. This list will kept in a locked file in office 1V-129 on the SCI unit.

DATA STORAGE: All study data will be securly stored in the SCI Research (1V-129) locked office. No data will be destroyed.

PUBLICATION OF DATA: No subject idetifiers will be disclosed in future publications.

Good Clinical Practice: The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, will be carried out by all study staff in keeping with Good Clinical Practice Guidelines and under the guiding principles in the Declaration of Helsinki. Any deviations fron the protocol will be fully explained and documented by the investigator and promptly reported to the IRB.

At the completion of this study all subjects will continue to receive their health care from their assigned SCI provider. All subjects will be identified by an assigned number and their initials. Subjects research charts will be kept in the PI's office 1V-129 which is locked. Only study staff listed on the Personnel List will have access to subject study records and medical information. All completed study files will be stored indefinitely according to VA policy in the PI's SCI research office 1V-129. If storage space is limited all records will be sent to Dunmar Storage Facility per direction of McGuire Research Institute. No research records will be destroyed.