Study Protocol and Statistical Analysis Plan

Study Title: Musculoskeletal Plasticity After Spinal Cord Injury

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Study Objectives

Patients with spinal cord injury (SCI) experience metabolic syndrome, diabetes, obesity, pressure ulcers, and cardiovascular disease at far greater rates than the general population. A rehabilitation method to prevent or reverse the systemic metabolic consequences of SCI is a pressing need.

The **purpose** of this study is to determine the dose of muscle activity that can enhance an oxidative muscle phenotype and improve clinical markers of metabolic health and bone turnover in patients with SCI. The **long-term goal** of this research is to develop exercise-based interventions to prevent secondary health conditions such as diabetes and to ultimately protect health-related quality of life (QOL).

Specific Aim 1: To compare changes in skeletal muscle gene regulation in individuals who receive high frequency (HF) active-resisted stance and low frequency (LF) active-resisted stance for 3 years.

> Hypothesis 1: The expression of genes regulating skeletal muscle metabolism will support that HF and LF both instigate a shift toward an oxidative muscle phenotype. A novel finding will be that LF is a powerful regulator of oxidative pathways in skeletal muscle.

Specific Aim 2: To compare changes in systemic markers of metabolic health and bone turnover in individuals with SCI who receive HF or LF for 3 years.

> Hypothesis 2: HF and LF will both reduce glucose/insulin levels and HOMA (homeostasis model assessment) score.

Secondary Aim: To measure subject-reported QOL using the EQ-5D survey metric.

> Hypothesis 3: HF and LF subjects will show a trend toward improved self-reported QOL after 3 years. There will be an association between metabolic improvement and improved perception of QOL. These observations will support that this intervention has strong feasibility for future clinical translation.

Design

Population: Individuals with motor-complete spinal cord injury (SCI) (AIS A – B), 21 to 60 years of age.

Exclusion Criteria: Pressure ulcers, chronic infection, lower extremity muscle contractures, deep vein thrombosis, bleeding disorder, recent limb fractures, any comorbid disease known to affect bone metabolism (such as parathyroid dysfunction), pregnancy, anti-osteoporosis medications, Vitamin D supplements, Metformin or other medications for diabetes

Enrollment: Non-random assignment

Study Arm 1: Acute gene regulation in response to single-session electrically induced exercise

Study Arm 2: Adaptations in gene regulation, metabolic markers, and subject-report metrics in response to up to 3 years of electrically induced exercise

Methods

Baseline Characteristics: Participants will provide information on age, sex, race, ethnicity, and level of SCI (quadriplegia, paraplegia)

Study Arm 1: Participants will undergo unilateral vastus lateralis skeletal muscle biopsy: they will be positioned in supine, the skin over the biopsy site will be sterilized, up to 3 mL of 1% lidocaine will be injected locally, and a $1/8^{th}$ inch incision will be made with a scalpel. The biopsy needle will then be inserted and four cores of muscle (~ 20 mg) will be obtained from four passes of the needle through the same puncture site. The incision site will be closed with steri-strips or a butterfly bandage and the subject will be told not to perform electrical muscle stimulation or wet the area for 48 hours. Muscle specimens will be immediately processed for microarray analysis. Participants will then undergo a single session of electrically-induced exercise to the quadriceps and hamstrings muscles of the non-biopsied limb. Stimulating electrodes will be placed over the quadriceps and hamstrings muscles, and electrical stimulation will be given to induce an isometric closed kinetic chain knee extension moment. Participants will rest for 3 hours and then the vastus lateralis biopsy procedure will be repeated on the limb that performed electrically-induced exercise.

Study Arm 2: Participants will complete a baseline assessment including the biopsy procedure described above, completion of the EQ-5D self-reported quality of life survey, and venipuncture. Venous blood will be collected into vacutainer tubes with anticoagulant for glucose, kept on ice in dark conditions. The blood will be centrifuged and analyzed with a blood auto-analyzer. A portion of the blood sample will be tested via enzyme-linked immunosorbent assay (ELISA). Insulin and glucose values will be entered into the homeostasis model assessment equation (HOMA) to yield a HOMA score. After two weeks, participants will begin electrically induced exercise training. They will perform electrically induced exercise as described above for up to 3 years. At the conclusion of training, participants will undergo repeat biopsy, EQ-5D survey, and venipuncture.

Outcome Measures: Microarray will be used to measure mRNA transcription levels for genes that regulate muscle atrophy (myostatin – MSTN), cellular adaptation to exercise (peroxisome proliferator-

activated receptor gamma coactivator alpha – PGC1-alpha), and muscle oxidative metabolism (pyruvate dehydrogenase kinase – PDK4, and succinate dehydrogenase-B – SDHB). Standard clinical assays will be used to measure levels of serum biomarkers for metabolism (fasting insulin, fasting glucose). Fasting insulin and fasting glucose will be used to calculate HOMA-IR, an index of insulin resistance. ELISA will be used to measure levels of osteocalcin, a biomarker of bone turnover. For the EQ-5D metric of health-related quality of life, self-reported numeric health state will be converted to Quality-Adjusted Life Years (QALY) using standardized tables.

Statistical Analysis Plan

Gene mRNA transcript levels will be expressed as Log_2 intensity (arbitrary units). Each blood biomarker will be expressed as its commonly-used clinical unit of measure (eg. mg/dL). EQ-5D health states will be expressed as QALY.

Change over time for each outcome measure will be assessed via repeated-measures one-way ANOVA. For study Arm 1, gene expression levels from the baseline biopsy will be contrasted to the 3 hour post-exercise biopsy. For study Arm 2, post-training values for all outcome measures will be contrasted to the baseline assessment point.