Effects of Electrical Stimulation and Vitamin D supplementation on Bone Health Following Spinal Cord Injury

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Background and Rationale: Neurogenic osteoporosis is a well-known complication of spinal cord injury (SCI). It results from bone demineralization due to the inevitable mechanical unloading of paralyzed limb[1-5]. Impaired skeletal muscle activity leads to muscle atrophy, loss of bone mass, bone porosity, fragility and increased incidence of fractures[6-10] Bone health is maintained by a continuous cycle of bone formation and resorption. However, when the cycle is interrupted or uncoupled, as seen in certain conditions like estrogen deficiency, changes in levels of parathyroid hormone (PTH), glucocorticoids, serum calcium and growth hormone, there is significant loss in bone tissue.[11] It is estimated that 200 million people worldwide are affected by bone loss or osteoporosis.[12-13] Osteoporosis is defined as a T-score of <-2.5 standard deviations (SD) below the bone mineral density (BMD) of a healthy young female, as measured by dual x-ray absorptiometry (DXA)[13]. In the United States, an estimated10 million Americans have osteoporosis and another 44 million more, have low bone mass or osteopenia.[17-20] Among able-bodied individuals, about 1 in 3 women and 1 in 5 men, over age 50, will suffer an osteoporotic fracture.[14-17]

Data from the Department of Veterans Affairs suggest that SCI affects an estimated 46,000 Veterans.[18] With about 18,000 new cases of SCI every year,[18-19] including a significant number of Veterans, the risk of osteoporosis and fracture is worth addressing. The first 2 weeks of SCI is the most critical for bone loss.[20] Thereafter, progression slows down and stabilizes in 2-5 years and in some cases, far beyond10 years[7,20-21] It is estimated that 50% -70% of individuals with SCI, will sustain a low impact fracture sometime in their lifetime.[11,20] The sites commonly affected are the distal femur and proximal tibia[21-22] Osteoporosis-related fractures involve significant health care expenditure. The medical cost for managing Veterans with SCI alone in 2016 was estimated at $30,770 - $62,563 per Veteran[23-24]; while cost in the acute phase could go above $140,000[25]. The cost of medical care for Veterans with SCI and osteoporosis-related fractures will naturally therefore, have significant impact on the finances of the VA healthcare system and those of Veterans and their families. It is estimated that health care cost for managing osteoporosis-related fractures, as a whole, will reach $25 billion by 2025 [23,25.] Treating and preventing osteoporosis in persons with SCI is challenging. Anti-resorptive drugs (bisphosphonate and denosumab) and anabolics (PTH peptides), have been shown to reduce fracture risk at vertebral and hip sites[11,21]. However, there is no consensus on whether they reduce fracture risk at the femur; the site of greatest relevance for persons with SCI. In contrast to anti-resorptive agents, sclerostin, which inhibits bone formation by up-regulating receptor activator of nuclear factor-kappa B ligand (RANKL) and down-regulating osteoprotegerin (OPG), with a net result of increased bone resorption, is emerging as a therapeutic target.[26] Anti-sclerostin drugs (e.g. romosozumab), have been shown to stimulate bone formation, reduce resorption and ultimately increase BMD[27]. Its use in the SCI population is however still being studied. It is only available in injectable form and has a black box warning for cardiovascular events/death, making it unattractive for use in a population already at high risk of cardiovascular events. Very importantly, electrical stimulation (ES) has been shown to partly reduce BMD loss in the femur if initiated early in SCI[28-29] and it presents no systemic side effects. Bélanger et al., observed that the distal femur and proximal tibia had almost 30% recovery after 24 weeks of daily one-hour ES

treatment to the quadriceps muscles 5 days a week.[30] However, there was no notable change in the mid-tibia. In another study of 24 persons with acute SCI receiving functional ES cycling exercises for 3 months, the rate of depletion in BMD in the distal femur, was significantly less in the study population compared to controls.[31] We recently adopted neuromuscular electrical stimulation (NMES) resistant training (RT) to evoke muscular activity in paralyzed lower extremity muscles.[32] Our preliminary data showed that just 16 weeks of **2 *times* a week** resulted in micro-architectural changes, suggesting that NMES-RT holds great promise as a therapeutic modality. We anticipate that a longer duration of treatment would yield even better outcomes. We therefore propose using telehealth to administer NMES-RT interventions over a 9-month period to improve the bone health of Veterans with SCI. Telehealth is an emerging utility that is gaining popularity. Using Telehealth for patient care has been shown to increase access and compliance.[33-34] In a pilot study NMES-RT induced muscle hypertrophy by Gorgey et al., all participants were compliant with keeping their telehealth appointments and participation in the intervention activities.[35] Additionally, vitamin D (Vit D) deficiency, defined as 25-hyroxyvitaminD 25(OH)D < 30ng/mL, has been implicated in decreased BMD, higher bone turnover, and higher incidence of bone fractures[36], whereas supplementation reduces fracture risk[11] when major circulation 25(OH)D is sufficient.

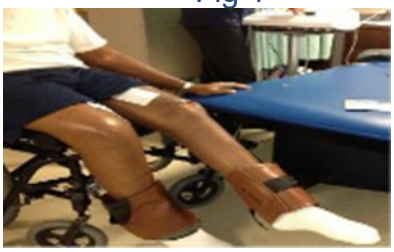
Vit D depletion in persons with SCI was first observed in the early 90s, when Zhou et al.,[37] compared serum levels of 25(OH)D of 92 individuals with SCI (50 paraplegics and 42 quadriplegics) with those of 28 able-bodied persons. Serum 25(OH)D was low in SCI participants compared to the control group. Ultraviolet radiation from sunlight is the primary stimulant for the elaboration of Vit D. Deficiency can easily develop in SCI patients who have limited exposure to sunlight. Bauman et al. demonstrated that a sizable proportion of Veterans with chronic SCI have Vit D deficiency compared to non-SCIs in the general population.[38] Although, Vit D deficiency is prevalent in the SCI population, the requisite amount of Vit D supplementation for correction of the deficiency has not yet been established. In a prospective drug intervention study, Bauman and Colleagues, used daily doses of 2000 IU of oral Vit D in Vit D deficient (< 20 mg/mL) subjects, plus 1.3 gram of elemental calcium to reach physiological levels of serum 25(OH)D, after 3 months[39]. Another study used 6000 IU daily dose of Vit D in a double-blinded study, to achieve physiological range within the same time frame[40]. The lower therapeutic dose in Bauman et al.'s study[39] may be explained by the fact that Vit D is only needed for control of body calcium homeostasis[41], to improve calcium absorption for bone health. Lani et al.[42] observed that calcium supplementation along with Vit D supplementation was necessary to achieve significant increase in bone mineralization in rat models. The authors suggested that even at higher doses, Vit D supplementation does not stimulate bone mineralization. Caution is however advised in the use of calcium supplementation in the SCI population, because of the risk of renal lithiasis and increased cardiovascular risks.

To our knowledge, no study has considered the combination of Vit D supplementation and NMES-RT for attenuation of bone loss after SCI. Prevention of osteoporosis and associated serious complications in the SCI population remains a major challenge and implementation of early interventions to mitigate complications and reduce health care cost is a worthwhile endeavor.

Significance of this research to the Veterans' population: The lack of weight bearing amongst Veterans with complete spinal cord injury and the prevalence of Vit. D deficiency, due to limited exposure to sunlight, is a significant problem. The consequence of these duo can result in low impact fracture leading to increased morbidity and mortality, as well as, high health care cost, increased care burden for the families and poor quality of life for the Veteran. The proposed study to use NMES-RT in conjunction with 2000 IU oral daily Vit D supplementation in Veterans with SCI of at least one-year post-injury, is a potential therapeutic strategy that may attenuate bone loss and decrease the frequency of low impact fractures after SCI. The fact that 50% of individuals with SCI will sustain low impact mechanical fractures during their lifetime, with an even higher incidence in Veterans, validates the need for this study. Additionally, the findings may help the VA healthcare system in the development of clinical guidelines for the prevention of osteoporosis in Veterans with SCI.

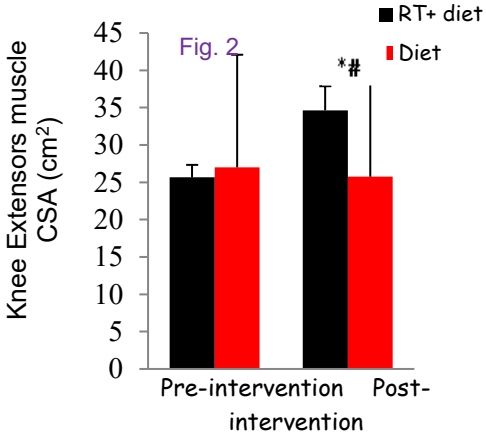
Preliminary Study: RT has been identified as a very important rehabilitation approach that increases lean mass and decreases fat mass[42-43]. After SCI, RT has also been used to exercise both paralyzed and non-paralyzed muscles of lower extremities. NMES has been used in individuals with chronic SCI to evoke exercise-induced RT using standard ankle weights.

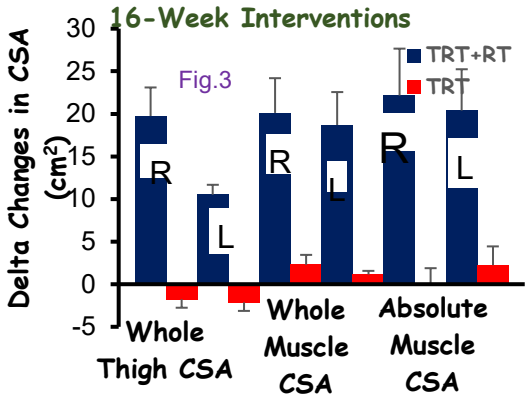
Fig 1



Mahoney et al.[44] showed that ***12 weeks*** *of* ***twice a week*** NMES-RT resulted in skeletal muscle hypertrophy by more than 40% and improved glucose tolerance years after injury. A follow-up study reported that ***fatigue resistance*** of the trained knee extensors increased by 33%. Gorgey noted that **12-weeks of twice weekly** NMES-RT elicited more than 35% increase in skeletal muscle size, improved muscle quality, and increased insulin growth factor-1 (IGF-1) by 25%. The authors implemented 12 weeks of NMES-RT, 40 contractions per leg per session, in 9 individuals with motor complete SCI (C5-T11). Participants were randomly assigned into one of two groups; RT + diet (n=5; 4 tetraplegic)

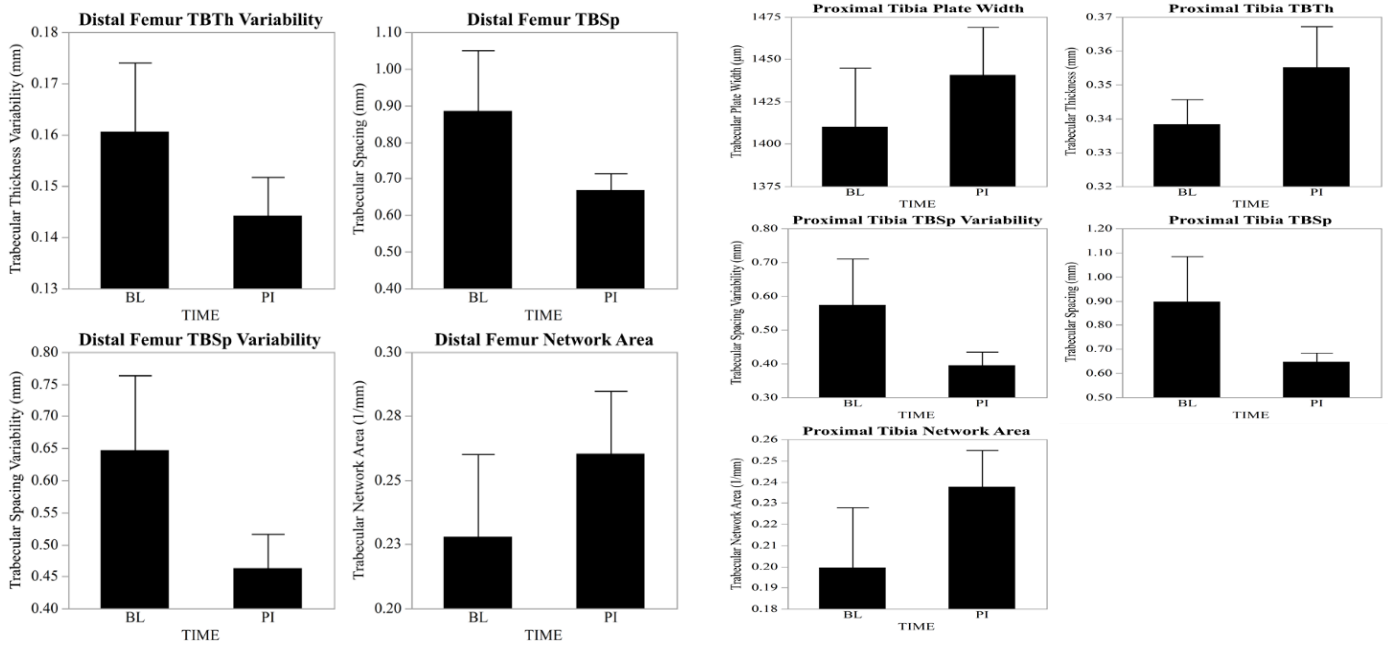
or diet only (n=4; paraplegic). Skeletal Muscle Cross-Sectional Area (CSA) was measured with T1-axial MRI at ***a)*** pre and ***b)*** post-12 week of NMES-RT. Thigh (28%) and knee extensor muscle CSA (35%) showed remarkable hypertrophy ***(Fig 2***). Another study demonstrated the effects of NMES-RT and Testosterone on cardio-metabolic risk factors after SCI[45]. In an open-label randomized clinical trial, we examined the effects of complementary approach of 16 weeks of surface NMES evoked RT and testosterone (TRT+RT) compared to testosterone replacement therapy (TRT) only (2-6 mg) on skeletal muscle size, body composition, and peak torque (muscle quality) in persons with motor complete SCI. Twenty-two individuals with chronic motor complete SCI (ages 18-50 years) were randomly assigned to either TRT+RT (n =11) or TRT (n= 11) for 16 weeks (***Fig3***)**.** In **fig. 3,** we determined the effects of TRT+ NMES-RT on whole thigh muscle CSA and whole thigh absolute muscle CSA after accounting for intramuscular fat. Compared to TRT only, TRT+ RT showed delta increase > 20 cm^2; demonstrating the efficacy of loading the paralyzed muscles in evoking muscle hypertrophy. Effects of NMES-RT with TRT on trabecular bone parameters of distal femur and proximal tibia were also measured. Nine participants with SCI were randomized into TRT+RT group for 16-week intervention. Each participant underwent MRI prior to BL and following the intervention PI. MRI microarchitecture techniques were used to evaluate trabecular bone quality, measured as plate width (PW; μm), trabecular bone thickness (TBTh; mm), trabecular bone spacing (TBSp; mm), and network area (NA; 1/mm) for the right proximal tibia (ProxT) and distal femur (DistF). Mixed models with random effects were used to calculate differences between BL and PI (MD ± SE) and were then transformed to effect sizes similar to Cohen's d (d, 95% CI [effect size]). Bar plots for trabecular measures of the distal femur depicting the difference for each measure across the intervention period. Small effect sizes were observed for distal femur TBTh variability, TBSp, and NA. A medium effect size was observed for distal femur TBSp variability. Mean ±SE. Bar plots for trabecular measures of the proximal tibia depicting the difference for each measure across the intervention period. A small effect size was observed for proximal tibia PW. Medium effect sizes were observed for ProxT, TBTh, TBSp, TBSp variability, and NA. Mean±SE. Following the intervention, ProxT PW (MD: 30.56 ± 22.52; d: 0.48, -0.21 to 1.17 [small]), TBTh (MD: 0.02 ± 0.01; d: 0.70, 0.01 to 1.39 [medium], and NA (MD:0.04 ± 0.02; d: 0.64, -0.05 to 1.33 [medium]) all increased from BL measures. In contrast, TBSp (MD: -0.25 ± 0.17; d: -0.52, -1.22 to 0.17 [medium]) decreased from BL. The DistF similarly presented with increases in bone quality from BL for PW (MD: 11.7 ± 42.70; d: 0.10, -0.60 to 0.79 [negligible], TBTh (MD: 0.01 ± 0.01; d: 0.29, -0.41 to 0.98 [negligible], and NA (MD: 0.03 ± 10/29/2019. *Fig. 4 & 5:* Due to the exploratory nature of the study on small sample size and short duration, we relied primarily in this trial on measuring the





effect size. Our results support the fact that the TRT+RT has a small effect size (<0.3) on parameters of trabecular bone at the distal femur and moderate effect size at proximal tibia (>0.5-0.8) compared to TRT only. Biostatistician advised that we should not run inferential statistics (***Holman et al. under review, Ost. Int****.-R2)*
Summary of preliminary data. There are two key questions that are yet to be answered before deployment of this intervention; ***1*****) Would NMES+RT +Vit D drive improvement in trabecular bone parameters compared to passive movement + Vit D in persons with chronic SCI? and 2) does skeletal muscle hypertrophy induce improvement in biomarkers of bone formation over bone resorption?** Our preliminary data is limited; however, it highlights the benefits of evoking skeletal muscle hypertrophy on trabecular bone parameters in persons with chronic SCI. We therefore posit that a home-based approach of 9 mos NMES-evoked RT and Vit D supplementation will enhance trabecular bone parameters and markers of bone formation compared to passive movement and Vit D supplementation.

Fig. 4



Research Design and Methods: A 2-year single site randomized controlled trial to investigate the efficacy of NMES-RT + Vit D versus passive movement + Vit D supplementation on trabecular bone parameters of distal femur and proximal tibia and on biomarkers of bone formation and resorption in persons with chronic SCI. All Recruitment will be conducted at CVHCS

Specific aims and Research Hypotheses:

Aim 1: **To determine the impact of home-based NMES-RT protocol plus oral Vit D supplementation, compared to passive movement plus oral Vit D on bone micro-architectural properties, as measured by MRI in Veterans with chronic SCI (> 1year post SCI)**. Both groups will receive 9 mos of prescribed treatment. We hypothesize that NMES-RT+ Vit D supplementation will result in improved trabecular bone parameters after SCI. We plan to compare changes in bone composition including bone marrow fat (MBF) and cortical porosity, at the distal femur and proximal tibia, as measured by MRI and DXA in the two groups, at enrollment and end of intervention (9 mos). We will analyze the effect of Vit D based on levels of 25-(OH)D, at enrollment; mid-intervention (4.5 mos), and at 9 mos. Participants with > 40ng/mL of 25 (OH)D will be excluded from the study.

Hypothesis: NMES-RT+ Vit D supplementation will result in improvement in trabecular and cortical bone parameters compared to passive movement and Vit D supplementation after 9 months in persons with SCI*.*

Aim 2: To determine the impact of home-based NMES-RT protocol plus oral Vit D compared to passive movement and Vit D supplementation on biomarkers of bone formation and bone resorption in Veterans > 1-year post SCI. Both groups will also receive 9 months of therapy. We hypothesized that NMES-RT will increase osteoblast activity and decrease bone demineralization compared to passive movements. We plan to measure serum 25(OH)D, PTH, Bone specific alkaline phosphatase (BALP), Procollagen Type 1Intact N Terminal Propeptide (P1NP), Hydroxyproline (HYP), Carboxy-terminal Crosslinked Telopeptide of Type 1 collagen (CTX), in participants' blood. The role of bone turnover markers in the evaluation of bone loss in persons with SCI has not been established. In able-bodied persons, serum CTX and serum P1NP are considered the best markers of bone resorption and formation, respectively[46-48]. Blood analysis will be done at enrollment, 4.5 mos and 9 mos, for both groups. We plan to account for dietary intakes of protein, sodium, calcium, and phosphorous on BMD. Each participant will turn in dietary logs of 3-days per week on a weekly basis. The dietary log of each participant will be analyzed by our registered dietitian using Nutrition Data System for Research (NDSR) software[49]. We will solicit the service of our dietitian who will contact participants once a month to advise them on balanced diet. Additionally, the World Health Organization quality of life (WHO-QOL) survey will be used to assess participants' physical and mental domains after intervention. The WHO-QOL is a survey instrument that rates six broad domains on health (physical, psychological, level of independence, social relations, environment, and spirituality/religion/personal belief), using a five-point Likert scale (1-5) to produce a total of 100 items in assessment. This survey will be administered at enrollment and at the end of intervention. It takes about 15 minutes to complete the survey. Data obtained from the survey will serve as preliminary data to determine whether exercise programs could also enhance the QOL for persons with SCI. It will be interesting to note any association between physical domain and bone parameters after SCI.

Hypothesis*:* Osteoblastic and osteoclastic activities undergo obvious dynamic changes favoring bone loss after SCI*.* NMES-RT +Vit D supplementation increases osteoblastic activity and decreases bone demineralization as observed by decreased biomarkers of osteoclastic activity compared to passive movement and Vit D supplementation.

Subjects: Twenty Veterans and non-Veterans with >1 year of SCI will be recruited from CVHCS and (VCU, if insufficient Veteran participants). The CVHCS SCI registry has ~1,800 Veterans and more than 298 meet the inclusion criteria of C8-T10 level of injury.

Inclusion Criteria: Participants should be 18-65 years of age; male or female, with traumatic motor complete SCI (≥1-year) and C8 to T10 neurological level of injury (NLI), by the International Standards for Neurological Classification (ISNCSCI) of SCI, serum 25(OH)D < 41ng/mL, be a wheelchair user for primary mobility, be able to receive written clearance from their medical Providers to ensure safety of participants, accessible caregiver capable of physically supporting participant throughout the trial and willing to be trained to apply intervention protocol in the home (placing weights and positioning the Veteran) throughout study duration, and have normal ECG. Participants' knee extensors must respond to standard surface NMES procedures (frequency: 30 Hz; pulse duration:450 μs and amplitude of the current < 200 mA), to ensure intact neural circuitry below the level of SCI. All participants will undergo NLI examination. Only those with American Spinal Cord Injury Impairment Scale (AIS) **A and B** will be included. Age limit of 65 years is to avoid cardiovascular challenges that could be provoked by 9 months of strenuous physical activity in older persons. Moreover, persons older than 65, have more than 2-fold risk of osteoporosis related fractures, which may confound the trial[50].

Exclusion Criteria: Participants with any of the following pre-existing medical conditions will be excluded from the trial: **1)** neurological injury other than SCI,(e.g. cauda equina or distal conus injuries resulting in limb or sacral areflexia; **2)** Unhealed fracture in either lower or upper extremities; **3)** Severe scoliosis, hip knee range of motion (ROM) > 20 degrees. or flexion knee contractures **4)** Uncontrolled hypertension (BP >140/90 mmHg) and severe orthostatic hypotension (BP drop > 20 mmHg) or inability to maintain a sitting position ; **5)** Other medical conditions like cardiovascular disease, uncontrolled type II DM, insulin-dependent DM, or symptomatic urinary tract infection; **6)** severe hypercalcemia (defined as Serum calcium >16 ng/mL)[51]; **7)** anti-coagulant or anti-platelet therapy, including aspirin. **8)** pacemakers and/or defibrillator devices in place, metallic implants, shrapnel or bullets in the vertebral column, or other contraindications for MRI; **9)** DXA total body-score < -2.5, knee BMD scores < 0.6 g/cm^2 ; **10)** Participants with spasticity that is severe or adjudged a contraindication by the site Physician or limited ROM will be excluded. **11)** Pressure ulcer of the trunk, pelvic area, or lower extremities of ≥ stage 3; **12)** Psychopathology history that may conflict with study objectives; **13)** Potential participants who are not wheelchair users and cannot transfer with sliding boards or portable lifts.**14)** serum Vit. D > 40 ng/mL; **15)** abnormal/elevated calcium level **16)** pregnancy (female participants). Medically accepted birth control is required to enter this study. This may include, but is not limited to, birth control pills, IUD's, condoms, diaphragms, implants, being surgically sterile, or being in a post-menopausal state. A blood pregnancy test will be conducted to rule out any pregnancy before the study at the McGuire VAMC. The test will be repeated every month during the course of the study. The blood samples will be sent to the McGuire VA pathology lab for analysis. **17)** Any condition that, in the judgment of the principal

investigator or medical provider precludes safe participation in the study and/or increases the risk of infection. A large percentage of patients in the department of VA are males. Should female participants be found, in order to ensure heterogeneity of samples between the two groups, postmenopausal and estrogen deficient females and men undergoing anti-androgen therapy or post orchiectomy, will be excluded. In addition, individuals with stage 3b or higher chronic kidney disease and those likely to have bone loss (age >65), will be excluded due to possible renal osteodystrophy and osteoporosis that may increase the risk of bone fractures from bone loading and ES to muscles. We will also exclude those with AIS C & D as they are already engaging in weight bearing activities which may confound the results of the trial. We have chosen inclusion and exclusion criteria for this study, to optimize homogeneity. Should the outcome be positive, it will be necessary to broaden inclusion criteria for future studies so that the methods can be used in a larger number of individuals with SCI.

Intervention for 9 months:

Vitamin D:
Participants will receive a 30-day supply of 2000 IU vitamin D to take by mouth once a day at about the same time every day. If baseline Vitamin D is low, we will supplement with Cholecalciferol for two weeks as follows:
Participants with Serum Vitamin D:
< 10 ng/mL, will receive 5,000 IU daily
11-20 ng/mL, will receive 2000 IU daily
21-40 ng/mL, will receive 1000 IU daily
Then, all participants will receive 2000 IU daily through the period of intervention. Vitamin D will be refilled every 25 days to ensure the participants do not run out of the intervention drug.
We will also call participants every 30 days for a pill count to ensure that they are taking the vitamin D.

Resistance training (RT) and Rowing Exercise:
Those assigned to the NMES-RT/Rowing with Vitamin D group will receive 4.5 months of electrical leg shocks with ankle weights (no ankle weights in the first week), twice a week. The intervention will be done while sitting in a wheelchair. Two adhesive patches (electrodes) will be placed on the skin over the knee muscles. Participants/caregivers will initiate and slowly increase electric current on the stimulator in 5-second intervals to cause full leg extension (leg straightens out). Once full knee extension is achieved in a sitting position, an extra 2 lbs. of weight will be added every week. Each session will consist of 4 sets of 10 knee extensions and will last for 30-40 minutes. Training will alternate between the right and left leg. In addition, the participant and a caregiver will train on how to set up the device for use at home.
After 4.5 months, each participant will receive a rowing machine (Murtisol Magnetic Rowing machine) to use twice weekly. The participant will be asked to transfer to the rowing machine (with assistance or supervision from a caregiver) for the intervention, using a sliding board or a portable lift. Participant/caregiver will place patches for NMES on both knee extensors (muscles that straighten out the legs) and flexors (muscles that

bend the knee) and initiate the electrical current. They will alternate the current 5 seconds on, then 10 seconds off on both muscle groups. Participants with upper body strength will be encouraged to use their arms to initiate the extension and flexion of both legs. If they do not have full trunk control, they will be provided with an adhesive belt to secure their trunk during an exercise and a special hand mitten to secure their hands to the rowing machine, if needed. Each session will consist of 4 sets of 10 knee extensions and flexions, and it will last for 30 - 40 minutes. Training will alternate between the right and left legs on the same day.

Passive range of motion or passive stretching:
Participants assigned to the passive exercise and Vitamin D group will have their caregivers perform passive ROM. Caregivers will be instructed to sit on a stool, cup the participant's leg above the ankle joint, and move it from 90-degrees of knee flexion to full knee extension. The leg will be maintained in extension for 5 sec and returned to flexion for 5 sec. The passive actions are repeated in the manner described in the RT protocol; 10 reps on the right leg followed by 10 reps on the left leg for 4 sets x 10 reps. This exercise training will take place twice a week. Each training exercise will last 30 – 40 minutes each day of the exercise.

Blood Pressure (BP) Monitoring: The caregiver will measure the participant's BP before starting the exercise, record the reading and leave the BP cuff in place. About 15 to 20 minutes into the exercise, another BP reading will be taken and the final reading at the end of the exercise to safeguard against Autonomic Dysreflexia (AD). Most individuals with high cervical and thoracic levels of SCI own BP machines. However, if they do not own one, they are eligible to get one from their Providers.

Caregiver Role
Caregivers will train on the use of neuromuscular electrical stimulation and passive range of motion (ROM). After the training, the caregivers will be responsible for placing and removing skin electrodes (patches) and checking the skin afterward for skin breakdown. The caregivers will also be responsible for adjusting the stimulator current. For those undergoing passive ROM, the caregivers will be responsible for moving the paralyzed legs (one at a time) while holding them above the ankle, from 90 degrees of knee flexion (bent knee) to full knee extension (straighten out). They will also be responsible for adding ankle weights during the exercise every week.
Additionally, the caregivers will assist with transfers (if participants were unable to do so). We will review transfer techniques with a sliding board or portable lift with caregivers if the participant or caregiver requests us to do so. Caregivers and persons with SCI are usually skilled in the use of these types of transfer equipment.

Home Video Telehealth Service:
Telehealth is a service offered by the VA for use to provide virtual care. Telehealth uses video conference calls. Suppose participants are not already enrolled in the VA Telehealth program. In that case, they will be given a registration form to complete to provide us permission to use VA Telehealth before they can participate. The participants will need to have a smartphone, a tablet, or a computer with a webcam and

internet access to be able to participate. However, if they not have one of these, they will be provided a tablet with a web camera and internet access, and we will help them create an e-mail account if they do not already have one**.**

The participants will need a quiet space in their homes for the training. The study team will make sure that the address is accessible to EMS in an emergency. If it is not accessible to EMS, they may not participate in the study. At the start of each session, the PI will verify the address and telephone number and an alternate contact person, and a phone number for use in emergencies or communication failure. Should the need arise, the study team will contact EMS or the National VA Telehealth Crisis Line for additional help.

Before starting the exercise training session, the microphone, speakers, and web camera will be tested to ensure things are working fine from both ends. We will request participants to be ready 20-30 min before the video call by asking the caregivers to place the electrodes on their legs, make sure the batteries of the stimulator are fully charged, electrodes are connected to the stimulator, and the ankle weights are positioned. Once the setup is completed, and the Telehealth connection is established, the training will begin.

All training sessions will be conducted twice weekly. At the end of each session, the PI will confirm the next appointment and request that the participant confirms his/her next appointment online 24 hours before the next session. If unable to keep the next appointment for any reason, we can reschedule the appointment for another day and time so long as participants can have two sessions in a week.

WHO-Quality of Life (QOL) Survey:
Participants will be given a survey regarding their health, wellness and QOL to determine whether exercise programs could enhance QOL after SCI. The WHO-QOL is a survey that rates six broad domains on health (physical, psychology, level of independence, social relations, environment, and spirituality/religion/personal belief), using a five-point scale (1-5) to produce a total of 100 items in the assessment. Participants will be given the survey at the beginning and at the end of the study. The survey takes about 15 minutes to complete.

Measurements: All training aspects and measurements will be conducted at the Central Virginia VA Health Care System (CVHCS) SCI Exercise Physiology & Body Composition Laboratory. All participants will undergo bone assessment measurements with MRI and DXA (**specific aim 1**); measurements of bone biomarkers and weekly dietary recalls (**specific aim 2**). All testing procedures will be conducted a week to training (**baseline**) and post-intervention for **Aim 1** and **baseline**, **4.5 mos** and **9 mos** for **Aim 2**. Will get MRI of thigh skeletal muscles to determine muscle CSA, trabecular bone parameters (including BMF), cortical porosity and CSA. To ensure consistency of dietary intake for protein, sodium, calcium, and phosphorous, team dietitian will contact participants once a month to advise on diet. Evaluation of participants will involve

cardiovascular assessments (EKG), heart and carotid auscultations, assessment of jugular venous pulse, peripheral pulses to identify any significant factors that may impact cardiovascular health, respiratory assessment (inspection, palpation, percussion, lungs auscultation, pulse oximetry and respiratory rate), and assessment of pain. Blood analysis will include: BALP, P1NP, CTX, 25-(OH)D, PTH, complete renal panel, calcium, magnesium and Hemoglobin A1c. While previous work from our institution used urinary NTX, the current standard for bone resorption test is considered to be serum CTX[48]. These markers will be obtained fasting at baseline, 4.5 months and 9 months; and would require no more than 10ml of blood sample (each time) from study participants.

Vit D: Each participant will be prescribed a 30-day supply of oral 2000 IU of Cholecalciferol (Vit D3), daily. The Endocrine Society guidelines recommend treating Vit D deficiency with Vit D3 to achieve a target 25(OH)D goal of 30 ng/mL[52]. Vit D deficiency is defined as 25(OH)D <20 ng/mL and insufficient as 25(OH)D 21 ng/mL to 29 ng/mL[53]. Participants with insufficient 25(OH)D3 will receive Vit D3 supplementation for 2wks as follows: Vit D < 10: 5000 units daily for 2 weeks; Vit D 11-20: 2000 units daily for 2 weeks; Vit D 21-40: 1000 units daily for 2 weeks. All participants will then receive 2000 units of Vit D for the rest of the study period. We chose to use 2000 IU of daily Vit D3 supplementation because prior studies have reported physiologic levels of 25(OH)D with this dose. Participants will be instructed to take their Vit D3 at about the same time every day. The prescription will be renewed every 25 days to allow for early mailing to participants. To monitor compliance, study participants will be called every 30 days for pill counts. Pre-treatment levels of 25(OH)D, will be measured at commencement of treatment and post treatment at 4.5 months and 9 months; participants with Vit D insufficiency will receive higher doses of Vit D3 supplement for 2 weeks prior to intervention. The values obtained will be entered in a spread sheet.

Resistance training (RT): Participants and caregivers will be trained on RT (application of electrodes, initiating and adjusting currents and the application of ankle weights). Resistant Training will be performed twice weekly **in an open-chain format (resistance is placed at the distal part of an extremity in a fixed position) in the first 4.5 mos, followed by 4.5 mos in a closed-chain format using surface NMES**. Initial open-chain format ensures muscle hypertrophy that is likely to cause modest bone adaptations[54], prior to it being subjected to significant loading during closed-chain format. This would ensure bone safety during the close kinematic exercise. No ankle weights in the first week to ensure full knee extension against gravity. Weekly increments of 2 lbs. will be used on the condition that full knee extension is achieved before any increase in load. NMES of the extensor muscles of the knee, for concentric-eccentric actions will be by surface electrodes as shown in ***fig 1.*** Two 8 X10 cm^2 (Uni-Patch, Wabasha, MI, USA) adhesive carbon electrodes will be placed on the skin over the knee extensor muscle group. Current from the stimulator will be increased at 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. Training will be performed remotely, twice weekly for 4 sets of 10 repetitions under full supervision from the PI **by telehealth**. Each RT session will consist of 4 sets of 10 repetitions of NMES-induced knee extensions for 60 min. A 5 sec/5 sec work/rest ratio will be used with 3 min rests between sets, 30 Hz, 1 ms pulses and current

sufficient to evoke full knee extension. To perform a closed kinematic chain exercise, participants will use a commercially available rowing machine (Murtisol Magnetic Rowing machine) to perform 4 sets x 10 reps twice weekly. NMES rowing has been shown to provide compressive loading on both distal femur and proximal tibia, further enhancing trabecular bone health in persons with chronic SCI. Participants will transfer to the rowing machine with assistance by the caregiver or significant other, using a sliding board or a portable lift if available at home. Most Veterans have a portable lift in their homes as part of their clinical care. Additionally, persons with paraplegia are trained to manage fall risks and get back to their manual wheelchair with minimal assistance from caregiver. This will provide them with an opportunity to transfer to and from their manual wheelchairs and rowing machine. Surface NMES will be applied to stimulate knee extensors for extension, alternating with the hamstrings for flexion, assisted by the upper extremities, with progression under full supervision by the trained caregiver. Electrodes will be placed bilaterally on both knee extensors and flexors and the current will be alternated 5 sec on/10 sec off on both muscle groups with frequency set at 30 Hz, pulse duration at 1 ms and reasonable amplitude to cause flexion/extension. This approach was previously adopted for our lab.

Passive range of motion (ROM) or passive stretching for the control group:
Participants and caregiver will be trained on passive ROM. A trained caregiver sits on a stool and cups participant's leg proximal to the ankle joint and move it from 90-degrees of knee flexion to full knee extension. The leg will be maintained in extension for 5 sec and returned to flexion for 5 sec. The passive actions is repeated in the manner described in the RT protocol; 10 reps on the right leg followed by 10 reps on left leg for a total of 4 sets x 10 reps. This will help to balance the design between the two groups and training will be conducted twice weekly.

Home Video Telehealth Service: Home exercise training will be conducted using Video Telehealth services, (known as, VA Video Connect (VVC)), under guidance from the Office of Connected Care with management and oversight from the VISN Telehealth Manager and Facility Telehealth Coordinator. The telehealth Program is located in the SCI&D service. The applicant is versed in the use of Telehealth for patient care as this has been the primary mode of patient evaluation and treatment during this COVID-19 pandemic. After obtaining participant's consent, the PI, together with the Telehealth Coordinator, will conduct a selection-consideration review for all participants, to ensure they agree to VVC for training, review clinical "video into home" worksheet and availability of appropriate technology. Participants without appropriate technology will be issued a tablet with web camera and internet access and assisted to create an e-mail account if they do not already have one. All participants who agree to provide a private, conducive environment for in-home training at addresses accessible to EMS, in emergency situations, will be enrolled. At the start of a session, the PI will obtain the address and telephone numbers of participants for use in emergency situations during VVC. Should the need arise, the PI can contact the local medical/telehealth emergency resources for back-up or contact the National Study Participant's Crisis Line for additional help. Before starting the exercise training session, the microphone, speakers and web camera are tested for efficacy in the home for both study participant and PI. The PI will ensure the availability of a telephone backup in the event of a communication failure via VVC. Participants will be advised to be ready 20-30 min prior

to the VVC by placing electrodes, ensuring the batteries of the stimulator are charged overnight, connecting the electrodes to the stimulator and positioning the ankle weights. Once the setup is completed and internet connection established, the participant is instructed to increase the current to bring the leg into full knee extension and then report the current in milliamps (mA) to the PI. The goal is for each participant to perform 4 sets of ten repetitions. Each exercise training session will be conducted for at least 30 min with 2-3 min of rest periods between each set and 5 sec between each repetition. At the end of each session, the PI confirms the next appointment, reminds participant to confirm next appointment online 24 hours before, and access their log in password. Our telehealth platform is well established with strong support from our IT department. It is unlikely to fail but should it fail, participant will be rescheduled for the next day.

Measurements: Specific aim 1. MRI: MRI with no contrast will be performed using a 3 Tesla magnet (GE). The skeletal muscle CSAs will be determined at baseline and 9 months, respectively. Both lower limbs will be strapped together using soft Thera-band to avoid any movement inside the magnet. Participants will be instructed to lie still inside the magnet and they will be provided with earplugs to protect their ears against the magnet noise. Duration of the whole scan including preparation time should not exceed 10 min. Images of both thighs will be obtained with the following scanning parameters (repetition time, 500; echo time, 14; field of view, 20cm; matrix, 256×256). Transaxial images, 8 mm thick and 4 mm apart, will be taken from the hip joint to the knee joint using a localized coil. A specific knee coil will be used to capture trabecular bone parameters of the distal femur and proximal tibia. Images will be downloaded and analyzed using X-vessel software for muscle/cortical bone CSAs, as previously conducted by our group.

Trabecular Bone Assessment: 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. A bilateral phased array coil (USA Instruments) will be used to obtain 30 contiguous1mm 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.

Assessment of Trabecular Microarchitecture: Within manually drawn regions of interest with the distal femur and proximal tibia trabecular bone, we will apply digital topological analysis and volumetric topological analysis to compute metrics of bone microarchitecture including trabecular thickness, trabecular separation, trabecular plate width, and network area density (1/mm) as in prior work, as well as BMF. In digital topological analysis, local connectivity and topological metrics are used to characterize different topological structures in a skeletal surface representation of a three-dimensional trabecular bone network. Volumetric topological analysis extends digital topological analysis and, through fuzzy skeletonization and unique manifold distance analysis approaches, will allow assessment of trabeculae on a continuum of plates to rods, permitting computation of individual trabecular plate width.

Dual energy x-ray absorptiometry (iDXA): iDXA will be used to measure bone composition including regional and total *Fat Mass (FM), Fat Free Mass (FFM*), cortical porosity, and BMD. Total body and regional scans will be performed using an iDXA scanner (Lunar Inc., Madison, WI) bone densitometer to determine regional BMD and total body T-score, Total hip BMD and knee BMD scores (Total body T-score less than -2.5. Total hip BMD T-scores < -3.5 and knee BMD scores of less than 0.6 g/cm^2, will be excluded). Testing is performed after lower extremity elevation for at least 20 min to minimize fluid shift. Scanning and analysis is certified by DXA operator. The participant is assisted to lie on a padded table with both legs strapped proximal to the knees and ankles. The arms and legs are properly aligned to allow participant to lie still for 10 min during the scan. Total and regional FM and FFM are determined with DXA software in order to control for confounding variables; fat mass and BMF may influence bone health[55]. The coefficient of variability of two repeated scans is < 3%.

Specific aim 2: We will assess bone remodeling with the following bone biomarkers: *BALP, P1NP and CTX,* 25(OH)D, PTH, renal panel, magnesium and calcium levels at baseline, 4.5 mos and 9 mos. HgbA1c will be obtained prior to and post intervention to determine glucose uptake benefit resulting from the intervention. Blood samples not exceeding 10 mls will be obtained after 8-10 hours overnight fasting. Samples will be processed at CVHCS Clinical Pathology Laboratory or Quest diagnostic depending on negotiated cost. All tests will be performed at the VAMC and participants transportation to and from test will be sponsored.

Pilot exploratory work for Both Groups

We will investigate the effects both groups on Toll-like receptors (TLRs), and Natural killer (NK) on T cell functionality (CD4+ T cells), cytokines, interferons, or chemokines, including IL-6, IL-10, IFN-α, IFN-γ and IP-10 in persons with chronic SCI. Peripheral Blood Mononuclear Cells (PBMCs) will be collected from all participants at three time points and isolated by Ficoll gradient centrifugation for immune analyses. For immune alternation of myeloid cells, the phagocytic and bactericidal function of circulating neutrophils in response to Staphylococcus aureus will be analyzed as described previously. Impairment in recognition of pathogen-associated molecular patterns by toll-like receptors (TLRs) and subsequently declining immune responses have been associated with immune dysfunction, PBMCs will be incubated with various ligands for TLRs, followed by assessment of production of cytokines, interferons, or chemokines, including IL-6, IL-10, IFN-α, IFN-γ and IP-10. To assess T cell functionality, PBMCs will be cultured in the presence of phorbol 12-myristate 13-acetat plus ionomycin and subjected to assays of IFN production by intracellular staining and flow cytometry. Natural killer (NK) cells represent the main components of lymphocyte-mediated innate immunity and play an indispensable role in combating bacterial and viral infections. These cells have been reported to be associated with SCI-induced immune deficiency. Expression of CD107a, the marker of NK cell cytotoxicity, as well as production of effector cytokines TNF-α and IFN-γ by NK cells will be examined. NK cell cytotoxicity will also be assayed using the target cell line K562 on the basis of lactate dehydrogenase release assay as described. Additionally, plasma collected from patients will be analyzed for immune-related cytokines including IL-10, which has been

identified to be a common candidate for immune suppression induced by stress and injury, including injury in the central nervous system. Samples will be analyzed at the VCU Immunology Department.

Pitfalls and Alternatives*:* Changes in trabecular bone parameters may be indeterminate because of the smallness and heterogeneity of sample size. We will rely on previous experience to calculate the effect size, and on that basis, adequately power future trials. **Failure to recruit proposed sample size**: The applicant's primary mentor (Dr. Gorgey) successfully conducted similar clinical trials in the past and demonstrated that use of telehealth facilitated recruitment and compliance among Veterans with SCI. Additionally, we may recruit non-Veterans to ensure completion of the study within the proposed timeline. Dr. Gorgey has a dual appointment with VCU and can successfully collaborate with VCU to ensure recruitment of the target sample size.

Statistical Analyses. After signing consent, the PI will randomly assign a number from 1-20 to participants using a computer program (nQuery). The order of enrollment will determine the participant's number based on level and duration of injury. Summary of information, including means and SD, frequencies and percentages, for all study variables will be calculated at all-time points. T-tests and Pearson chi-square tests will be used to compare characteristics that may act as confounders, including age, level and time since injury.

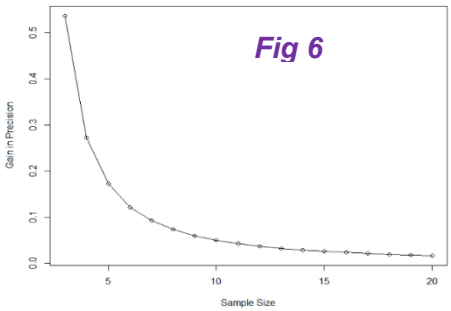


Fig 6

Sample Size Justification: Being a pilot study, consideration of sample size is just to allow determination and examination of preliminary evidence of efficacy and effect size estimation towards a larger future randomized trial. The proposed sample size of 10 per group is adjudged sufficient to accomplish these goals. Although Julious recommends a sample size of at least 12 per group on the basis that gain in precision for estimates of variance becomes relatively small on reaching a sample size of 12,[56] we are unable to meet that target because of cost of the intervention and testing. Our sample size still allows for adequate accuracy. As shown in ***Fig 6***, the gain in precision is still limited with a sample size of 10 per group. Although testing efficacy is not the primary goal, we will have 80% power to detect a standardized mean difference of d=0.9 using an alpha of 0.25 corresponding to a large effect size. Type I error rates larger than 0.05 are often used and recommended for pilot studies where tests of efficacy are not the primary aim.

Data Analysis Plan: IBM SPSS statistical software version 26 will be used. Being a pilot study, primary analyses will be mainly descriptive. Means and SD will be estimated for utilization (number of sessions completed), with each outcome. Finally, analysis of covariance (ANCOVA) will be used to test differences between groups post intervention for each outcome, using the baseline score as a covariate. If normality of groups appears to be violated in the sample normalizing transformations or non-parametric statistics will be considered.

Human Subject Section:

I. Human Subjects Involvement and Characteristics:

Twenty (20) individuals with chronic (>1-year post-injury), motor complete Spinal Cord Injury (SCI) with American Spinal Injury Association Impairment Scale (AIS) score A or B will be recruited to participate in the proposed Telehealth study. Participants will be recruited from the Central Virginia VA Health Care System (CVVHCS) Spinal Cord Injury & Disorders (SCI&D) registry. The SCI&D registry has approximately 1,800 Veterans (men and women), with more than 298 of the Veterans meeting the criteria for neurological level of injury (NLI) of C8-T10 and AIS A or B, which is our target study population. The SCI population is however, predominantly male based on natural disposition, since men tend to join the military than do women.

The SCI service at CVVHCS offers inpatient and outpatient services. The inpatient service provides acute rehabilitation services for Veterans with new SCI, annual evaluation for those with motor complete injury who are unable to use outpatient level of care, Veterans with pressure ulcers that are not responding to outpatient level of care and those requiring respite care to relieve caregivers of care burden. Veterans needing minor surgical procedures are also admitted for short inpatient stay, for example, for cystoscopy or colonoscopy procedures. In 2019 fiscal year, there were a total of 1022 Veterans, ages 18 to 70 years, within the Richmond catchment area and 298 reside in Richmond. Over 800 were admitted to SCI inpatient service for various reasons and over 600 Veterans used outpatient level of care. The tables below show some of the common reasons for inpatient and outpatient care, by number of Veterans, for fiscal year 2019:

Common reasons for Inpatient Admission for fiscal year 2019:

Reasons for Admission	Number of Veterans
Acute Rehabilitation	~87
Annual Evaluation (chronic SCI)	~368
Wound Care	~269
Respite Care	~154

Common reasons for outpatient visits in fiscal year 2019:

Reasons for Visit	Number of Veterans
Annual or new evaluation (chronic SCI)	472

The recruitment for the proposed study will be drawn from the SCI population within the Richmond catchment area (this includes Veterans who live outside Richmond but use CVVHCS for their SCI yearly evaluation). After informed consent, each participant will undergo a complete physical examination to be done by the PI or a board-certified Physiatrist. The physical examination will include:

1. General: Race, gender, mood
2. Vital signs:

a. Temperature
b. Pulse
c. Respiration rate
d. Pulse oximetry
e. Blood Pressure
f. Pain level
g. weight

3. Skin inspection for:
    a. Rashes
    b. Eruptions
    c. Pressure sores
4. Head, Eyes, Ears, Nose, and Throat (HEENT)
    a. Head-size and shape; tenderness
    b. Eyes-conjunctiva; sclera, pupil size, shape and reactivity; extraocular muscle movements; visual fields
    c. Ears-hearing test, tenderness, discharge, external canal, tympanic membrane.
    d. Nose-symmetry; palpation of sinuses for tenderness; flaring; grunting
    e. Throat-lips, teeth, gums, tongue, pharynx
5. Neck:
    a. Range of motion; tenderness, jugular vein distension, lymph nodes, thyroid, carotid bruits, Hepatojugular reflux
6. Chest:
    a. Symmetry of movement with respiration
    b. Palpation for tenderness
    c. Breath sounds
7. Heart:
    a. Rate
    b. Palpation of precordium for point of maximal impulse and thrill
    c. Auscultation for heart murmurs
8. Abdomen:
    a. Shape
    b. Auscultation for bowel sounds and bruits
    c. Percussion for tympanic masses
    d. Palpate for tenderness/guarding; organomegaly
9. Genitalia:
    a. Inspect for lesions, swelling, masses, hernias
10. Rectal:
    a. Inspect/palpate for hemorrhoids, fissures, tags, sphincter tone
    b. Note presence or absence of stool
    c. Male- prostate size and texture, nodularity, tenderness
11. Musculoskeletal:
    a. Range of motion
    b. Absence of limbs/deformities
    c. Swelling
    d. tenderness

12. Neurological assessment:
    a. Mental status
    b. Cranial nerves
    c. motor strength and sensory level based on the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI), to determine level of motor and sensory impairment.
    d. Reflexes
    e. Modified Ashworth scale (measure of spasticity)
    f. Penn Spasm Frequency Scale (self-reported measure of perceived frequency and severity of spasticity).

Eligibility to participate will be based on physical examination findings. Body weight will be measurement using a wheelchair weighing scale and subtracting the wheelchair weight from the total weight to obtain participants' absolute weight. A baseline electrocardiogram (ECG) to rule out any concerning abnormal heart rhythm or myocardial ischemia will be obtained. All participants will also undergo bone mineral density (BMD) measurements using Dual-energy X-ray absorptiometry (**DXA)** focusing on the distal femur and proximal tibia and MRI using a 3 Tesla magnet (GE) to determine skeletal muscle and cortical bone CSAs (specific Aim 1) and fasting blood work (specific Aim 2), at no cost.

Inclusion criteria. To be included, participants will have to:

1. Be 18-65 years of age
2. Have history of traumatic SCI (≥1-year) with NLI of C8-T10 and AIS A or B (confirmed by AIS examination performed by PI or a qualified Provider.
3. Have a caregiver who is available and willing to be trained to apply intervention protocol in the home (placing weights and positioning the Veteran) throughout study duration
4. Be a wheelchair user for primary mode of mobility
5. Have Knee extensors that must respond to standard surface electrical stimulation procedures (stimulation frequency procedures, 30 Hz; pulse duration:1 ms and amplitude of the current of less than200 mA)).
6. Be able to receive written clearance from their medical Providers to ensure safety of participants.
7. **Be a Veteran (male or female),** however, the Department of Veteran Affairs has limited number of female Veterans, especially those with motor complete injury at level C8-T10 (currently, there are only 3 female Veterans with the target NLI in our SCI registry). Hence, inclusion of women Veterans may be challenging due to this disparity in the SCI population. If we fail to recruit 20 Veterans, we may seek an IRB approval to recruit non-Veterans to meet our target sample size.
8. Have normal ECG
9. Commit to undergo 9 months of trial; 4.5 months of open-kinematic chain resistance training followed by 4.5 months of closed-kinematic chain using simple rowing approach + Vit. D supplementation (Experimental group) **or** 9 months of passive movement +Vit D supplementation (control group).

Rationale for conducting the trial up to 9 months:
The preliminary study of Veterans with motor complete injury demonstrated that 16 weeks (i.e. 4 months) of surface Neuromuscular electrical stimulation (NMES) evoked resistant training (RT) impacted skeletal muscle size positively, which will convey skeletal loading benefits to the bones. Additionally, the increase in muscle mass has the potential to increase peripheral blood flow and increase blood glucose uptake that may delay the onset of Type 2 diabetes or/and improve diabetes control for those who are already diabetic. However, the 16 weeks study was only exploratory and bone adaptation are likely to take longer time period, especially in persons with SCI. The primary rationale was that the duration of previous work that effectively used either electrical stimulation or Vit D ranged from 6 to 12 months. Since we are unaware of any study that has determined the combined effects of both interventions, we chose to average 6 and 12 months to arrive at a 9 months trial. We believe that compared to the initial exploratory trial for 4 months, the proposed 9 months trial will set the basis for future clinical trials that are adequately powered to enhance bone parameters in persons with chronic SCI.

Rationale for recruitment outside Richmond and non-Veterans:
The target population for this study is Veteran with SCI but are no different than SCI individuals in the general population. Therefore, in the event, that we are unable to meet the target sample size within study period, we may reach out to Virginia Commonwealth University (VCU), as previously discussed.

Inclusion of women Veterans:
There is inherently gender disparity in the Veteran population based on the fact that more men than women tend to join the military. However, the study team will make every effort to recruit female Veterans but may be limited due to scarcity of female Veterans with SCI.

Exclusion criteria: Every effort will be made to ensure participants' safety and any participant with active medical condition that may confound the result of the trial will be excluded from participation.
Potential participants will be excluded if they exhibit any of the following:

1. Neurological injury other than SCI
2. Older than 65 years of age as they may likely have considerable amount of bone loss at that age.
3. Have severe osteoporosis because loading porous/fragile bone by electrical stimulation may result in bone fracture.
4. Those classified as AIS C & D, as they may already be engaging in weight bearing activities that may confound the results of this trial.
5. Unhealed or unstable fractures in either lower or upper extremities.
6. Severe scoliosis, deformities in the hip, knee, or ankles OR impaired range-of-motion, as these could be a barrier to safe positioning on the rowing machine, and on MRI or DXA tables.
7. No caregiver or family member/significant other, willing to help with placing weights and positioning participants' lower extremities on the rowing machine.

8. Untreated or uncontrolled hypertension (systolic blood pressure (BP) > 140 mmHg; diastolic BP > 90 mmHg), and/or sudden hypotension upon transferring from bed to wheelchair, characterized by a drop in BP by 20 mmHg (especially in persons with tetraplegia) or heart rate > 100 beats per minute.
9. Anti-coagulation or anti-platelet therapy (including aspirin)
10. Implanted pacemakers, implanted defibrillator devices or any metallic implants including knee or hip implants.
11. Presence of bullets in vertebral column or shrapnel anywhere in the body that may interfere with MRI procedure.
12. Other medical conditions including cardiovascular disease, uncontrolled type II DM, active deep vein thrombosis (DVT), uncontrolled autonomic dysreflexia, use of insulin for DM management, pressures injuries of stage 3 or higher, or active urinary tract infection.
13. Severe hypercalcemia (serum calcium > 16mg/dl), stage III-V kidney disease, post-menopausal or estrogen dependent female, and men undergoing anti-androgen therapy or are post orchiectomy.
14. DXA total body T-score less than -2.5. Total hip BMD T-scores < -3.5 and knee BMD scores of less than 0.6 g/cm^2.
15. Untreatable severe spasticity bearing on potential participants' activities of daily living, such as transfers from bed to wheelchair or maintaining position in wheelchair.
16. Any psychiatric illness confounding judgment or cognitive impairment in participant or caregiver who is expected to help participant in the trial.
17. Those with prosthetic lower limbs
18. Any condition that, in the judgment of the PI or other medical Providers, preclude safe participation in the study and/or has the potential to expose/increase participant's risk of infection.
19. Unable to tolerate increasing either electrical stimulation current or weights to the lower legs for any reason.

Sources of Materials:

Electronic medical records (EMR) will be reviewed for each potential participant enrolled by IRB-approved investigators (PI or Co-PI; Dr. Ifon and Dr. Gorgey, respectively). All research data will be stored in the PI's office in a locked cabinet. No VA research data will be destroyed.

II. Study Procedures:

The study procedures include: consenting process, description of study activities and expectations. Consent to participate will be obtained as described under process of obtaining informed consent. Informed consent will be obtained prior to enrollment in the study. After consenting, the participant will undergo 1-hour of history and physical examination that will be done by the PI or a certified SCI physician (Dr. Timothy Lavis) at CVVHCS. The screening examination will include history and physical examination and assessment of inclusion/exclusion criteria previously stated.

Detailed Strategies for the Recruitment Plan.

Efforts will be made by the applicant (PI), to implement a successful recruitment plan, and to avoid any source of coercion during the actual process. Twenty Veterans (10 in

intervention group vs. 10 in control group) with motor complete SCI involving C8-T10 and AIS A or B classification, will be recruited to participate in a 9 months trial. The primary goal of this work is to ensure that our procedures are safe and effective for persons with motor complete SCI.

The following timeline will be implemented to meet our recruitment goals. We plan to recruit 20 Veterans/non-Veterans over a 2-year period (24 months). We plan to start participants enrollment in the 1st quarter of year-1 and finish in 1st quarter of year-2 (see recruitment table below). We will continue to collect data and finish analysis by the 4th quarter of year two. This is feasible considering our previous experience recruiting for the parent study. Also, we do not anticipate attrition due to prior established compliance with telehealth home-based study. We plan to offer resistant training to participants on a flexible schedule so as to accommodate their lifestyle, to avoid attrition. Participants will be randomized to either intervention or control group.

Recruitment Table:

Research Activities Year	**2021**									**2022**												**2023**					
Months	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
IRB process	x	x	x																								
Purchase of Equipment & Supplies				x	x																						
Dissemination of recruitment flyers				x	x	x	x	x	x	x	x	x	x	x	x	x											
Participants enrollment (from start to finish)						2	2	2	2	2	2	2	2	2	2												

															2 →											
Data Collection						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Progress Report																										
Data entry/coding/cleaning						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Data analysis and interpretation														x	x	x	x	x	x	x	x	x	x	x	x	x

Feasibility of recruitment:

There is an emphasis on feasibility of the trial recruitment to ensure we reach our target sample size, especially considering the 9-months commitment requirement for each participant. Below is a step-by-step description of our recruitment strategies to demonstrate feasibility:

- Prior to submission of the current application, 3 participants were already identified and were willing to participate in our preliminary work.
- We will contact every SCI physician, nurse and rehabilitation staff (including those at the Spokes sites) about our ongoing protocol through our weekly clinical rounds, grand rounds, and friendly e-mail reminders.
- We will send an email blast through our secured network to our 13 Spokes sites and encourage them to refer potential participants to our research team.
- We will send electronic flyers and the PI will present at our SCI monthly meetings, PVA monthly meeting, SCI quarterly newsletters, hub and spoke annual conferences, and SCI national Chief teleconference meetings.
- Our SCI&D service has a designated wheelchair van that we have been using on a regular basis for transportation of our participants back and forth during the course of clinical trials, assuming they reside in the Richmond area. This accessible transportation has encouraged several participants to be enrolled in previous VA trials and we plan to use this method too to encourage participation in the proposed trial; they will only need to come to the center at the beginning of the trial, mid-term and at the end of the trial for imaging and blood work.
-

Process for obtaining informed consent:

The PI will seek informed consent from eligible participants with an IRB approved consent form. Determination of a participant's capacity to consent will be made by the PI. Input may be solicited from participant's SCI Provider on record, who knows the potential participant well. Potential participants will be given full verbal explanation of the study protocol by the PI. If they voice an interest in the study, they will receive the written consent form and will be provided ample time to consider their participation. All concerns and questions will be answered by the PI. The PI will place emphasis on explaining all study procedures. Family members will be included in the discussion as desired by the participants. Participants will be made aware that participation is voluntary and that they can withdraw at any time with no impact on the care they currently receive or future care from the VA. Participants will be made aware that they can discuss the study participation with their primary SCI Provider, if they chose. Potential participants who take the consent forms home will be given a returned addressed envelope with postage paid in which they can mail back the consent form once signed. All signed consents will be scanned into the VA Computerized Patient Record System (CPRS); a warning note indicating participation in an ongoing research study will be enter in the "Crisis Notes, Warning Notes, Directives (CWD)," section of CPRS to alert CPRS users of participants' enrollment in an ongoing study. In addition to a consent note entered into CPRS for all participants, progress notes of their active participation will also be entered.

Limitations and Alternatives:

1) Those with incomplete SCI (AIS C or D) as they may already be engaging in some weight bearing activities that convey some benefits of bone health, hence may confound the results of the study.
2) Those with marked osteopenia and severe osteoporosis as electrical stimulation and loading to already weakened limbs may increase the risk of sustaining stress fractures.
3) It may be difficult to recruit female Veterans because of limited access as they are fewer in number compared to male Veterans.
4) Ability to recruit and retain the required sample size from current SCI & D registry. However, since the primary mentor had previously recruited and retained almost half of the proposed sample size in the parent study, we anticipate similar success rate.
5) Frequent blood sampling; this procedure has been previously used in IRB approved funded studies without recorded incidence.
6) concerns about **retaining participants** to finish the 9 months study; transportation could be a major impairment to participants' compliance in the SCI population. Fortunately, the study involves limited travel since we are going to use Telehealth (a home-based) approach for the intervention and participants would only be required to travel at enrollment, mid-term and at the conclusion for testing procedures at no financial cost to them. Participants affiliated with the Spokes site can complete these tests at their local VAMC.

Risk to Subjects.

Every effort will be made to minimize potential harm to individuals during the study. There will strict adherence to the protocol's inclusion/exclusion criteria. However, the following may pose potential risk to study participants:

Potential source of risk	Potential Risk	Possibility of occurring
Pressure to skin from weights or skin irritation from weights or during exercise	Pressure injury (break to skin)	Occasionally but no more than usual from daily activities
DXA/MRI	No significant risk This research study will involve exposure to radiation from 2 whole body DEXA scans and MRI with no contrast. This radiation exposure is not necessary for medical care and is for research purposes only. All radiation exposure increases the risk of developing cancer in the future. The total amount of radiation that would be used in this study is equal to less than one day of exposure from natural background radiation. Permission would be obtained from the CVVHCS Radiation Safety Committee, who will review the use of radiation in this research study, involving minimal risk and necessary to obtain the desired research information. Participants will be instructed to tell their Providers if they have participated in other research studies or received any other medical care involving radiation in the recent past.	Unlikely
Phlebotomy	Phlebotomy can cause pain, bruising at the site of the venipuncture, nerve damage, hematoma or infection	Rare
Electrical stimulator/weights (Risk of fracture)	SCI is commonly associated with bone weakness and weight bearing on the participant's limbs while exercising on the rowing machine may increase risk of fracture to already weakened bones.	Rare

Autonomic Dysreflexia (AD)	Weights and exercise to limbs of participants may pose as noxious stimuli that may lead to symptoms of AD (headache, pounding in the head, ringing in the ears, dizziness, blurry vision, sweating, anxiety, flushing, tightness in the chest, stuffy nose, heart flutters, or difficulty breathing), especially in those with cervical level of injury and high thoracic level of injury (T6 and above).	Rare
Falls	Falls could occur during training or transfer unto testing equipment (MRI/DXA table or in the home during transfer to/from wheelchair unto rowing machine.	Rare
Risk of Fracture	SCI is commonly accompanied by bone weakness and weight bearing on the participants' limbs may increase their risk of sustaining stress/low impact mechanical fracture.	
Medication (Vitamin D)	Vitamin D toxicity can lead to hypercalcemia and kidney stones. To reduce this risk participant's serum level of 25(OH)D will be monitored closely and re-assessed at mid-term and at end of intervention. Hypercalcemia is usually not seen unless the D level is > 100 ng/ml.	Rare
Rowing equipment	It is possible that the rowing machine may malfunction or cause stress injury to the weakened limbs	Rare
Implanted device malfunction due to use of MRI	Implanted metallic device may pose risk for using MRI device, for example, implanted stimulator or intrathecal pump device may malfunction following MRI exposure. These are reprogrammable if they occur.	Rare

Protection Against Risk:

Every effort will be made by the PI and the study team to minimize potential risks. Any procedures will be carried out in accordance with established standard operating procedures and necessary precautions will be taken to mitigate any potential risk. There will be strict adherence to the inclusion & exclusion criteria for the protocol. Appropriate

health screening and strict compliance with the established exclusion criteria will minimize attrition due to medical reasons. Participants will be closely monitored by the PI at all visits. All participants will be provided a 24-hour contact information for the PI and instructed to call for any concerns. Unscheduled visits may be performed if required for evaluation of safety. If a participant exhibits an adverse event, more frequent visits may be necessary.

- Protection of skin integrity during intervention

Skin will be closely inspected with the help of the caregivers for dose with caregivers, otherwise, by the PI via the webcam during each intervention session to ensure maintenance of skin integrity.

- Protection from risk associated with transfers

To minimize risk, a caregiver will be available to assist in all transfers and provide a sliding board or any other assistive devices needed for those without good upper body strengths. For those without caregivers, safe transfer techniques would have been validated prior to enrollment.

- Protection from risk associated with radiation exposure

To minimize risk, only the required scans will be performed. Testing will be performed early in the day and after lower extremity elevation for at least 30 minutes to minimize lower extremity edema. An overhead lift system is installed in the exercise lab to ensure safe transfers on and off the DXA scanner.

A possible health problem seen with radiation exposure is the development of cancer later in life. This extra cancer risk is higher at younger ages and for girls and women. The extra lifetime risk of dying of a fatal cancer due to the radiation exposure from this research is very low. At such low radiation exposures, scientists disagree about the amount of risk. These estimates are very uncertain, and there may be no extra risk at all.

- Protection from falls during transfers

Falls could occur during transfers with sliding board or portable lift from bed to wheelchair and from wheelchair unto intervention equipment (rowing machine). This requires careful use of equipment. To avoid this kind of accident, transfer techniques will be reviewed with participants and caregivers (where applicable) at the beginning of the study. However, this rarely occurs after a Veteran has successfully completed rehabilitation treatment post injury.

- Protection against risk of fracture

Every effort will be taken to minimize possible bone fracture; DXA scan will be performed as detailed earlier prior to enrollment in the trial. Persons with T-score less than -2.5 SD at the hip joints or with bone mineral density less than 0.6 gm/cm^2 at the knee joints will be excluded from the trial.

- Protection from autonomic dysreflexia

Blood pressure (BP) will be monitored closely during the entire intervention and established AD protocol followed should participant experience AD symptoms (patients and caregivers usually receive training on AD management at home, during their rehabilitation course). Any significant rise in BP greater than 20 mmHg above resting BP, the intervention will be suspended immediately and AD protocol instituted. The PI will remain on video connection until AD symptoms resolved and participant returns to normal state.

- Protection against medications adverse effects

Medication associated with the current trial can cause allergic reactions, such as, rashes, nausea, vomiting, and hypercalcemia. The PI will obtain allergy history (prior reactions) and monitor the patient and blood parameters closely during the course of the trial to avert any serious drug reaction.

Study removal criteria may include: Participants may be withdrawn from the study by the research team for the following concerns:

1. New medical diagnosis of carcinoma of the breast or prostate as these would may have bone health implications. Deep venous thromboembolism as this will hinder intervention and may increase risk of pulmonary embolism; stroke, hypertension, serious heart problems, heart failure, myocardial infarction, ventricular arrhythmia, exertional chest pain, insulin dependent diabetes, hypercholesterolemia, hypertriglyceridemia, impaired liver function, hypercalcemia, or significant renal dysfunction.
2. Hospitalization for acute medical problems (e.g. Liver enzymes (AST / ALT) >1.5 times normal upper limit).
3. Serum calcium greater than 16mg/dL or symptoms of hypercalcemia
4. Severe peripheral edema, classified as 2+ or higher
5. Any SAE that the SCI physician, Dr. Lavis, deems necessary for withdrawal for participant's safety.

Protection against possible participants' coercion:

The $300 paid to each participant is to compensate for time and inconveniences associate with the commitment to participate in a 9 months trial and would be paid in 2 installments ($150 will be disbursed at the end of the 4.5 months testing and the end of the study, respectively) to protect against coercion. Furthermore, the PI will work closely with the IRB to avoid any coercive influence on any of our study participants.

Adverse Events:

All participants will be monitored for any changes in health status from baseline. The SCI Provider on record for each participant will be notified of any changes that may require attention. An adverse event is any experience that has taken place during research project, which, in the opinion of the investigators, was harmful to the research participant, 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 period via physical examinations, vital signs, laboratory tests, review of medical charts, and verbalized concerns by the participants, caregivers, significant other or family member. Any adverse event will be documented in each participant's file. Files will be reviewed for adverse events, protocol violations, and reasons for dropouts/withdrawals every ten days. The PI will provide CVVHCS IRB an annual summary of adverse events. All serious adverse events (SAEs), such as those that are life-threatening or involve hospitalization, will be promptly reported to CVVHC IRB Research Subjects Protection Program. Serious adverse event is defined as the unexpected medical occurrence associated with the research procedure that may lead to death, life-threatening medical condition, inpatient hospitalization for or longer than 24 hours, persistent or significant incapacity or substantial disruption of the ability to engage in normal activities of daily living.

During the study period and at the completion of the study, all participants will continue to receive their usual health care from their assigned SCI Provider and/or other Providers within and outside VHA health care system. When study procedures begin, the importance of reporting any perceived problem, adverse event or change from baseline health will be stressed to the participants. Examples of potential problems requiring immediate medical attention or a call to the PI will be described to the participants. Above all, it will be stressed to the participants that direct phone access to the PI is available 24hrs/day, 7 days a week and in the event of any questions or concerns, they are to call the PI with the phone number that they were given. All participants will be identified by an assigned number. Participants' research charts will be kept in the PIs office which is locked inside a locked file cabinet. Only the PI and Co-PI will have access to participants' study records and medical information. A master sheet of the full names will be kept in the locked file in the PI's office. All completed study files will be stored in the mentor's SCI research office 1V-129. At the end of 4-year period, all records will either be stored in the SCI Research Exercise Laboratory locked room or sent to Dunmar Storage Facility based on space needs.

Subject's burden:

The current study ***was designed to reduce and minimize participants' burden*** including transportation and participation time. Although participation may impose a significant burden, every effort will be taken by the study team to reduce the stressors and burden associated with study participation over the study period. Each intervention session would last approximately 60 minutes. The current study is designed to allow participants to participate from the comfort of their homes using video telehealth technologies. This should provide some level of control over most SCI factors that may otherwise impact the outcome of the study, for example, stress associated with travel burden that could lead to attrition. Each participant will have the liberty to miss up to

15% of scheduled Telehealth visit, due to unanticipated events, such as, short duration of acute illness, medical appointments, or vacation, without being asked to withdraw or disenrolled from the study. We believe that we can retain participants through the duration of the study (9 months) by:

a) Providing free transportation for tests at the medical center (most Veterans already have this benefit).
b) Providing flexible hours to accommodate education and work-related schedules.
c) During data collection, we will provide sufficient time for participants to have their meals and use medications.
d) Using a comprehensive team approach that would allow participants to engage in recreational activities and avail themselves of Psychology (if desired) and Nutritional services.

Benefits:

Potential benefits of research to Veterans and others:

The proposed study is geared towards improving both bone and muscle strengths and will benefit the participants in increasing muscle blood flow, blood glucose uptake as well as decrease bone demineralization. The anticipated benefits of the current trial should be viewed in the light that the proposed work will serve as a pilot work for future protocols by providing supporting preliminary data to adequately power future clinical trials.

We hope findings from this work will provide supporting evidence to establish the resources necessary to **accelerate research and development of therapies for bone loss**

attenuation and improved functionality and quality of life for persons with chronic SCI. It is anticipated that this will reduce the costs associated with managing low impact fractures secondary to bone loss after SCI. The PI will make every effort to disseminate the knowledge gained from this trial through presentations at local, national and/or international scientific meetings. And also, by manuscript preparations and submission to high impact peer-reviewed journals for publications. The intervention in this study may directly benefit participants physiologically, aside from the financial compensation, by increasing muscle mass and improving blood flow. However, if there are no direct benefits to participants, the information gained, has the potential to help others in the future.

Importance of Knowledge to be gained

The prevalence of individuals with SCI has been estimated to be 250,000-400,000 with an estimated 18% growth in the prevalence since 1988. Of the more than 46,000 Veterans with SCI-related disability, more than 50% will develop osteoporosis in the first year of injury if the process is not mitigated. The incidence of osteoporosis in this population gets even higher and exceeds 80% with increased years since injury and predisposes persons with SCI to low impact mechanical fractures. In fact, 50% of

individuals with SCI will sustain low impact mechanical fractures during their lifetime. It is worrisome that the cost of managing osteoporosis-related fractures may cost about $25 billion by 2025. Fortunately, mechanical loading of paralyzed limbs has been shown to attenuate bone loss providing hopes for mitigation of bone loss after SCI, thereby slowing down progression to osteoporosis and associated complications. In addition, electrical stimulation has been shown to partly reduce the loss of bone mineral density in the femur when treatment was initiated early post SCI. Therefore, this study proposes to combine loading and electrical stimulation in addition to vitamin D supplementation, in an attempt to attenuate bone loss in persons with SCI. Considering the time, cost and potential associated-risks, we are proposing the use of both electrical stimulation and paralyzed limb loading strategy plus vitamin D supplementation to achieve bone attenuation. Assuming that our protocol is successful in attenuating bone loss, many of the 46,000 Veterans with SCI stand to benefit as does VHA in health care costs savings.

Clinical Trial Requirements: This is a drug trial and registration with Clinical Trials is mandated. We are aware of this requirement and are experienced in complying with all regulatory requirements.

Data and Safety Monitoring Plan.
The PI will monitor and review the protocol as well as data collection every month to evaluate participants' safety, data quality, and study progression and execution. The PI will review the protocol for any major concerns prior to implementation. The local data monitoring board will meet every 6 months to review/discuss the study. The data monitoring board will have the option to request stopping or cessation of participants' enrollment and all research procedure, if deemed necessary. It is unlikely that they would find significant improvement in collected bone parameters at mid-intervention (4.5 months) in the intervention group because bone requires a longer time for adaptation, especially in persons with SCI. However, should there be significant improvement (s) in the intervention group compared to the control group, the data monitoring team could terminate the study early. The PI will also assess the performance of overall study operations and any other relevant issues, as necessary. The Data and Safety Monitoring Board (DSMB) is responsible for overseeing the safety of the research and report observations/findings to the IRB on an annual basis. The data monitoring team will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. They may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps necessary to protect the safety and well-being of human participants until the IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB. A letter will be drafted from the DSMB and will be attached with the annual summary report to the IRB. Elements in the letter will include the followings:

- Evidence of study-related AEs

- Evidence of efficacy according to pre-established statistical guidelines, if appropriate;
- Data quality, completeness, and timeliness
- Performance of the specific research center
- Adequacy of compliance with goals for recruitment and retention
- Adherence to the protocol
- Factors that might affect the study outcome or compromise the confidentiality of the trial data (such as protocol violations)

Factors external to the study such as scientific or therapeutic developments that may impact participant safety or the ethics of the study.

The Data Safety Monitoring Board (DSMB) will include

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