**DETAILED PROTOCOL**

PROTOCOL TITLE:
An exercise intervention to Reduce Neuropathic Pain and Brain Inflammation after Spinal Cord Injury
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## I. BACKGROUND AND SIGNIFICANCE

Traumatic injuries to the spinal cord commonly result in neuropathic pain. Persistent neuropathic pain, affecting >1/2 of those with SCI, furthers cardiovascular risk, increases depression, interferes with rehabilitation[1], decreases pain inhibitory capacity[2], and appears to cause more extensive atrophy and reorganization of the central nervous system (CNS)[3]. This is initiated by direct effects of spinal nerve damage and immediate secondary inflammatory responses. The CNS changes continue via adaptive and maladaptive plasticity, persistent reactive microglia, altered pain perception, and physiological consequences of living with paralysis.

In non-disabled individuals, physical inactivity in and of itself rapidly leads to insulin resistance, increased fat, muscle atrophy[4], lower pain inhibitory capacity[2], and reduced frontal and hippocampal gray matter volume[5]. This last potentially caused by decreased hippocampal neurogenesis[6] and persistent neuroinflammation. Preclinical work indicates SCI leads to changes in the brain opioid receptor system[7], and we observe elevated glial response in the hippocampus both in humans and in our animal SCI model.

Aerobic exercise may reduce chronic inflammation and pain in SCI, and animal studies indicate that high intensity exercise training could increase hippocampal neurogenesis, reduce hippocampal neuroinflammation, and increase endogenous opioid transmission. Chronic neuropathic pain is a dire consequence of SCI and exercise training is a main component of rehabilitation in SCI, but there is a paucity of data on the mechanism by which exercise training could improve neuropathic pain after SCI. Aerobic exercise reduces risk for metabolic and cardiorespiratory diseases[8], and reduces pain in SCI[9]. Two main pathways exercise influences pain are increased beta-endorphin transmission in the periaqueductal gray (PAG)[10], and/or a downregulation of neuroinflammation[11].

Exercise interventions appear most effective in the sub-acute stage, prior to the establishment of chronic neuropathic pain[12]. Hence, aerobic exercise holds promise as a safe, effective, and low-cost analgesic, neuroprotective, and neurorestorative early intervention. Importantly, the intensity of exercise, but not its frequency, is related to recovery from sensory hypersensitivity in neuropathic pain[13]. However, muscle paralysis in SCI restricts the ability to achieve high intensity exercise. The two limitations to achieving sufficient aerobic work in those with SCI are that the largest muscle mass is denervated and that cardiovascular capacity is compromised. Arm exercise can increase maximal oxygen consumption and power output[14] but the small muscle mass is insufficient to produce more than modest aerobic work.

In contrast to other forms of exercise (arms only, Functional Electrical Stimulation (FES) leg cycling, or FES leg cycling with arm exercise), FES Row Training (FES-RT) couples volitional arm and electrically controlled leg exercise. FES-RT is unique in that the non-innervated legs are integral for the performance of this whole-body exercise. We have

found that FES-RT can be done safely as early as 3 months after SCI, and our preliminary data indicates that high intensity aerobic exercise significantly reduces pain in subacute SCI.

This study proposes to investigate the effect of whole-body hybrid FES-RT, an intensive aerobic exercise intervention, in a randomized cross-over study, with microglial and opioidergic Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) measures. Our overall goal is to define how glia, opioid receptors and brain structure respond to the exercise intervention. Outcome measured are pain interference, hippocampus volume, hippocampus 11C-PBR28 uptake (glial activation), and periaqueductal gray (PAG) 11C-diprenorphine binding potential (opioid receptor density). We are further interested in understanding how exercise training may affect brain resting state network connectivity, and cervical spinal cord cross-section atrophy.

Aerobic exercise may improve outcomes, inhibit maladaptive plasticity, and could potentially reduce opioid use in SCI rehabilitation. Defining underlying mechanisms will aid bedside-to-bench target validation and may translate to other neurotrauma conditions such as traumatic brain injury and stroke, and also to neurodegenerative conditions.

## II. SPECIFIC AIMS

Aim 1- To determine the effect of a 3-month intensive aerobic exercise intervention (FES-rowing training) on **pain interference and conditioned pain modulation**, compare to control (usual treatment)

Aim 2- To determine the effect of a 3-month intensive aerobic exercise intervention (FES-rowing training) on **spinal cord cross-section, hippocampus volume and brain resting state networks** compare to control (usual treatment)

Aim 3- To determine the effect of a 3-month intensive aerobic exercise intervention (FES-rowing training) on **hippocampus glial activation**, compare to control (usual treatment)

Aim 4- To determine the effect of a 3-month intensive aerobic exercise intervention (FES-rowing training) on **periaqueductal gray (PAG) opioid binding potential**, compare to control (usual treatment)

## III. SUBJECT SELECTION

Fifty-five subjects will be enrolled. Subjects will be medically stable and inclusion criteria are SCI at or below neurological level C2 with American Spinal Injury Association grade A, B, C or D within 3-72 months after injury and aged 18-60 years old. Exclusion criteria are pregnancy or breast feeding, contraindications to MRI and PET, current or past history of major medical, neurological, or psychiatric illness, major kidney or liver problems, use of tobacco or recreational drugs (before or during the study), an abnormal physical exam (e.g., heart murmurs or peripheral edema). Contraindications to FES-rowing will also be exclusion criteria including unresponsive to FES stimulation, heart disease, physical limitations to FES-RT success, blood pressure>140/90 mmHg, significant arrhythmias, cancer, epilepsy, current use of cardioactive medications, current grade 2 or greater pressure injuries at relevant contact sites, other neurological disease, peripheral nerve compressions or rotator cuff tears that limit the ability to row,

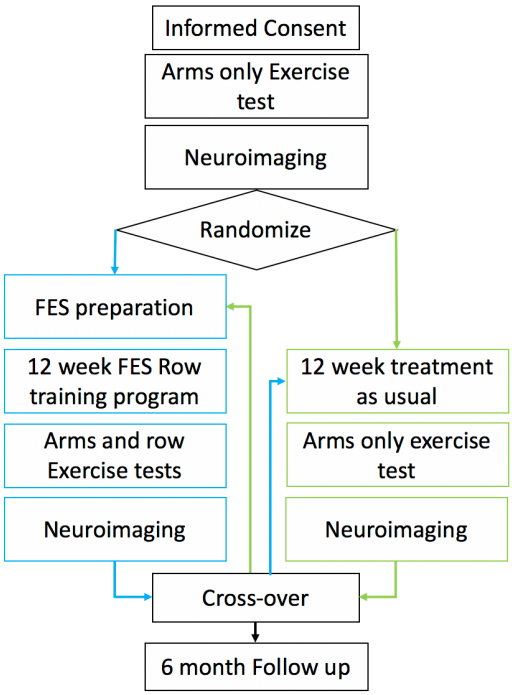
and history of bleeding disorder. The participants further need to be able to transfer on to the MRI table with assistance.

## IV. SUBJECT ENROLLMENT

Research study staff will obtain subject consent in accordance with guidelines established by the Institutional Review Board during the first visit to the Laboratory or via video conference. Participants will be sent the consent form at least 48 hours in advance so they have ample time to read and ask questions. Participants are encouraged to ask questions and are reminded that participation is strictly voluntary and will not affect their current or future care at Spaulding Rehabilitation Hospital or any of its affiliates.

## V. STUDY PROCEDURES

After consenting, subjects will undergo baseline assessments including: arms-only maximal exercise tests, pain and psychometric tests, and neuroimaging. Then, the participants will be randomized to either 12-weeks of FES-row training or a 12-week wait-list (no FES-rowing). Subjects will then cross over to the other arm. After completion of each arm, baseline assessments will be repeated. Participants in the FES-rowing arm will also perform FES-rowing maximal exercise tests before and after training. After completion of the study, subjects will be followed up at 6 months for study questionnaires.




### Consenting visit

Eligible subjects will be recruited to participate in a 2-hour consenting session. The session will include obtaining informed consent, detailed health history, weight measurements, a saliva sample, seated resting blood pressure, resting heart rate, FES device test (response to FES), and administration of study questionnaires (see Psychometric measures below).

*PBR affinity test:* A saliva sample will be collected from all subjects in order to have them genotyped for the Ala147Thr TSPO polymorphism in the *TSPO* gene (rs6971). About 10% of human subjects show low binding to PBR28[16]. Therefore, testing for the Ala147Thr polymorphism has been suggested to predict low affinity for [$^{11}$C]PBR28 in all organs, including the brain. If subjects have the low-affinity genotype, they may still be included in the study, but will not undergo PBR28 PET investigations.

*Urine drug test:* To exclude users of illicit drugs, we will perform a urine test to screen for use of non-prescribed opioids and illicit drugs (including amphetamine, barbiturates, cocaine, etc.). using monoclonal antibodies kit test (One Step Drug of Abuse Test).

A standard physical exam (International Standards for Neurologic Classification of SCI, ISNCSCI exam and Modified Ashworth Scale) will be performed by a physician unless one has been recently performed (<1 month) , in this case, medical records may be used.

Subjects may also be asked to provide additional medical records or may need further testing to determine their eligibility and the safety of their participation in this program which includes regular vigorous exercise (typically in the case of suspected cardiovascular disease). These additional tests may or may not be covered by insurance. The subjects will be responsible for obtaining any additional testing and providing the program with medical records or results.

**Arms-only Exercise test**

This incremental exercise test of arms only rowing determines maximal oxygen uptake, exercise capacity and aerobic power from on-line computer-assisted open circuit spirometry. Subjects will row with increasing resistance every 2 min until volitional fatigue. To ensure attainment of maximal exercise capacity, at least three of these criteria will be met: *I)* $O_2$ consumption plateaus despite increasing workload, *II)* respiratory exchange ratio >1.10 at end exercise, *III)* age-predicted maximal heart rate is achieved, *IV)* perceived exertion is rated at least 17 on the Borg scale of 6-20, and *V)* peak lactate >8 mmol. Immediately after completion of the exercise test, a finger top pin-prick blood lactate test (< 1 ml of blood) will be collected to confirm lactate level.

Pulmonary Function Tests: On the exercise test day, spirometry will be used to measure lung function, specifically the measurement of the amount (volume) and/or speed (flow) of air that can be inhaled and exhaled. Specifically, Forced Volume Vital Capacity (FVC) and Maximum Voluntary Ventilation (MVV) tests will be administered.

A *saliva sample* may be collected prior to and immediately after the exercise test to measure exercise induced changes in salivary protein expression.

**Neuroimaging**

***Set-up***

All neuroimaging study procedures will be performed at MGH Martinos Center for Biomedical Imaging. As SCI subjects are paraplegic to varying degree, special care will be taken when transferring subjects from their wheelchair to an MR safe wheelchair, and when positioning subjects in the PET-MR scanner so as to avoid discomfort. Our staff has a track record of performing these types of studies in SCI subjects without complications, including ensuring efficient transfer to the MR table, a comfortable position (to reduce motion and risk of pressure injuries), and emptying bladder before procedure.

Two to 3 days prior to scheduling subjects for PET-MR scans, participants will be asked if they have any new symptoms potentially indicative of urinary tract infection (fever, cloudy urine, urinary incontinence)[19], and if so, they will be asked to conduct a self-screen of urinary tract infection (UTI), using an over-the-counter test strip (AZO Test Strips Urinary Tract Infection Test). Subjects will be provided with tests and instructed on how to use them. While the self-test is not conclusive in SCI subjects (87% specificity, 93% negative predictive value)[20], it is done to lower the risk of scheduling subjects with an UTI, as this may cause cancellation and also may impact imaging results. Should the screen be positive, subjects will be instructed to contact their primary care provider.

Prior to scan session, subjects will complete a Screening Checklist for MRI and PET to again check for contraindications and urine sample may be repeated for drug and UTI testing. Female participants of childbearing age will be asked to have ~3mL of their blood drawn in order to perform a serum pregnancy test on the day of the scan (super stat testing).

Before and after the scan session, some study questionnaires (see **Psychometric Measures** below) will also be administered.

Study data will be collected and managed using REDCap electronic data capture tools hosted at Partners Healthcare Research Computing, Enterprise Research Infrastructure & services (ERIS) group. REDCap (Research Electronic Data Capture) Is a secure web-based application designed to support data capture for research studies.[53]

At the beginning of the scan session, an intravenous catheter will be placed in the participant's antecubital vein of the arm for ~3mL blood draw for testing of serum chemistries, interleukins, and protein levels and for subsequent tracer administration. Once positioned in the scanner, subjects will be instructed to remain relaxed and still for the total duration of the scan. Physiological monitoring (fingertip pulse oximetry and breathing frequency) will be collected during scanning [21,22].

The total duration of each PET-MR day will be up to 6 hours, with ~45min for nursing, ~120 min inside the scanner, and ~60min for observation after the scan. An additional ~1.5hr is required to perform pregnancy test in women of childbearing age. Ample time will be given to subjects to take breaks during this visit.

***Imaging***

MR-PET scanning will be performed at 3 Tesla, using a Siemens whole-body MRI, whole-body PET camera (Biograph MMR, Martinos Center Bay 7), or the BrainPET combined MRI PET scanner (Martinos Center Bay 6). MRI data will be acquired simultaneously to the PET data.

For opioid imaging, $^{11}$C-diprenorphine will be injected intravenously. Dynamic data will be collected over the brain for 90 minutes. The dose will be up to 12mCi, which is equivalent to ~2.5 mSv.

For microglial imaging, $^{11}$C-PBR28 will be injected intravenously with a slow bolus over a 30s period [23]. The catheter will be flushed post-injection with 0.9% saline solution. Dynamic data will be collected over the brain for 90 minutes. After collecting brain data, the MR-PET table may be moved and [$^{11}$C]PBR28 uptake will be measured over the spine region for up to 30 minutes. This way, we will obtain data for both the brain and the spinal cord using a single [$^{11}$C]PBR28 injection, thereby minimizing subjects exposure to ionizing radiation. The dose will be up to 15mCi, which is equivalent to ~3.7 mSv.

$^{11}$C-diprenorphine PET-MR and $^{11}$C-PBR28 PET-MR may be obtained on different days.

Each subject will undergo up to three $^{11}$C-diprenorphine investigations and up to three $^{11}$C-PBR28 across the study, i.e. at baseline, at 12 weeks and at 24 weeks. The total radiation exposure of the study will be $3 \times 2.5$ mSv + $3 \times 3.7$ mSv $\leq$ 18.6 mSv, which is within the FDA regulatory limits of 50 mSv per year for research-related exposure (Title 21 CFR Part 361).

MRI data using standard protocols will be acquired simultaneously with the PET data.

*Spinal cord imaging:* After the brain PET-MR, the patient may be moved so that the PET gantry covers the region of spinal cord injury. The total duration of spine MR imaging will be less than 50 minutes. High-resolution MRI imaging of the spinal cord using T1 and T2 weighted imaging. Novel spine optimized MRI sequences, developed at the Martinos Center for Biomedical Imaging, may also be used for evaluating the extent of spinal lesions

**Conditioned Pain Modulation Test**

Conditioned pain modulation (CPM) is considered a method to reliably evaluate the individual capabilities to inhibit pain[25]. CPM is based on the “pain inhibits pain” phenomenon when Stimulus A (test-pain) given together with Stimulus B (conditioning pain) is perceived less painful than when Stimulus A was given alone. Stimulation and recordings are done on the masseter and head [26] as cervical spinal cord injured subjects may have altered innervation of the hands and arms. Briefly, subjects will sit in a chair or wheelchair and pressure pain threshold (PPT) and pressure pain tolerance (PPTol) are recorded for right and left masseter muscles with a pressure algometer (Wager instruments). PPT is defined as the pressure at which the subject’s perceived sensation changed from pressure to the first sensation of pain. PPTol is defined as the maximum pressure pain that the subject can tolerate. The PPTs at each site is determined in triplicate at baseline. PPTol is determined once at each site.

For the conditioning stimuli, a headband comprising a series of plastic bags filled with ice + water having a temperature of approximately 3°C is used. The headband lightly covers the forehead, temple region, and back of the head, and is kept in position up to 10 minutes, or evoking a visual analogic scale (VAS) pain of 3 to 7. PPT and PPTol are then recorded again and the headband is removed.

For the entire study, CPM testing will be performed up to 5 times: After each $^{11}$C-diprenorphine neuroimaging session (3 times), and two times during a normal FES-exercise session: prior to and immediately after each exercise in order to also establish the magnitude of exercise induced analgesia during FES-rowing.

**FES row training program**

Subjects with SCI may require two to six weeks of preliminary strength-training prior to FES-row training. Electrodes are placed over motor points of the quadriceps and hamstrings and intensity of the stimulus is set at the level producing full knee flexion-extension. Training is performed until subjects can produce strong enough knee extension contractions to be able to FES-row train.

Once subjects are able to row, the row training program will begin: Exercise sessions will be performed 3 times per week for 12 weeks. The initial training sessions will be individualized based on subjects responses to training but typically include 6 sets of FES-rowing for 5 min at 60% of $VO_2$ peak with a work-to-rest ratio of 2:1. Participants

unable to row continuously for 5 min will row for 2-4 min with 30-second breaks incorporated until they achieve sets totaling 30 min. To maintain the proper training stimulus at the same relative intensity, a maximum FES-rowing test will then be performed at baseline for intensity to be determined. The maximum FES-rowing test procedures are identical to the arms-only exercise test described above, with the addition of incorporating legs. For subjects with strong extensor tone, a pneumatic system may be used to aid in return of the rowing seat to the forward seat position.

The goal is for each subject to achieve an exercise intensity of 70-85% maintained for a total of 30-40 min performed 3 times each week. A maximum FES-rowing test, and an arms-only exercise test will also be conducted at the end of the 12-week training program.

Adherence is easily tracked since all exercise sessions are performed in our facility on ergometers with log cards capturing exercise intensity (i.e., watts and heart rates) and duration. We have found that individuals with SCI who seek to participate in FES-RT are highly motivated. We will employ our current strategies to maximize adherence in the proposed trial, including weekly follow-ups with subjects to determine reasons for missed sessions, regular feedback to participants on progress, and goal-setting for maximal attendance and training intensity/duration (e.g., tracking and comparing/competing via 2000-meter rowing times). If subjects are unable to exercise 3 times a week for 12 weeks due to scheduling or health difficulties, they may still continue participating in the study.

### 12-week treatment-as-usual wait list

During the 12-week treatment as usual program, subjects will not participate in FES-rowing. After 12 weeks, subjects will undergo an arms only exercise test and then be scheduled for neuroimaging.

### Cross-over

After the first 12 weeks, subjects will cross over to either wait-list, or to the FES-rowing program, as described above.

### Follow up

Approximately 6 months after the completion of the two study arms, subjects will be contacted to complete some **Psychometric measures**.

### Psychometric measures

Our primary pain outcome measure is the PROMIS Pain Interference questionnaire.[27] The PROMIS(r) Pain Interference instrument measure the self-reported consequences of pain on relevant aspects of a person's life and may include the extent to which pain hinders engagement with social, cognitive, emotional, physical, and recreational activities. Pain Interference also incorporates items probing sleep and enjoyment in life.

In addition, following the National Institute of Neurological Disorders and Stroke SCI Common Data Elements recommendations, we will obtain:

- International SCI Upper Extremity Basic Data Set [28]. This standardized exam collects information about upper extremity status.

- Spinal Cord Independence Measure [29]. The SCIM has been developed to address three specific areas of function in subjects with spinal cord injuries (SCI). It looks at self-care (feeding, grooming, bathing, and dressing), respiration and sphincter management, and a patient's mobility abilities (bed and transfers and indoors/outdoors).
- International SCI Pain Basic Data Set Version 2.0 [30] This instrument contains 0-10 ratings of pain interference, and asks about the three worst pain types experienced along with pain type and severity.
- PROMIS 57 Questionnaire[33]: The PROMIS 57 questionnaire will be used to assess changes in subject's views on their physical, psychological, and social well-being. The PROMIS 57 scales include eight items from seven primary PROMIS domains: depression, anxiety, physical function, pain interference (and one pain intensity item), fatigue, sleep disturbance and satisfaction with social roles.
- NIH Toolbox Meaning and Purpose Short Form[34]: is a 7 item questionnaire that measures a person's sense of meaning and purpose in life over the past 7 days.
- SCI Exercise Self- Efficacy Scale (ESES)[35]: is a reliable and valid measurement of self-efficacy, or belief and confidence in one's skill and ability, to engage in physical activity.
- Numerical Rating Score of Pain. [36]. This widely used scale is used to rate pain of pressure probe and of the headband ice cooling during the conditioned pain modulation. It asks subjects to numerically rate their current pain from 0 to 10, no pain to worst imaginable pain.
- The Pain Catastrophizing Scale (PCS) [40]*:* It is a 13-item self report scale which measures pain-related Rumination, Magnification and Helplessness.
- The American Spinal Injury Association (ASIA) International Standards for Neurological and Functional Classification of Spinal Cord Injury [41]. The protocol consists of two sensory examinations, a motor examination and a classification framework (the impairment scale) to quantify the severity of the spinal cord injury.

## VI. BIOSTATISTICAL ANALYSES

Hypothesis testing for the four primary outcomes will be performed using a general linear mixed model (Laird and Ware 1982) with Hotelling-Lawley, α =0.0125 (Bonferroni corrected 0.05 for 4 outcomes).

***Primary outcomes***

Pain Interference PROMIS Pain Interference - Short Form 6b V1.0.

*Structure:* Hippocampal volume will be defined using the longitudinal pipeline in the Freesurfer image analysis suite[43-54]. Specifically, the Freesurfer 6.0 hippocampal longitudinal model will be used[55,56]

*Glial activation:* $^{11}$C-PBR28 PET data will be modeled as Standardized uptake value (SUV) normalized to whole brain mean (SUVR) from 60 to 90 min post time of injection to assess TSPO expression. The average voxel value across the hippocampus will be extracted for each time point in each subject.

*Periaqueductal gray PET $^{11}$C-diprenorphine Non Displaceable Binding Potential* ($BP_{ND}$) Uptake will be modeled as per our recent study (Linnman et al 2017) [61] on 90 min of

dynamic PET data using the simplified reference tissue model 2 [62] with the occipital cortex used as the reference tissue.

***Secondary outcomes***

*Resting state fMRI*: data processing will be carried out using the CONN toolbox [63].

*Spinal cord thickness:* Metrics on spinal cord cross-sectional area at the C2 level will be defined using the Spinal Cord Toolbox [64].

## VII. POWER ANALYSIS

***Sample size***

The aim of this study is to define the impact of 12 weeks of FES-RT on four primary outcomes: Pain interference, hippocampal volume, hippocampal glial activity, and periaqueductal gray opioid receptor binding potential. As we hypothesize change in any of these variables, significance is set to $\alpha = 0.0125$ (0.05 Bonferroni corrected for 4 outcomes). We based our sample size on the biggest sample size analysis which was found for *Hippocampal gilal activation.* We recently investigated PBR28 uptake in 10 subjects with chronic lower back pain and 9 healthy subjects. [58] This study found hippocampal uptake of PBR28 to be 1.065 (±0.058) and 0.982 (±0.074) (SUV). This approximately 8% SUV difference yields an effect size of 1.25, requiring a sample size of 28 subjects per group for 80% detection power at p=0.0125 (i.e. 0.05 Bonferroni corrected for 4 outcomes) to *define cross-sectional differences*. However, in our case study, we observed a 5% decrease in left-, and a 4% decrease in right-hippocampal PBR28 uptake after 12 weeks of FES-RT. Hence, to be conservative, a total of >31 subjects need to enter into this two-treatment crossover study. The probability is 80 percent that the study will detect a treatment difference at a two-sided 0.012 significance level, if the true difference between treatments is 5%. This assumes that the within-patient standard deviation of the response variable is 5.5%. We thus settle on a final **n = 32**.

***Enrollment***

To reach a complete sample of 32 participants, we have set a target enrollment of 55 subjects. Indeed, there is 10% occurrence of the low affinity TSPO polymorphism (rs6971), that is approximately 5 subjects who may not be eligible for PBR28 PET neuroimaging session. Moreover, adherence to FES-RT has been high in our prior studies, but some sub-acute SCI subjects are anticipated to have medical complications. In our experience, 35% of participants will drop out due to health issues. Of note, drop-out may be asymmetric, and more common in subjects that cross-over from the FES-RT arm to the WL (i.e. participants may want to continue to exercise and cannot ethically be prevented from doing so). Hence, we anticipated a total of n = 55 enrolled individuals, enable a n = 50 participants who will be randomized to FES-rowing or wait list first (see table below).

| | |
|---|---|
| Screened | 70 |
| 20% ineligible (illicit drug use, MR incompatible, and other exclusion criteria) | -15 |
| Enrolled: consented and genotyped | 55 |
| Ineligible for PBR28 PET (rs6971 polymorphism) | -5 |
| Randomized | 50 |
| 35% dropout between baseline and 24 weeks | -17 |
| Final sample | ≥ **32** |

## VIII. RISKS AND DISCOMFORTS

*FES-rowing training and testing*

Moderate risks are associated with exercise testing and exercise training. Some discomfort and feeling of fatigue will be experienced during these tests. There are other risks associated with these tests including abnormal blood pressure responses, fainting, irregular, fast or slow heart rhythm, and in rare instances, heart attack, stroke, or death. Wearing the face-mask during the VO2max testing may cause feelings of claustrophobia. Exercise training may induce some muscle or joint discomfort when beginning an exercise program and could result in tendonitis and/or musculoskeletal overuse injuries over time. The electrical stimulation unit can cause tingling, pins and needles sensations on the skin, skin irritation, increased muscle spasticity and autonomic dysreflexia (signs include headache, nausea, rise in blood pressure, sweating, and goosebumps).

There is some discomfort with the lancet used on the finger for lactate measurement and there is a small possibility of swelling and bruising at the site(s), there is also a slight chance that individuals become dizzy and even faint. Subjects may feel lightheaded or short of breath during the pulmonary function tests, there is also a slight chance that individuals become dizzy and even faint. The study physician will be contacted if serious events occur.

*PET/MRI Imaging*

The U.S. Food and Drug Administration (FDA) recently gave the first regulatory clearance of a hybrid PET/MRI scanner in the U.S. Additionally, FDA considers investigations of MRI software and hardware operating within FDA specific parameters as non-significant risk device studies. All studies will adhere to these FDA approved safety levels for the Siemens system. These safety parameters include static magnetic field, time varying magnetic fields (dB/dt), specific absorption rate (SAR), and acoustic noise levels.

The radiation exposure in this study will be within established limits for research and there is no evidence that it represents a major health risk. If subjects have participated in other research studies in the past 12 months that have involved radiation exposure, they will be asked to inform the investigators or study staff. If it is determined that their prior medical or other radiation exposure exceeds current guidelines, they will not be allowed to participate in this study.

Intravenous catheter. The subject will feel a slight pinprick, similar to a bee sting, and may feel some discomfort and have some bruising or bleeding at the site where the needle goes in. Depending on the length of time the catheter is in place, a bruise may last for a day or so. The risks associated with having blood drawn include bruising, local discomfort, or infection at the site of the needle puncture. Rarely an infection may occur at this site, and if an infection does occur, it will be treated.

We will use [$^{11}$C] tracers (PBR28 and Diprenorphine) produced by the cyclotron/radiochemistry/radio-pharmacy facility at the A. A. Martinos Center for Biomedical Imaging. We will follow the safety standards approved by the Radiation Safety Committee for the use of radioligands. The IV injection will be administered by a licensed nuclear medicine technologist. Should there be an adverse event, Dr. Linnman will inform the study physician (Dr. Tsitsilianos), the independent study monitor (Dr. Maleki). Averse event will also be reported to the IRB within the stipulated time frame.

*Conditioned Pain Modulation Test*
This test involved painful pressure to the masseter muscle and wearing a head-band with cold water packs inducing cold pain to the head. Pressure pain is slowly increased, and subjects may interrupt it at any time and also remove the cold head-band. The procedures induce mild to moderate pain and are safe and commonly used in clinical populations [72] including SCI.[71]

*Psychometric measures*
Subjects will be instructed to complete the questionnaires to the best of their ability but will have the option to leave any question(s) blank. In the unlikely event that evidence of physical or psychological disorder is found, with the individual's permission, the information will be shared with his or her primary care physician who can direct care as needed. If suicidal ideation is expressed or suspected, subjects will be referred to the study physician for immediate follow-up.

*Confidentiality*
There is a small risk that information about subjects could become known to people outside of this study, and this risk is identified in the informed consent form. Subject data is stored under coded name without identifying features, and only the study PI holds the subject key on a locked and encrypted computer.

## IX. POTENTIAL BENEFITS:

Subjects will have the opportunity to participate in a supervised exercise program, which may improve their overall cardiovascular fitness and endurance during activities of daily living as well as decrease risk of chronic disease. It is unlikely that individual subjects will benefit from the imaging study. Findings from these studies will help advance our understanding of the pathophysiology of neuropathic pain after SCI. As such, we envision that in the future the information obtained from the proposed research will enhance the diagnosis and management of a SCI and may be used as a marker of therapeutic interventions. Moreover, by defining the hypothesized beneficial effect of aerobic exercise on brain health and pain after SCI, the exercise intervention may be more widely adopted, and the intervention may be translated to a wider variety of chronic pain conditions.

## X. REMUNERATION:

Subjects will be paid by check at the completion of the study for their participation. Payments will be as follows:
$150 for each MR-PET scanning visit.
Females of childbearing potential will receive an additional $25 for the added burden of blood-draws for pregnancy testing prior to PET-MR investigations.

If during the imaging visit(s) we cannot inject the subject with the radioligand (e.g., due to a failure in radiosynthesis, or to issues with the scanner) he/she will receive $50.

If the subject will need to stop the scan early for any reason, he/she will still receive $50 for his/her time. Additionally, transportation costs (<$100) and parking fees will be covered as needed.

## XI. MONITORING AND QUALITY ASSURANCE

The Principal Investigator will monitor the informed consent procedures in accordance with the Informed Consent Compliance Checklist of Partners HealthCare Systems HRQIP. The subject's data/protocol adherence will be monitored by the research coordinator at each step in the study. The research coordinator will be responsible for monitoring the completeness of all data and source documents.

The team will evaluate the progress of the study, verify that the rights and well-being of the subjects are protected, verify that the reported study data are accurate, complete and verifiable from source documents, and the conduct of the study is in compliance with the approved protocol and amendments. Outcome monitoring and adverse events will all be reported through appropriate channels of the Human Studies Committee.

*FES-rowing training and testing*
The Cardiovascular Research Laboratory Medical Emergency Safety Plan will be followed in the case of an adverse medical event. Any clinical/health related issues would be immediately presented to the subject (i.e. abnormal EKG, etc) to determine appropriate notification (ie. current physician or appropriate specialist)

At each visit, study staff will prompt the participant to report any occurrences of health changes or problems. Any serious or non-serious adverse event will be recorded in the participant's study folder and adverse event log and reviewed by the PI. Serious and non-serious adverse events will also be reported to the Human Research Committee in accordance with Human Research Committee reporting guidelines, following the timeframes specified by the Partners Investigator's Guidelines. In case of a serious adverse event appropriate diagnostic and therapeutic measures will be taken, as determined by the investigator and study physician.

*Imaging*
The Siemens MR-PET scanners have a built in self monitoring system that automatically shuts off if parameters exceed safe levels. Quality assurance of the scanner's performance is obtained by a daily quality assurance protocol. More extensive quality assurance protocols are performed monthly under the commercial service contract with Siemens Medical Systems.

Subjects will be informed about minimal risks of routine high magnetic field and non-ionizing RF radiation involved in MR imaging, PET procedure and the risks associated with exposure to radiation. In addition, they will be informed about that if they feel uncomfortable with the study, that they can choose to terminate the study at any time with no effect on care and treatment received by them at Partners now or in future and that their personal information will be protected as per the HIPAA guidelines.

As SCI subjects are paraplegic of varying degree, special care will be taken when placing them in the scanner so as to avoid discomfort and pressure sores. Imaging will be stopped should any untoward reaction be observed during the imaging session or if the participant so requests for whatever reason. Subjects will be required to use earplugs to decrease the noise perceived while in the scanner.

*Adverse events*

Adverse events and unanticipated problems involving risks to subjects or others will be reported to the PHRC in accordance with PHRC adverse event and unanticipated problems reporting guidelines. In addition, we will involve the MGH Human Research Committee and Radioactive Drug Research Committee/Radiation Safety Committee in consultation if any additional potential risks arise and particularly with the use of the radioactive drug [21CFR361.1(d)8].

*Oversight*

An independent research monitor, Dr Nasim Maleki, Department of Psychiatry, Massachusetts General Hospital, has been appointed. Dr. Malekis functions include:

- Overseeing that recruitment and enrollment procedures and the consent process for individuals is adhered to.
- Overseeing study interventions and interactions.
- Overseeing data matching, data collection, and analysis

- The research monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research
- The research monitor has the authority to stop the research protocol in progress, remove individual human subjects from the research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report
- The research monitor has the responsibility to promptly report their observations and findings to the IRB or other designated official and to the NIH and/or FDA as appropriate.

## XII. REFERENCE LIST


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