Effects of Remote Ischemic Conditioning on Hand Use in Individuals With Spinal Cord Injury and Amyotrophic Lateral Sclerosis: A Preliminary Study

NCT03851302

Study Protocol and Statistical Analysis Plan

24 November 2023

Research Strategy

Significance

Between 250-350,000 individuals live with spinal cord injury (SCI) in the United States. Among this population, ~ 60% have injuries at the cervical level[1]. Impairments of arm and hand function in individuals with cervical SCI (cSCI) greatly reduce quality of life and adversely impact the level of independence[2]. Previous research on the needs of individuals with cSCI has shown that the improvement of hand function is as important as regaining bladder and bowel function, and more important than walking[3]. Most spinal cord injuries are not fully transected, indicating that there are residual nerve circuits after injury[4]. Rehabilitation interventions such as physical training and neural stimulation have been shown to reorganize motor pathways in the brain, corticospinal tract, and at the spinal level, a process called neural plasticity[5–8]. Thus, neural plasticity after SCI could improve function by enhancing the excitability of residual neural circuits[9–14].

However, both physical training and neural stimulation require a large number of repetitions, and even so, the retention of the intervention effects may be fleeting. To enhance the magnitude and duration of neuroplasticity, investigators are supplementing physical training with methods that augment training effects and lengthen the retention of functional gains. Transcranial direct current stimulation (tDCS) and acute intermittent hypoxia (AIH) are two such approaches. Some preliminary research has shown that either tDCS or AIH coupled with task-oriented physical rehabilitation enhances the learning effects of task-specific motor training after SCI[15,16]. However, tDCS requires not only an electrical stimulation device but also the optimization of electrode placement, which is controversial. AIH requires an hypoxicator system to provide prolonged systemic low oxygen exposure. Further both tDCS and AIH are costly, demand highly trained staff and require sophisticated technological devices. **Therefore, the need remains for a simple, effective approach to synergistically improve neuroplasticity in combination with physical rehabilitation.**

In this proposed study, we will investigate a potential approach called remote ischemic conditioning (RIC). Ischemic conditioning occurs when a specific organ or tissue is exposed to one or more transient events of sublethal ischemia. This leads to several protective reactions against subsequent ischemia on that organ or tissue[17–19]. Studies have demonstrated that those endogenous protective effects are not limited to ischemic organ/tissue alone – they are transferrable to other organs or tissues[20–22]. This phenomenon is called remote ischemic conditioning[23,24]. For example, cardioprotective effects can be provoked simply by transiently restricting blood flow of one limb using a tourniquet. This simplicity makes RIC more attractive, less expensive and practical. The mechanisms of cardioprotection induced by RIC are not entirely clear, but evidence suggests that RIC has extensive effects via humoral and neural factors that may aid in cardioprotection[24]. Among the potential mechanisms supporting the cardioprotective benefits of RIC, two effects might potentially promote neuroplasticity: induction of hypoxia-inducible factor 1α (HIF-1α) and anti-inflammatory factors. H1F-1α may have neuroprotective effects via triggering the expression of genes related to oxygen transport, glycolytic metabolism, and apoptosis[25]. Albrecht and colleagues found that upper limb RIC induced H1F-1α accumulation and activation in the right atrial tissue in patients undergoing cardiopulmonary bypass[26]. Whether the activation of H1F-1α induced by RIC extends to corticospinal areas and regulates neural excitability is unknown. Also, it has been speculated that H1F-1α expression helps to overcome the negative environment for transplanted neural stem cells due to ischemia and inflammation after SCI[27]. On the other hand, RIC also reduces inflammation[28,29]. Inflammation has been shown to attenuate expression of brain-derived neurotrophic factor (BDNF) in the brain[30–33]. Since individuals with SCI show signs of chronic systemic inflammation[34,35], we hypothesize RIC's ability to dampen inflammation could improve the expression of BDNF, which might further promote neuroplasticity.

Cherry-Allen and colleagues recently published the first study testing the synergistic effects of RIC on motor task learning[36]. Able-bodied adults (n=18) were randomly assigned into active or sham RIC groups to undergo seven consecutive weekday sessions of RIC followed by stability platform balance training or training of a cognitive task dependent on the hippocampus. One active or sham RIC (5 cycles of 5-min inflation and 5-min deflation) was conducted before training each day. The authors noted significantly improved performance on the stability platform task in the active RIC group compared to the sham group, immediately after the completion of seven-day training sessions and even at the 2- and 4-week follow-up visits. The mechanism of improvement was unclear in their study, since they did not find any significant changes in serum BDNF, cognitive learning, or generalized muscle activation measured by finger flexor EMG activity and grip force. **However, their study did**

not investigate changes of corticospinal excitability. We will focus on the acute effects of RIC on corticospinal excitability and systemic inflammatory mediators in this proposal.

Innovation

We propose a proof of concept study to investigate the acute synergistic effects of active versus sham RIC on motor task training, especially in persons with chronic cSCI. **The primary outcome measure will be change in corticospinal excitability after RIC combined with a single bout of isometric hand exercise.** The advantage of RIC compared to other current modes of inducing neuroplasticity, such as tDCS and AIH, is that RIC is a more approachable application, because RIC is simple and low-cost. **Yet, published studies of RIC regarding neuroplasticity do not exist for populations of individuals with neurologic injury or disease.** If synergistic effects of RIC with physical training can be demonstrated in this study, then effects of RIC coupled with various other rehabilitation interventions can be tested in future studies. In addition, we expect the analysis of neurophysiology and inflammatory mediators before and after RIC to provide some preliminary information regarding the mechanism by which RIC promotes neuroplasticity and improves effects of training. Finally, as a safety precaution, we will monitor beat-to-beat changes in heart rate (HR), blood pressure (BP) and oxygen saturation (SaO_2) during RIC, since damaged autonomic nervous system (ANS) in individuals with SCI might dysregulate those hemodynamic responses toward the ischemic stimulation. **As far as we know, this will be the first study in the SCI population (1) to investigate the synergistic effects of RIC with physical training on corticospinal excitability, (2) to measure changes in inflammatory mediators after RIC, and (3) to observe in real time the responses of HR, BP and SaO_2 during RIC.**

Approach

Overview

We propose a randomized crossover study to compare active versus sham RIC combined with one bout of isometric hand exercise. Participants will be randomly assigned the order of the two experimental sessions: active or sham RIC. The isometric hand exercise will be performed in both sessions. The washout period between the two experimental sessions will be two to three weeks to prevent any carry-over effects. Figure 1 outlines the experimental protocol. Each session will consist of a pre-test measurement (baseline), active/sham RIC, post-RIC measurement, an isometric hand exercise and a post-exercise measurement. The total time in each session is around 3 hours including preparation. At the beginning and end of active/sham RIC, blood samples will be collected to measure changes in inflammatory mediators.

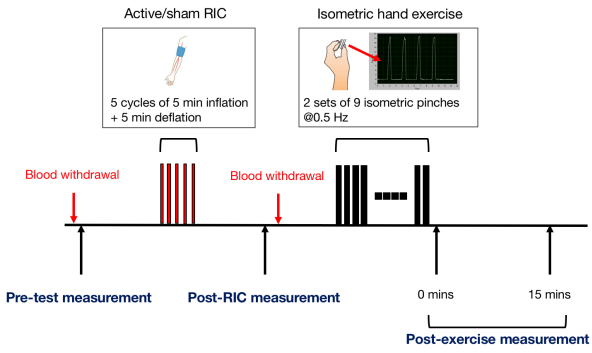


Figure 1. The experimental protocol.

Participants with chronic cSCI (n=8, ASIA Impairment Scale (AIS) A-D) and age-matched (within ±5 years) able-bodied controls (n=8) will be recruited. Participants with cSCI must have at least partial ability to make volitional finger opposition movements (thumb and index finger) in at least one hand. Participants will be excluded if they have concurrent serious medical illness, traumatic brain injury, cardiovascular or pulmonary complications, seizure history, deep vein thrombosis, or any other issues that would increase risk of magnetic or electrical stimulation required for outcome measure assessment. We will also document medications that participants are currently using for systemic inflammation, spasticity, anxiety, or depression, which could affect nervous system excitability. One screening session will be conducted to confirm eligibility and determine the target arm in each participant. The arm with consistent responses on abductor pollicis brevis muscle (APB) induced by magnetic or electrical stimulation will be the target arm. Transcranial magnetic stimulation (TMS) will be performed to determine each subject's optimal scalp location for hand motor cortex stimulation, and to determine resting motor threshold for the APB muscle. Participants will be withdrawn if TMS-induced motor evoked potentials (MEPs) are unable to be detected at the APB muscles on either hand at stimulus intensity below 90% of maximal stimulator output. More details regarding inclusion and exclusion criteria are listed in the human subject section.

The RIC protocol involves 5 cycles of 5-min inflation and 5-min deflation on the non-target arm. RIC will be achieved via blood pressure cuff inflation to 200 mmHg for active RIC[37–40] and 10 mmHg below the subjects' diastolic blood pressure for sham conditioning. This method replicates RIC as it has been applied in many other studies[23,38,41].

The primary muscles assessed in this study are APB and the first dorsal interosseous (FDI) muscle on the target arm. The electrophysiological variables include intra-cortical facilitation/inhibition, corticospinal excitability and peripheral nerve conduction profile. Electrophysiological variables will be measured at pre-test measurement (baseline), post-RIC measurement (post-RIC), and post-exercise measurement (immediately after the isometric hand exercise (post-RICx-0) and 15 minute later (post-RICx-15)). The blood samples will be collected at baseline and post-RIC to measure gene expression for inflammatory mediators related to Toll-like receptor (TLR) signal pathway.

For the isometric hand exercise, subjects will be instructed to pinch a dynamometer (Anyload) with thumb and index finger at different intensities and durations. Performing volitional movements at varying intensities stimulates corticospinal circuits[42,43]. The intensities of pinch force will be 10%, 25%, and 50% of the maximal voluntary contraction (MVC). For each intensity, durations of 2, 4, and 6 s will be employed, which resulted in nine different combinations delivered in pseudorandom order. Participants will perform 2 sets of the isometric hand exercise (18 pinches in total). The interval between each pinch will be 2 seconds, with 30 second intervals between each set.

Aim 1: To determine the effects of active versus sham RIC prior to one bout of muscle contraction exercise on motor corticospinal excitability.

Hypothesis 1A: Motor evoked potential (MEP) amplitudes at the APB muscle (Primary Outcome) will significantly increase after active RIC plus isometric hand exercise compared to sham RIC plus isometric hand exercise. Active RIC alone will not significantly increase motor evoked potential amplitudes. These trends will be similar in both cSCI and able-bodied participants.

Hypothesis 1B: Secondary electrophysiological outcomes at the APB muscle will significantly change after active RIC plus isometric hand exercise compared to sham RIC plus isometric hand exercise. Short-interval and long-interval cortical inhibition will decrease; intracortical facilitation will increase. Active RIC alone will not significantly change these outcome measures. These trends will be similar in both cSCI and able-bodied participants.

We hypothesize that RIC might synergize with motor task learning via increased corticospinal excitability at supraspinal level. In order to test our hypothesis, we select an isometric hand exercise as the motor training task in this pilot study. The reason is that several studies have demonstrated immediate and transient increased MEPs as a result of post-exercise facilitation in able-bodied participants after a short period of repetitive contraction exercise in thenar muscle[44], wrist muscle[45], forearm[46] and leg[47,48]. Additionally, the various combinations of the intensity and the duration of pinch movements in our study, as well as the use of an intrinsic hand muscle, theoretically involves more cortical attention, which should magnify the likelihood of supraspinal neuroplasticity[42,43,49]. We will test MEPs at baseline, post-RIC, post-RICx-0 and post-RICx-15. We expect to observe further increased MEPs of the APB thenar muscle at post-RICx due to the synergistic effects of active RIC on isometric hand exercise compared to sham RIC. Likewise, we expect that intracortical inhibition and intracortical facilitation of the APB muscle will decrease and increase, respectively. We also anticipate similar responses of all electrophysiological outcomes in both participants with cSCI and able-bodied participants.

Method: We will conduct electrophysiological measurements at four time points: baseline, post-RIC, post-RICx-0 and post-RIC-15. The post-RIC measurement allows us to first observe whether RIC-alone acutely affects corticospinal excitability. In addition, the 15-minute follow-up measurements (post-RICx-15) will allow us to investigate if the exercise effects will last longer due to active versus sham RIC. In order to localize changes on the pathway from the motor cortex to muscle, the electrophysiological outcomes include changes of intra-cortical facilitation/inhibition, peak-to-peak amplitude of MEPs at 120% resting motor threshold (RMT), and peripheral nerve profile including M/F waves. We have and already use all the major equipment necessary for outcome assessments, including a MagPro X100 TMS system (MagVenture), Digitimer peripheral nerve stimulators (Digitimer), surface EMG pre-amplifiers (Motion Lab Systems), and data acquisition boards (National Instruments).

Peak-to-Peak Amplitude of Motor Evoked Potentials at 120% intensity of Resting Motor Threshold (MEP_{120}): The optimal scalp location for APB will be determine at the screening session and stored in our Brainsight TMS Navigation system (Rogue Resolutions) for later use in each experimental session. After navigating to the optimal stimulation spot, the RMT, used for calculating single- and paired-pulse stimulation of the APB will be determined by the lowest TMS intensity at which an MEP could be induced with at least 5 of the 10 stimuli with peak to peak amplitude of 50-100 uV at rest. The single-pulse TMS will be applied at 120% RMT to induce and record peak-to-peak amplitude of MEP_{120}.

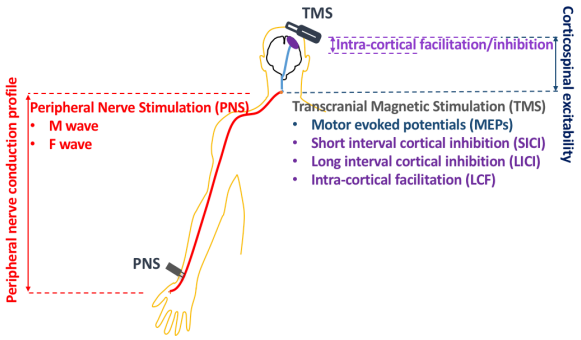


Figure 2. Illustration of electrophysiological outcomes used to localize changes of excitability from cortex to peripheral nerve.

Cortical inhibition and facilitation: Paired-pulse TMS will be used to measure short interval cortical inhibition (SICI), long interval cortical inhibition (LICI) and intra-cortical facilitation (ICF)[50]. Paired-pulse TMS includes a conditioning (CS) and test stimulus (TS) separated by a specified interstimulus intervals (ISI). The configuration for measuring SICI, LICI and ICF in this study are as follows[51–54]:

- SICI: CS = 90% RMT, TS = 120% RMT, ISI = 3 ms
- LICI: CS = 120% RMT, TS = 120% RMT, ISI = 100 ms
- ICF: CS = 90% RMT, TS = 120% RMT, ISI = 12 ms

Peripheral Nerve Profile: Supramaximal electrical stimulation will be delivered at median and ulnar nerves at wrist level. The peripheral nerve profile includes the latency and the peak-to-peak amplitude of the M/F waves. The peak-to-peak amplitude of the M waves will be used to normalize the MEP_{120} at each time point to ensure that changes of corticospinal excitability are not due to peripheral variation[50].

Data Analysis:

Corticospinal Excitability: The primary outcome will be **MEP_{120}** on APB (primary outcome muscle) and FDI muscles. **The MEP_{120}** will be first normalized to peak-to-peak amplitude of M waves to take account of the peripheral variation. The changes of MEP_{120} at post-RIC, post-RICx-0 and post-RICx-15 will be expressed as percentage of the changes compared to baseline MEP_{120}. The SICI, LICI and ICF will also be expressed as percentage of the changes compared to baseline in each value.

Statistical Analysis: Electrophysiological outcomes will be compared after RIC and after the isometric hand exercise relative to baseline values. Descriptive analysis will be computed first for all outcomes variables to test the distribution of the data and correlation tests will be performed to check the independence among the outcome variables. A three way 2 (cSCI, able-bodied) by 2 (RIC, sham) by 3 (post-RIC, post-RICx-0, post-RICx-15) mixed-ANOVA will be used to analyze MEP_{120} and intra-cortical facilitation/inhibition. If the data is not normally distributed and the assumptions of the ANOVA are not met, Wilcoxon signed rank tests will be used instead. Post hoc pairwise comparisons will be performed using the Bonferroni adjustment, if there are any main or interaction effects within the three independent variables (group, active/sham RIC and time points).

Potential Pitfalls/Alternative approaches/Future Directions: Applying RIC on motor task learning is relatively new area with little preliminary data – therefore, our primary hypothesis that RIC may promote motor task learning via increasing corticospinal excitability is highly speculative. Additionally, the effects of RIC and acute post-exercise facilitation have mostly been shown in able-bodied participants, and there are no published data in the SCI population that measured corticospinal excitability as a mechanism for acute post-exercise facilitation. Therefore, individuals with SCI might demonstrate uncertain responses toward RIC and the isometric hand exercise than that exhibited in able-bodied participants. The inconsistent injury level and the residual nerve circuits among participants with cSCI might result in the wide variability of the outcomes. Nevertheless, although our hypotheses are speculative, the proposed study is designed to provide clear answers via observing the responses in participants with cSCI and able-bodied participants. If this brief isometric hand exercise cannot lead to significantly increased MEPs in participants with cSCI, our next step might test RIC in combination with more advanced interventions such as non-invasive paired stimulation, a technique that our research team is actively studying in other protocols. Finally, if the pilot study shows a significant synergistic effect of RIC on the isometric hand exercise task, our future goal will be coupling RIC with more prolonged rehabilitation training to promote long-term effects.

Aim 2: To investigate effects of active versus sham RIC on systemic inflammatory mediators in individuals with cSCI.

Hypothesis 2A: The gene expression of inflammatory mediators will significantly decrease after active RIC compared to the sham RIC in participants with cSCI.

Hypothesis 2B: The trends of inflammatory mediator change will be similar in both participants with cSCI and able-bodied participants.

Our collaborator Dr. Bloom and others have demonstrated elevated inflammatory mediators in persons with chronic SCI[55–57]. Our objective here is to profile systemic immune responses to determine if RIC has anti-inflammatory effects in cSCI and able-bodied participants. Dr. Bloom (see letter of support) recently demonstrated broad changes in inflammatory gene expression in persons with chronic SCI, which was particularly evident in persons with higher level injuries[57]. Specifically, they found elevated members of the Toll-like receptor 2/4 (TLR) signaling pathway, metalloproteinases (ADAM10), caspases (CASP1, 3, 8) and chemokine gene families. Previously, it was shown in able-bodied adults that RIC (3x5 min) resulted in reduced levels of inflammatory mediators in the blood at 15min and 24h later[58]. Specifically, members of the same genes and gene families elevated in persons with chronic SCI[57] were reduced by RIC in able bodied individuals: TLR signaling pathway, TNF receptor pathway, MAP kinases, apoptosis pathway (CASP8), chemokines, T cell signaling molecules, metalloproteinases and leukocyte adhesion moleculesref[58] Here, we will test if RIC in the current experimental paradigm has a similar anti-inflammatory effect in persons with SCI as has been documented in the able-bodied population.

Methods: Blood samples (3cc) will be collected before the active/sham RIC cycle and 15 minutes after the end of RIC at the post-RIC measurement. According to previous research, the alteration of inflammatory mediators should occur after 15 minutes[58].

Data analysis: Given the previous data from the Bloom Lab indicating that TLR signaling is elevated in persons with chronic SCI and the data from able bodied persons that RIC reduces TLR signaling, we will first profile expression of genes related to TLR signaling in the current participants using qPCR profiling. Briefly, RNA will be isolated from whole blood collected in PAXgene tubes (PreAnalytix, BD), using standard methods and the manufacturer's protocol (Qiagen QIAcube, Venlo, The Netherlands). RNA quality and quantity will be determined using the Bioanalyzer (Agilent). As per standard Qiagen's instructions, RNA will be converted to cDNA using the RT^2 First Strand Kit will be used to prepare cDNA, which is then mixed with RT^2 SYBR Green Mastermix. We will use the PCR Array for Human Toll-Like Receptor Signaling Pathway (Qiagen, USA) on the Roche Lightcycler 480 (384-well block). Relative gene expression will be determined using the delta delta Ct method.

Statistical analysis: A three-way 2 by 2 by 2 mixed model ANOVA will be performed for 2 within group factors (baseline vs. post-RIC) (active vs. shame) and one between group factor (able-bodied vs. cSCI) to compare the gene expression intensity. If the data is not normally distributed and the assumptions of the ANOVA are not met, Wilcoxon signed rank tests will be used instead. Post hoc pairwise comparisons will be performed using the Bonferroni adjustment, if there are any main or interaction effects within the three independent variables (group, active/sham RIC and baseline vs. post-RIC).

Potential Pitfalls/Alternative approaches/Future Directions: We propose to use standard gene expression profiling techniques and we do not anticipate technical difficulties with the approach. If we do not observe changes in gene expression of the TLR signaling pathway, we can use RNA from the samples for RNA-seq to obtain a broader and unbiased gene expression profile. Initial RNA-seq runs could be limited to 8 million reads/sample, 100bp single end, which would yield a general overview of relative changes in gene expression of the high to moderately expressed genes. If the initial pilot data look promising, then we would seek funding to perform a fuller RNA-Seq profiling.

Aim 3: To determine changes in heart rate (HR), blood pressure (BP) and oxygen saturation (SaO_2) during active versus sham RIC in individuals with incomplete cSCI and able-bodied subjects.

Hypothesis 3A: There will be no difference in HR, BP and SaO_2 (pulse oximeter on the finger of the target arm) responses among baseline, inflation phase, deflation phase and post-RIC during active and sham RIC in persons with SCI and able-bodied controls.

Although RIC has been shown to be safe in the healthy able-bodied population as well as in individuals with heart disease and critically ill patients with subarachnoid hemorrhage[39,40,59], there are no data describing the safety of RIC in persons with SCI. The SCI population, particularly those with cSCI, have widespread sensory impairment, including a limited ability to feel pain/discomfort. In addition, damage to the ANS after cSCI contributes to cardiovascular dysregulation and may alter hemodynamic responses to RIC. Several studies[60–63] have reported stable HR and BP responses before and after RIC not just in healthy participants but also in participants with heart diseases or vascular stenosis. The BP responses even remained unchanged during the whole course of RIC in able-bodied participants and in participants with stable angina pectoris[60,62]. However, the sympathetic hypoactivity after cSCI might result in altered or delayed hemodynamic responses, possibly leading to fluctuation of HR and BP. Therefore, we will real-time record HR, BP and SaO_2 on the contralateral arm and document the pain scale and any adverse effects during RIC.

Method: During the 50-minute active and sham RIC (5 cycles of 5 min inflation plus 5 min deflation), beat-to-beat HR, RR and SaO_2 will be monitored in real-time and digital signals will be stored on a computer hard-drive for subsequent analysis. **Electrocardiogram** - A 3-lead ECG (UFI: model RESP 1, Morro Bay CA) will be used to measure beat-to-beat HR during testing. Electrodes will be placed at the right and left clavicle and in the V-5 position; data will be recorded from V-5. **Finger Arteriolar Blood Pressure -** Beat-to-beat BP will be continuously monitored from the left middle or ring finger using photoplethysmography (FMS: Finometer, Pro; Amsterdam, Netherlands). HR and finger arteriolar BP will be viewed in real time on a computer screen and will be stored for offline analysis using customized programs written with LabView graphical software. **Brachial Blood Pressure** and **Oxygen Saturation (SaO_2)** - Brachial BP and SaO_2 will measured by a trained technician using a standard adult BP cuff (GE Healthcare Information Technologies, Milwaukee, WI) with a finger pulse oximeter at 1 minute intervals during administration of the sham and RIC conditions. The peak HR, BP and the minimal SaO_2 in baseline, inflation phase, deflation phase and post-RIC will be reported for statistical analysis.

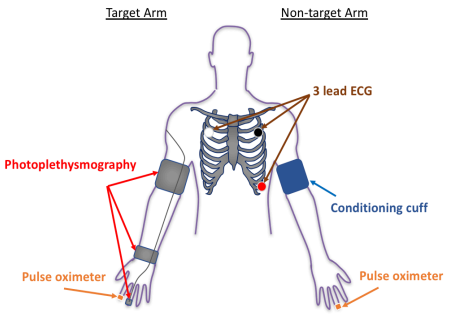
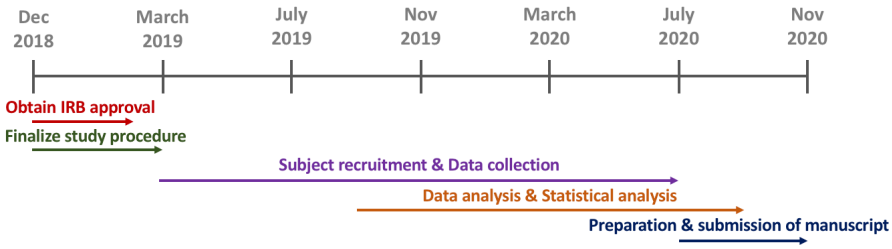


Figure 3. The equipment configuration of testing HR variability, respiratory rate and blood pressure changes during RIC/Sha conditioning.

Statistical analysis: Changes from baseline in HR, BP and SaO_2 will be compared following RIC and Sham conditions in the cSCI and Able-bodied groups separately using a 2 (RIC, Sham) by 4 (baseline, inflation, deflation and post-RIC) repeated measures ANOVA to measure the hemodynamic responses for the within subject variability, and to determine main and interaction effects with Tukey post-hoc analyses. If the data is not normally distributed and the assumptions of the ANOVA are not met, Wilcoxon signed rank tests will be used instead. Post hoc pairwise comparisons will be performed using the Bonferroni adjustment, if there are any main or interaction effects within the two independent variables. The pain scale will be also compare with the same ANOVA model.

Project timeline



The IRB protocol will be submitted within the first month of funding notification. We expect to obtain the full approval within two months. We estimate one participant with SCI and one able-bodied control every two months, so the total enrollment period would be completed in about 16 months. We will begin the data analysis after the first two or three participants and continue to examine subject responses and modify the protocol if and when necessary. During the final quarter of the funding period, we will prepare a manuscript to disseminate the findings.