Title: Diaphragm Pacing in Individuals with Spinal Cord Injuries

NCT 04179799
Study IRB-Approved Protocol

2/3/22

Protocol #: 201902285
IRB version: 10/09/2019
PI version: 2/3/2022

1. Project Title:

Diaphragm stimulation after human spinal cord injury: Effects on respiratory neural drive and function

2. Investigators:

PI: Emily J. Fox, PT, DPT, PhD	UF Dept. of Physical Therapy & Brooks Rehabilitation
Co-I: Alicia K. Vose, PhD, CCC-SLP	University of Florida
Co-I: Brian Yorkgitis, D.O.	UF Health Shands Jacksonville
Co-I: Kathryn Doughty, DPT	Brooks Rehabilitation
Co-I: Clayton Wauneka, PhD	Brooks Rehabilitation
Co-I: Amy Redding, M.Ed., CCC-SLP	Brooks Rehabilitation
Study Coordinator: Jennifer Mull, MSN, RN, CCRC	UF Health Shands Jacksonville
Study Coordinator: Yohan Diaz Zuniga, MD	UF Health Shands Jacksonville
Study Coordinator: Hannah Snyder, MS, ACSM-EP	Brooks Rehabilitation

3. Abstract:

Respiratory dysfunction is the leading cause of death in individuals with spinal cord injuries (SCIs). Nearly one-quarter of all SCI cases involve injury to the upper spinal cord segments which impairs neural activation of the diaphragm muscle and compromises breathing. Although mechanical ventilation can be life-saving after cervical SCI (C-SCI), it also triggers rapid and profound diaphragm muscle atrophy, thereby complicating (or even preventing) ventilator weaning. Intramuscular diaphragm stimulation, or diaphragm pacing, was developed to replace long-term ventilator support and is now used acutely post C-SCI (<4 months following injury) to promote ventilator weaning. Our group is part of a national team that initially reported 80% of C-SCI patients who underwent diaphragm pacing were successfully weaned from mechanical ventilation. Moreover, following electrode placement, gains in respiratory function were reported; remarkably, 36% of patients who received diaphragm pacing recovered independent breathing, enabling removal of the pacing electrodes. More recently, our team assessed the effects of diaphragm stimulation (pacing) longitudinally, from days to weeks after onset of diaphragm pacing demonstrating improvements in respiratory outcomes and EMG diaphragm activation (details below)**.** However, there are clear gaps in how such data can be interpreted to determine the unique effects of diaphragm pacing since many factors affected outcomes in our longitudinal assessments (i.e. patient comorbidities, secondary complications after SCI, variances in ICU care, etc). Thus, these newly proposed experiments will examine the effects of diaphragm stimulation within **carefully controlled test sessions and examine effects immediately after a controlled period of stimulation**. This will allow us to determine the unique effects of diaphragm stimulation. Specifically, we will measure respiratory function and diaphragm activation prior to and following two ~1-hour time blocks and a single ~12-hour time block of diaphragm stimulation in patients with traumatic SCI. Our team is experienced in working with these patients and the conduct of these measurements. Overall, the proposed experiments will allow us to assess the effects of diaphragm stimulation on respiratory neural drive and breathing function in individuals with acute, traumatic C-SCI.

4. Introduction & Specific Aim:

Respiratory dysfunction is a leading cause of death in individuals with spinal cord injuries; indeed, individuals with cervical lesions are at the greatest risk for respiratory complications leading to mortality.[1-3] Cervical spinal cord injury (C-SCI) causes primary neurological impairment of the respiratory muscles. Since 40% of C-SCI cases involve C1 to C5 injury, phrenic nerve activation will be impaired, weakening diaphragm contractions. Although mechanical ventilation can be lifesaving after C-SCI, it triggers rapid and profound diaphragm muscle atrophy, thereby complicating or preventing ventilator weaning.[4,5] Intramuscular diaphragm stimulation, or diaphragm pacing, was developed to replace ventilator support and is now used *acutely* to promote ventilator weaning in individuals with C-SCIs (< 4 months post C-SCI).[6] Our group is part of a national team that reported that the majority (80%) of patients with C-SCI who received diaphragm pacing were subsequently weaned from mechanical ventilation. Diaphragm pacing may improve respiratory function since 36% of patients receiving diaphragm pacing **recovered independent breathing**, enabling removal of the pacing electrodes.[6]

More recently, our team assessed the effects of diaphragm stimulation (pacing) longitudinally, from days to weeks after onset of diaphragm pacing and showed improvements in respiratory outcomes including increased tidal volume, forced vital capacity, and maximum inspiratory pressures. Preliminary analyses also highlight increased EMG diaphragm activation evident after use of pacing. Thus, accumulating evidence suggests that diaphragm pacing exceeds the current standard of care (*i.e.* mechanical ventilation), and may represent a rehabilitation strategy to improve and even restore lost respiratory function**.** However, there are clear gaps in how such data can be interpreted to determine the unique effects of diaphragm pacing since many factors affected outcomes in our longitudinal assessments (i.e. patient comorbidities, secondary complications after SCI, variances in ICU care, etc). Thus, a primary goal in this protocol is to examine the effects of diaphragm stimulation within carefully controlled test sessions. Here, we propose to systematically evaluate the impact of diaphragm pacing on diaphragm neuromuscular activation (EMGs) and breathing capacity after two ~1-hour time blocks and a single ~12-hour time block of diaphragm stimulation in adults with acute, traumatic C-SCIs. This work represents an essential step in determining the efficacy of intramuscular diaphragm stimulation and its effects on respiratory function and neural control of the diaphragm following C-SCI.

Research AIM:

The aim of this protocol is to test the hypothesis that diaphragm pacing enhances neuromuscular activation of the diaphragm in adults with C-SCIs and improves respiratory function. We will test the hypothesis by recording activity of the diaphragm muscle (electromyograms; EMGs) from intramuscular pacing electrodes. Recording from these surgically implanted electrodes allows comparisons of electromyogram recordings before and after short periods of diaphragm pacing to better isolate the unique contribution of diaphragm pacing on neuromuscular diaphragm activation. This approach, in association with respiratory assessments, will enable us to better determine the ***unique*** impact of diaphragm pacing in patients with acute, traumatic C-SCIs.

5. Background & Preliminary Data:

***SCI and respiratory dysfunction*.** One of the most serious and direct consequences of C-SCI is impaired breathing function. Indeed, respiratory dysfunction is a leading cause of death after SCI, and individuals with cervical injuries have the greatest risk for respiratory complications.[1-3] C-SCI interrupts descending spinal pathways to respiratory motor neurons, thereby causing respiratory muscle paralysis or paresis.[12] These primary respiratory control deficits are

exacerbated by inadequate respiratory defense and high rates of respiratory infection, atelectasis and altered breathing mechanics.[13] In 40% of C-SCI cases, the upper cervical segments (C1-C5) are involved,[3] causing severe respiratory impairment; these individuals often require mechanical ventilation. More than two-thirds of acute C-SCI patients will require ventilatory support (usually mechanical ventilation), and 40% will require continued ventilatory support after discharge from acute care.[14]

***Mechanical ventilation*.** Mechanical ventilation can be lifesaving and is the current standard of care when C-SCI patients cannot sustain independent breathing.[2,15] Unfortunately, mechanical ventilation causes rapid and profound diaphragm muscle atrophy, thereby complicating or even preventing ventilator weaning.[4] Ventilators essentially assume the role of respiratory muscles, leading to diaphragm muscle inactivity and profound atrophy and weakness. Numerous studies have demonstrated that mechanical ventilation induces diaphragm dysfunction,[16] a syndrome known as ventilator-induced diaphragm dysfunction or VIDD.[17,18] VIDD has been observed in animal models[19] and humans[18,20,21] with prolonged ventilatory support. Even brief periods (18-69 hours) of mechanical ventilation cause atrophy.[20,22] SCI patients on mechanical ventilation experience greater rates of respiratory infections and complications, primary determinants in the duration of hospitalization.[24] Moreover, ventilator-dependent individuals with SCI are 39 times more likely to die during the first year post-SCI, and 3.5 times more likely to die after the first year post-injury.[25,26]

***Intramuscular diaphragm stimulation (diaphragm pacing)*.** Intramuscular stimulation of the diaphragm, or diaphragm pacing, was pioneered by Onders and colleagues to replace long term mechanical ventilation.[11,31,32] The method is now used *acutely* post C-SCI to promote ventilator weaning and avoid ventilator-associated complications.[6] Compared with mechanical ventilation, patients using diaphragm pacing experience fewer respiratory infections exhibit improved speech and olfaction and report overall better quality of life.[10,33-35] Moreover, accumulating evidence, including both animal and humans reports, indicates that intramuscular diaphragm stimulation mitigates the negative impact of combined ventilatory support and SCI-induced inactivity.[36,37] Dr. Martin and colleagues extended this work, demonstrating that even brief, intermittent stimulation (1 min/ per hour) improves muscle fiber mitochondrial respiration in the stimulated hemidiaphragm in humans undergoing mechanical ventilation during cardiothoracic surgery.[23]

Recent Data

Since this report, our group recently studied longitudinal outcomes in 11 adults with chronic C-SCI who received surgical placement of a diaphragm pacer due to failure to wean from mechanical ventilation. Of the 11 participants, all had a traumatic cervical SCI, C1-C4, ASIA impairment scale A or B, motor complete (except 1 individual whose injury was classified as AIS C). A brief summary of respiratory and EMG outcomes is below.

Of the 11 adults who participated in this study, 9 of the 11 were successfully weaned from the ventilator and 6 out of those 9 were successfully weaned from the diaphragm pacer, meaning they resumed independent respiration and the diaphragm pacer was able to be stopped and in many cases, removed. Preliminary analyses of respiratory outcomes from these individuals have focused on comparing two early tests to determine the impact of diaphragm stimulation. Early tests were conducted ~1-7 days after pacer implant (Early 1) and then ~5-7 days later (Early 2). We also include data from the participant's final test, though the number of test sessions varied since some participants transferred to inpatient rehabilitation where testing was ongoing and others transferred to other facilities or remained in acute care due to complicated

discharge planning or medical needs. Figure 1 highlights the improvement in respiratory outcomes (tidal volume, forced vital capacity, and max inspiratory pressures) over the course of these time points. Also highlighted are pilot results from 3 individuals showing increased diaphragm activation indicated by greater EMG activity from Early 1 to Early 2 time points (Figure 2).

Figure 1

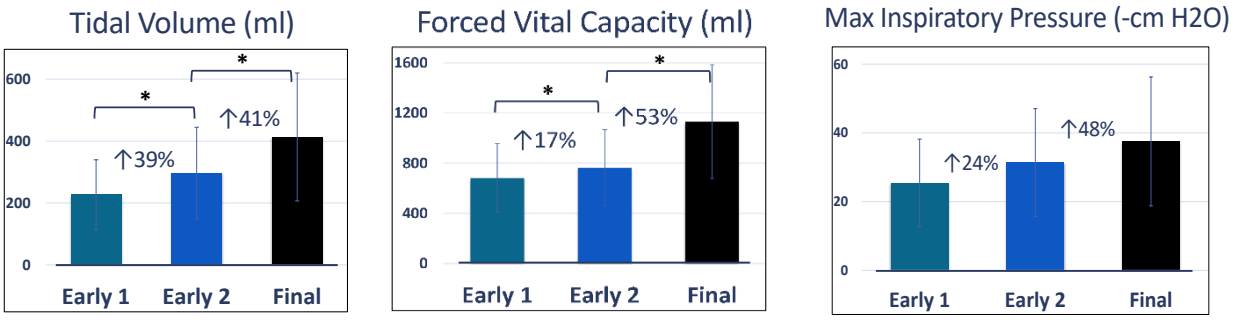
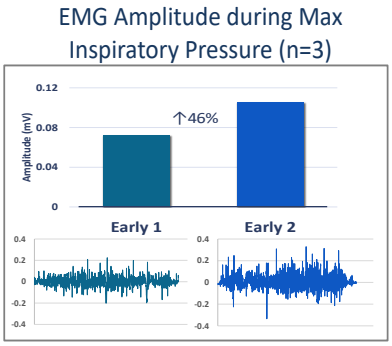


Figure 2



Summary: Overall, these data combined with previous evidence suggests the possibility that diaphragm pacing is a potentially legitimate rehabilitation strategy to improve and even restore respiratory function.[6-8,23] **The goal of this current protocol is to further examine the unique effects of diaphragm pacing in carefully controlled sessions shortly after implantation of the pacer.** This is an essential and critical next step in determining the efficacy of intramuscular diaphragm stimulation and its effects on respiratory neural drive and function after SCI.

6. Research Plan:

Inclusion and exclusion criteria.

Ten adults will be recruited who are ≥18 years old with acute, traumatic cervical spinal cord injuries (C-SCIs), classified according to the American Spinal Injury Association (ASIA) Impairment Scale (AIS) as A-C (complete SCI (A); motor complete SCI (B); motor incomplete with minimal motor function (C)),[58] affecting C1-C6 spinal cord segments, and who have been scheduled to undergo implantation of a diaphragm pacer, or who have recently received (in past 5-days) implantation of intramuscular diaphragm pacing electrodes due to severe respiratory impairments and dependence on mechanical ventilation. The inclusion criteria are based on our prior work with SCI patients and the injury characteristics of SCI patients with intramuscular pacing electrodes per clinical indications.[6,7]

Exclusion criteria include: progressive neuromuscular diseases such as multiple sclerosis and myasthenia gravis, history of neurologic injuries such as stroke or prior SCI, chest wall injuries or deformities likely to influence breathing, pregnancy or cognitive impairments limiting study participation.

Subject recruitment. Patients with acute, traumatic C-SCIs, scheduled to receive intramuscular diaphragm pacing OR who have recently received a diaphragm pacer (within past 5-days) will be recruited from UF Health Shands Jacksonville, a Level-1 trauma hospital serving North Florida. Potential study participants will be identified and informed of our study by Dr. Brian Yorkgitis (study Co-I and expert in surgical diaphragm pacer implantation). UF Health Shands provides care to more than 500 acute, traumatic SCI patients annually, including 100 C-SCI patients. From this population, more than 50 patients are estimated to have C-SCIs categorized as AISA-C and are potential candidates for intramuscular diaphragm pacing. We aim to complete successful tests in 5 individuals. To complete these tests, we anticipate enrollment of 10 individuals who meet study inclusion criteria. Study procedures will be conducted at UF Health Jacksonville. Dr. Yorkgitis routinely provides medical and surgical care to this study population.

Of note, we will aim to recruit and enroll participants once they are scheduled to undergo diaphragm pacing (i.e. before pacer placement) so that the baseline tests can be performed prior to implantation and post-implant assessments can be performed shortly after implantation of the diaphragm pacer. We recognize that this process may not always be feasible due to complexities in the patient's medical condition or status. In some cases a patient may undergo implantation of the diaphragm pacer and the device may not be turned on for days. We, therefore, may enroll participants up to 5-days following pacer implantation. This strategy will help ensure that we meet enrollment goals and include those who would like to participate but had delayed use of the pacer.

Dr. Yorkgitis will inform potential participants about the study and study-related procedures. Because Dr. Yorkgitis provides medical care and/or performs surgical diaphragm pacer implantation to this C-SCI population, he will identify potential participants who meet the inclusion/exclusion criteria. The specified criteria (determination of SCI injury level and status, respiratory function, medical history) are routinely part of this patient population's medical care and medical history. If a potential participant would like to learn more about the study, detailed information will be provided to the patient and their caregivers/family. The informed consent document will be used to guide this process. Dr. Yorkgitis also will notify the PI, Dr. Fox or a study Co-I that a patient would like to learn about the study and may be potentially interested in enrolling. Study coordinators, Jennifer Mull or Yohan Diaz Zuniga, who work closely with the study physicians, also may notify Dr. Fox and study Co-I. Based on this, Dr. Fox and/or a study Co-I will discuss the study with the patient and caregivers/family.

Study design & overview.

A single-factor quasi-experimental time-series study design is proposed. We will systematically evaluate diaphragm activation from EMGs recorded from intramuscular pacing electrodes and test inspiratory muscle strength and respiratory function in a small group of patients with acute, C-SCIs who have undergone implantation of diaphragm pacing electrodes.

An overview of the assessments is below, with details outlined in the subsequent sections.

Assessment Overview: We will study a small cohort of patients with acute C-SCIs following implantation of intramuscular diaphragm pacing wires. All individuals will meet inclusion criteria and will complete the informed consent process. Experiments are designed to determine if diaphragm stimulation (pacing) can produce neuroplastic changes in respiratory neural drive to the diaphragm and increase respiratory function. Neural drive to the diaphragm will be assessed by recording electromyograms (EMGs) from the intramuscular pacing wires following each of 3 blocks of diaphragm stimulation (Figure 3). Concurrent with these recordings from the diaphragm, respiratory function will be assessed using standardized clinical respiratory measures.

Diaphragm EMGs will be recorded from the intramuscular diaphragm pacing electrodes and respiratory function will be assessed during non-stimulated respiration (diaphragm pacing unit turned off). Diaphragm EMGs will be recorded from the intramuscular pacing electrodes with a custom connector that attaches to the external pacing electrode wires and interfaces with an EMG acquisition system. Concurrent with EMG recordings, each assessment will include tests of maximal inspiratory pressures to assess diaphragm muscle strength and maximal activation. Respiratory function also will be assessed by measurement of standard spirometry (tidal breathing, forced vital capacity or FVC).

To classify the level and severity of each subject's C-SCI, a clinical ASIA neurologic examination will be performed in accordance with the International Standards for Neurological Classification of Spinal Cord Injury. This standardized clinical examination will allow us to characterize segmental sensory and motor function.

Baseline Assessments

After obtaining informed consent for participation in the study, Dr. Fox or designated members of the study team (Alicia K. Vose, PhD, Kathryn Doughty (Cavka), DPT) will review the participant's medical records to obtain study-related medical and demographic data. Information obtained from the medical record will include information pertaining to health and medical history, details pertaining to the SCI, reports of medications and test results that could influence the individual's participation in the research procedures.. Additionally, clinical tests of sensory and motor function will be conducted to classify the level and severity of the individual's SCI. The tests of sensory and motor function are part of the American Spinal Injury Association (ASIA) Impairment Scale and the International Standards for Neurological Classification of Spinal Cord Injury. These clinical assessments are part of standard clinical care and include tests of sensation (dermatomes) and muscle strength via manual muscle testing (myotomes). These routine, standardized tests are done to clinically determine which segmental levels of the spinal cord demonstrate normal or impaired function. These tests also aid in determining if the SCI is clinically complete or incomplete. Determination of complete vs. incomplete is based on the sensory and motor function in the lowest sacral segments which control sensation and motor function at the anal sphincter. These standardized clinical tests are part of routine examination following spinal cord injury.

Baseline respiratory testing will be initiated once per day, up to 3 times on the days prior to diaphragm pacer implantation (Figure 3). Baseline respiratory tests include measurements of standard spirometry (tidal breathing, forced vital capacity or FVC) and maximum inspiratory pressures. We recognize that enrolling patients prior to diaphragm pacer placement may not always be feasible and we, therefore, may enroll participants up to 5-days following pacer implantation and will not obtain baseline (pre-implant) respiratory measures in these individuals.

*Pre-stimulation Assessment (post-diaphragm pacer placement)**

Following diaphragm pacer placement, the pre-stimulation assessment (prior to turning **on** the diaphragm stimulation) will be initiated approximately 18-24 hours after pacer implantation, once the patient is medically stable and able to participate. Waiting 18-24 hours after pacer implantation is standard of practice based on the procedures used by the diaphragm pacing surgical team (Dr. Yorkgitis). For individuals enrolled within 5-days after pacer implantation, the pre-stimulation assessment will occur as soon as possible after enrollment. The pre-stimulation assessment will include clinical respiratory tests and recording of diaphragm EMGs which are detailed in sections below. Pre-stimulation assessment will take approximately 2 hours to complete. To obtain a clear pre-stimulation assessment, two sets of tests will be conducted, about 1 hour apart. Ample time for rest breaks will be provided. The participant will be closely monitored and testing will be adjusted for each participant's comfort and medical needs.

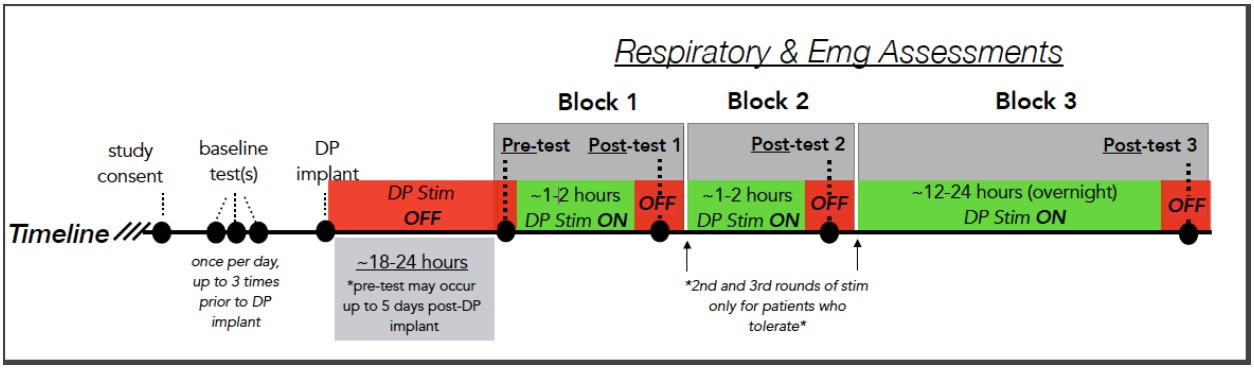
*Post-tests (following periods of diaphragm stimulation)**

Post-testing will occur after 3 different blocks of diaphragm stimulation, and will occur with the stimulator turned off (Figure 3). These blocks are contiguous, meaning block 2 will begin immediately after post-testing in block 1, and block 3 will begin immediately after post-testing in block 2. See Figure below. Of note, turning the stimulator off and on is a typical occurrence in the medical setting. This allows patients to get used to the stimulation and also is necessary to change batteries in the control box. This externalized control box is easily accessible and has simple controls for turning the unit on and off. Post-tests will include the same battery of clinical respiratory assessments and diaphragm EMGs as pre-testing and will take approximately 1 hour to complete. The duration of stimulation varies for each block:

Block 1: ~1 hour of stimulation (no longer than 2 hours*) followed by post-test 1

Block 2: ~ 1 hour of stimulation (no longer than 2 hours*) followed by post-test 2
Block 3: ~ 12 hours of stimulation (no longer than 24 hours*) followed by post-test 3

Figure 3



Dr. Yorkgitis will provide medical oversight of procedures. Assessments will be conducted by the PI, Dr. Fox and/or Alicia K. Vose, PhD and Kathryn Doughty (Cavka), DPT. A study research assistant may assist with the assessments. The study team has extensive experience with these assessments. Assessments are conducted in the intensive care unit, with the patient's care team easily accessible.

Given the medical condition of these participants, we will adjust timing or omit a test if necessary, as to not interfere with the participant's medical care. Furthermore, the timing of tests and duration of stimulation may vary slightly given the constraints of patients being in the intensive care unit (ICU) and coordination with nurses, physicians, and ICU scheduling. Additionally, these parameters may vary based on the safety and medical monitoring of each subject as directed by Dr. Yorkgitis.

During the assessments, photographs and/or video recordings may be obtained. These recordings will be done with the participant's knowledge and consent for the type of recording being obtained. Photographs and video recordings will be used to communicate study procedures with the study team and to monitor the assessment process. Video and photographic data will be used to assess how individuals with SCIs perform and respond to the testing and to communicate the study procedures. Video and photographic information will be used in a manner based on the level of consent obtained from each participant.

Diaphragm Stimulation: This protocol will not alter the implantation of the diaphragm pacer. This study will assess those who have received or who are planned to receive the device based on their medical needs and prior medical assessments (not associated with this study). Diaphragm stimulation settings are determined intraoperatively or immediately postoperatively by the surgeon (Dr. Yorkgitis) to maximize stimulation effectiveness and also to ensure patient comfort. Parameters such as stimulation amplitude, frequency, and breaths per minute are adjusted within a pre-set range based on the device usage guidelines. Typical values are 15-25mA at a frequency of 30 or 50Hz, and at a rate of 12-18 breaths per minute;[39,40] we expect stimulation parameters to be in this range.

Assessment of neuromuscular activation of the diaphragm. Neuromuscular activation of the diaphragm will be assessed by recording diaphragm EMGs from the surgically-implanted intramuscular stimulating electrodes.[11,75] This approach will allow for comparisons of EMG recordings across time and reduces the methodological limitations associated with surface or percutaneous EMG approaches.[9] EMGs will be recorded during non-stimulated respiration (diaphragm pacer turned off) and simultaneously with assessments of respiratory function. These assessments may include maximal inspiratory pressures and assessments of resting tidal breathing and measurement of flow volumes using standard spirometry.[76,77]

Primary outcomes to characterize neuromuscular activation of the diaphragm and assess changes in diaphragm activation associated with intramuscular diaphragm stimulation will include: amplitude and timing characteristics (raw, filtered EMGs and normalized);[76,77] neural respiratory drive to evaluate diaphragm muscle activation during tidal breathing relative to activation capacity during maximal inspiratory maneuvers;[77,78] and time-frequency characteristics of the diaphragm EMGs (wavelet analysis).[75,79-81] Methods pertaining to diaphragm EMG signal acquisition and processing are explained below.

Diaphragm EMG acquisition and signal processing. The PI has extensive experience with EMG acquisition and analyses in humans.[69,70] EMGs will be recorded from the externalized pacing wires which connected to each of four intramuscular electrodes (2 in each hemidiaphragm). The implanted, remote sub-cutaneous electrode will serve as the ground.[11] A custom-connector (Synapse Biomedical, Oberlin, OH) will be used to link the exposed pacing wires to our EMG acquisition system. EMG signals will be amplified and band pass filtered (10 Hz to 1000 Hz), acquired at a sampling frequency of 2 kHz, and saved to a computer for off-line analysis.

To acquire the diaphragm EMG signals, the pacer will be shut off to allow connection to the EMG acquisition system. It is feasible to shut off the diaphragm pacer for several reasons.

First, most of the individuals with SCI have residual respiratory control and are able to maintain sufficient respiration for a short time period. It is part of standard procedures to turn off the pacer on a regular basis to change the pacer batteries. Consistent with this process (turning off the pacer when changing the batteries), supplementary respiratory support will be provided as needed (i.e. mechanical ventilation) and oxygen saturation will be monitored at all times to ensure adequate respiration (>90% O2 saturation) during the assessments. Second, all assessments will be overseen by the participant's clinical medical team (ICU) with oversight from the participant's physician. All assessments will include cardiorespiratory monitoring. Finally, an assessment will be halted at any time the patient indicates distress or indicates that he or she would like to stop the assessment. In this case, the pacer will be re-connected and turned on and respiratory support will continue until the patient is comfortable.

Neuromuscular activation outcomes. Raw, filtered EMGs, as well as normalized signals will be assessed for timing and amplitude characteristics. Normalization will be based on maximal amplitude of the signals recorded during tests of maximal inspiratory pressure and sniff nasal inspiratory pressure.[76,78,89] Signals will be full-wave rectification and amplitudes calculated based on the root mean square or integral of the signal. Comparisons of normalized signal amplitudes, burst timing, and burst durations will provide evidence of changes in neuromuscular activation of the diaphragm in association with intramuscular stimulation of the diaphragm and following C-CSI. Increased EMG amplitudes are indicative of greater motor unit recruitment and suggest gains in muscle force generation.[65,75,90]

To determine the relationship between diaphragm muscle activation during resting tidal breathing and diaphragm activation capacity during maximal inspiratory maneuvers, the relative level of "neural respiratory drive" will be calculated from the normalized diaphragm EMGs. Neural respiratory drive is a measurement approach used to quantify the respiratory load/capacity relationship and is a marker of respiratory disease severity.[78,89,91,92] Changes in neural respiratory drive are associated with changes in respiratory function and breathlessness during exercise in patient populations.[78,92] Neural respiratory drive will be calculated based on the root mean square of the diaphragm EMG signals during resting tidal breathing normalized to the maximal EMG values obtained during the maximal inspiratory maneuvers.[89]

Finally, time-frequency characteristics of diaphragm EMGs will be assessed using wavelet analysis.[75,80,93] Continuous wavelet analysis using a Morlet wavelet will be used to decompose the diaphragm EMG signals into individual frequency components as a function of time. The Morlet wavelet will be used because it provides a reasonable balance between time and frequency localization.[94] Wavelet transforms of the EMG signals will be calculated using a base algorithm in Matlab (The Mathworks, Natick MA) developed by Torrence and Compo and available at URL: http://paos.colorado.edu/research/wavelets.[95] Comparisons of the frequency characteristics of the EMGs recorded during different respiratory maneuvers and longitudinally will provide novel insight regarding motor unit recruitment, firing frequencies, muscle fiber changes, as well as central activation of phrenic motor neurons.[75,80,81]

Higher frequencies of the EMG suggest greater recruitment of larger muscle fibers and faster conduction velocities. For instance, a shift toward higher frequencies during more forceful maneuvers or over-time would suggest recruitment of larger, more forceful motor units with higher recruitment thresholds.[96,97] Such changes would support our previous observations[8,23] and others[11,36] that intramuscular stimulation promotes neural and muscular adaptations.

Assessments of diaphragm muscle strength and respiratory function.

Diaphragm muscle strength. PI, Dr. Fox, has experience with diaphragm and respiratory assessments. Maximal inspiratory maneuvers are typically used as an index of diaphragm muscle strength.[98] Measures will be obtained in accordance with the American Thoracic Society testing guidelines[100] and adjusted for SCI patients.[101] Inspiratory pressures will be acquired using a digital respiratory pressure meter or a clinical respiratory monitor connected to a laptop computer. Subjects will exhale to residual volume and then attempt a maximal inspiration for at least 2s in duration. Subjects requiring mechanical ventilation will use a manometer connected to the subject's endotracheal tube or tracheostomy and attached to a one-way valve, allowing only expiration.[102] All maneuvers will be performed ~3-5 times with a minimum of 60s intervals between tests or rest breaks as needed by the participant.

Respiratory function. In addition to the above assessments, respiratory function will be assessed by measurement of standard spirometry to measure forced vital capacity (FVC) and tidal breathing. A digital spirometer or clinical respiratory monitor connected to a laptop computer will be used to obtain forced flow volume curves/loops and to assess tidal breathing. Testing will be performed in accordance with the American Thoracic Society guidelines and adjusted for SCI patients.[100,101] Percent predicted values will be calculated based on uninjured adults of the same age, gender and height.[103]

Overall, a battery of clinical respiratory assessments will be used to measure inspiratory or diaphragm muscle strength, inspiratory pressures, and respiratory function. Testing procedures will be standardized (consistent test positions, standardized procedures, randomized testing order) and conducted in accordance with established guidelines to allow for appropriate comparisons.[100,101] Results from this battery of respiratory assessments will allow us to characterize changes in inspiratory strength and respiratory function across time and in association with diaphragm pacing, and to determine the association of such changes with diaphragm activation.

Safety monitoring. Our protocol will not alter medical care. Oversight of study procedures will be provided by Dr. Yorkgitis, and study collaborators and trauma surgeons who provide acute SCI care, including diaphragm pacer implantation. The PI, Dr. Fox, will oversee all assessment procedures and will maintain regular communication with the study physicians and study team.

Medical monitoring during assessments in the ICU hospital setting will include bedside monitoring of vital signs including heart rate, blood pressure, oxygen saturation, and may include electrocardiogram. If the patient is receiving ventilator support, ventilator settings and pulmonary mechanics also will be monitored. Additionally, subjects will be monitored for signs of autonomic dysreflexia which include complaints of headache, sweating above the lesion level, elevated blood pressure and bradycardia. Standard guidelines when autonomic dysreflexia is suspected will be followed[104] and appropriate medical care and monitoring will be provided.

Statistical analysis. As mentioned above, we are conducting an initial proof of concept experiment in a small (n=10) sub population of individuals with C-SCI. Summary statistics will be provided for demographic and clinical characteristics. We do not expect the data to have a normal distribution and therefore plan to use non-parametric statistical approaches. A Mann-Whitney U will be used to test for differences in the EMG and respiratory outcomes pre and post stimulator activation.

Interpretation and possible outcomes If study outcomes are consistent with our preliminary data[6-8,23] and a published report,[11] we expect to demonstrate increased neuromuscular activation of the diaphragm and improved respiratory function associated with intramuscular

stimulation of the diaphragm. This outcome would suggest a rehabilitative effect and that diaphragm pacing is a therapeutic strategy to promote respiratory function after C-SCI. Further, changes in neuromuscular activation would provide evidence that diaphragm pacing activates myogenic pathways and could be a trigger for spinal respiratory plasticity. These outcomes would justify and inform a larger diaphragm pacing trial and identify candidate mechanisms underlying restoration of function.

It is possible (and perhaps likely) that some individuals will demonstrate gains in respiratory function and others will not. This outcome may be associated with variations in SCI lesion characteristics and spared spinal pathways mediating changes in respiratory control. This result would afford the chance to begin looking for determinant factors associated with 'responders' to diaphragm pacing and 'non-responders', i.e. individuals who benefit from pacing by weaning from mechanical ventilation, but who do not demonstrate gains in respiratory function or diaphragm activation. Some individuals also may exhibit changes in neuromuscular activation, but no apparent gains in respiratory function. This outcome also could suggest that changes in neuromuscular activation occur before an overt change in respiratory control is detectable.

Participant compensation To compensate participants for their time and involvement in the assessment procedures, study participants will be paid fifty ($50.00) for each assessment. Payments will be made through the U of Florida Human Subject Payment system using payment cards. Since up to 8 assessments may be conducted (7 EMG/respiratory assessments and the ASIA sensory/motor exam), each participant may receive up to $400.00 for completion of study procedures.

7. Possible Discomforts and Risks:

There is a low risk of harm associated with the assessments in this protocol. All procedures will be closely monitored and fully explained to the participants (using lay-language, understandable terms) so that they understand what to expect and the reasoning for the procedures. They will be informed that they may refuse testing or discontinue study participation at any time. For participants who have difficulty or who are unable to communicate verbally, a communication system will be implemented. For instance, the study team will monitor facial expressions and use eye blinking by the participant to respond yes/no or signal discomfort. Participants may be at risk for autonomic dysreflexia, although the incidence is low in patients with acute injuries. Participants will be informed to alert the study team if they notice symptoms consistent with autonomic dysreflexia (head ache, sweating above the lesion, elevated blood pressure). The study team will closely monitor the participants and is trained to assess and respond to signs of dysreflexia. All procedures will occur in the ICU setting with the participant's medical team immediately available.

The clinical ASIA snesory and motor examination of neurologic function is part of standard clinical assessments after SCI and is non-invasive. There is no specific risk of this assessment which includes standard tests of sensation and motor function (strength). Participants may feel frustrated or uncomfortable with the testing because they are unable to feel sensations and move their limbs as they did prior to SCI. This may upset some individuals. It may be uncomfortable for some participants to undergo testing in the peri-anal region. This testing is part of the standard care after SCI. Some participants may feel pain or uncomfortable sensations during the testing. The procedures will be adjusted to be as comfortable as possible and the participants will be closely monitored.

The respiratory assessments also are part of standard clinical care after SCI and in individuals with respiratory impairments. These assessments may cause anxiety and may challenge an individual's breathing and cause participants to feel out of breath. These discomforts will be explained. Participants will be closely monitored as previously described. At any time the assessments may be discontinued and breathing support provided as indicated (i.e. re-connect to the ventilator) or turn back on the diaphragm stimulator or supplemental oxygen support.

Recording of diaphragm EMGs from the external pacing wires is not part of clinical care. This procedure does not pose direct risk or likelihood of harm. It is a non-invasive procedure since the pacing wires are already implanted and externalized. The participant may feel breathless or uncomfortable with the device turned off. Some participants may feel nervous about turning the pacer off. However, as previously described, this is part of standard procedures during regular battery changes and cleaning of the connectors. Our team is experienced in working with patients and helping them feel 'OK' and safe with the pacer turned off. The participant will be closely monitored and supplemental respiratory support will be readily available. At any time the assessments may be discontinued and breathing support provided as indicated (i.e. re-connect to the ventilator or turn back on the diaphragm stimulator or supplemental oxygen support).

8. Possible Benefits:

There is no direct benefit to participants enrolled in this study. This study will involve assessments to determine the effects of diaphragm pacing in adults with C-SCIs. The implementation of this intervention (diaphragm pacing) will not be altered by this study. Our goal is to monitor and study the effects of this intervention after short duration, carefully controlled sessions of stimulation. The outcomes from this study may benefit future individuals with C-SCIs or other clinical populations with respiratory impairments.

The risks associated with this study are reasonable because overall the risks are minimal and there is potential benefit to future individuals with C-SCIs, which is a severe catastrophic condition. This study is an essential step in determining the efficacy of intramuscular diaphragm stimulation and its effects on respiratory function after SCI. Outcomes from this study may alter and/or improve respiratory care post-C-SCI. Currently, respiratory illness and complications are the leading cause of illness and death after SCI. Advancements in respiratory care and long-term management are needed to improve outcomes post-SCI and improve health and quality of life after SCI.

9. Conflict of Interest:

The investigators associated with this research project have no known conflicts of interest.

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