Study Protocol and Statistical Analysis Plan

Study Title: Long Duration Activity and Metabolic Control After Spinal Cord Injury

NCT03139344

Document Version: 11/3/2022

Study Objectives

Skeletal muscle is the largest endocrine organ in the body, playing an indispensable role in glucose homeostasis. Spinal cord injury (SCI) prevents skeletal muscle from carrying out this important function. Dysregulation of glucose metabolism precipitates high rates of metabolic syndrome, diabetes, and other secondary health conditions (SHCs) of SCI. These SHCs exert a negative influence on health-related quality of life (HRQOL). New discoveries support that a low level of activity throughout the day offers a more effective metabolic stimulus than brief, episodic exercise bouts.

The **purpose** of this study is to translate this emerging concept to the population of individuals with SCI by using low-force, long-duration electrical muscle stimulation to subsidize daily activity levels. The **long-term goal** of this research is to develop a rehabilitation strategy to protect the musculoskeletal health, metabolic function, and health-related quality of life of people living with complete SCI.

Specific Aim 1: To compare acute changes in skeletal muscle gene regulation in individuals who receive a single session of low frequency (LF) or high frequency (HF) exercise.

> Hypothesis 1: The expression of genes regulating skeletal muscle metabolism will support that HF and LF both initiate a shift toward an oxidative muscle phenotype.

Specific Aim 2: To compare changes in skeletal muscle gene regulation and systemic biomarkers of metabolic health in individuals with SCI who receive LF or HF for 6 months.

> Hypothesis 2: LF and HF will both yield gene expression changes indicating a shift toward a slow-oxidative muscle phenotype.
>
> Hypothesis 3: LF training will yield systemic biomarker changes indicating improved glucose homeostasis and reduced systemic inflammation.

Specific Aim 3: To measure subject-reported HRQOL using the PROMIS survey metric.

> Hypothesis 4: LF training will yield improved self-reported QOL via PROMIS physical and mental health scales.

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 a single session of low frequency electrically induced exercise

Study Arm 2: Acute gene regulation in response to a single session of high frequency electrically induced exercise

Study Arm 3: Adaptations in gene regulation, systemic metabolic markers, and patient-report metrics in response to training with low frequency electrically induced exercise

Study Arm 4: Adaptations in gene regulation in response to training with high frequency electrically induced exercise

Study Arm 5: Comparator participants will undergo selected outcome measures to provide comparison values for Experimental arms.

Methods

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

Study Arms 1 and 2: 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 low frequency (Arm 1) or high frequency (Arm 2) 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 3: Participants will complete a baseline assessment including the biopsy procedure described above, completion of the PROMIS 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. After two weeks, participants will begin low frequency electrically induced exercise training. They will perform electrically induced exercise as described above for 6 months. At the conclusion of training, participants will undergo repeat biopsy, PROMIS survey, and venipuncture.

Study Arm 4: Participants will complete a baseline muscle biopsy assessment using the procedure described above. After two weeks, participants will begin high frequency electrically induced exercise training. They will perform electrically induced exercise as described above for 6 months. At the conclusion of training, participants will undergo repeat biopsy.

Study Arm 5: Comparator participants will complete the PROMIS self-reported quality of life survey.

Outcome Measures: Microarray will be used to measure mRNA transcription levels for genes that regulate cellular adaptation to exercise (peroxisome proliferator-activated receptor gamma coactivator alpha – PGC1-alpha), muscle oxidative metabolism (pyruvate dehydrogenase kinase – PDK4, actin binding Rho activating protein – ABRA, and nuclear receptor subfamily 4 group A member 3 – NR4A3), and muscle fiber type (myosin heavy chain 6 – MYH6, myosin light chain 3 – MYL3, myosin heavy chain 7 – MYH7, actin 3 – ACTN3).

Standard clinical assays will be used to measure levels of serum biomarkers for metabolic control (fasting insulin, fasting glucose, fasting glucose-insulin ratio, fasting hemoglobin A1c) and systemic inflammation (c-reactive protein – CRP).

For the PROMIS participant-report metric of health-related quality of life, raw scores will be used to calculate the physical health t-scale and the mental health t-scale.

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). PROMIS physical and mental health scores will be expressed as t-scores, which are anchored to the U.S. population mean (50) and standard deviation (10).

Change over time for each gene-related outcome measure will be assessed via one-way ANOVA. For study Arms 1 and 2, gene expression levels from the baseline biopsy will be contrasted to the 3 hour post-exercise biopsy. For study Arms 3 and 4, gene expression levels from the pre-training biopsy will be contrasted to the post-training biopsy.

For study Arm 3, change over time in pre-training and post-training values for systemic biomarkers and for PROMIS scores will be assessed via 2-sided t-tests.

Pre-training values for PROMIS scores in study Arm 3 will be contrasted with the comparator cohort (study Arm 5) via 2-sided t-tests.