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TITLE: Acute Intermittent Hypoxia and Respiratory Strength Training to Improve Breathing Function After SCI

PRINCIPAL INVESTIGATOR: Emily Fox

CONTRACTING ORGANIZATION: University of Florida, Gainesville, FL

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14. ABSTRACT

Background: Spinal cord injury (SCI) disrupts neural pathways to respiratory motor neurons, causing muscle paralysis and decreased breathing capacity. Since respiratory impairment is the major cause of illness and death with SCI, it is critical to devise new strategies to restore breathing function. One promising strategy to restore breathing capacity following SCI is to strengthen spared neural pathways by inducing spinal neuroplasticity. Our research group has developed novel methods to induce spinal respiratory plasticity in rats via repetitive exposure to brief episodes of low oxygen (acute intermittent hypoxia or AIH). In rats with incomplete SCI, repeated AIH restores lost breathing ability. These preclinical findings translate to humans with SCI; a single day of AIH, or daily AIH for 5 days (dAIH; 1-2 min of 9% oxygen, 1 min intervals), induces recovery of respiratory and non-respiratory motor function (such as walking or hand function). We demonstrated that AIH increases respiratory function in humans with chronic SCI. However, additional pre-clinical studies demonstrate that AIH-induced functional benefits are enhanced by combining AIH with task-specific training. Unfortunately combined dAIH and task-specific respiratory training has not been studied despite the promise of this novel therapeutic approach. It is essential to fill this knowledge gap as we work to translate this simple, safe and effective treatment modality to restore breathing function in Veterans with breathing impairment due to SCI. Central Hypothesis: Combined dAIH and respiratory strength training will elicit greater and more sustained gains in respiratory function than either treatment alone in people with chronic SCI.

Aim 1: Test the hypothesis that combined dAIH and respiratory strength training improves respiratory function more than dAIH or respiratory strength training alone. In adults with chronic, incomplete SCI and demonstrated breathing impairment, the effects of combined dAIH (5 days; 15, 1.5-min episodes of 9% oxygen; 1-min room air intervals) and respiratory strength training (inspiratory and expiratory training; 4 sets of 6-10 breaths per day; pressure-threshold device set to 80% of each individual's maximum) will be compared to the effects of each treatment alone and sham protocols.

Aim 2: Test the hypothesis that functional improvement from combined dAIH and respiratory strength training elicits more sustained effects than either treatment alone. Functional respiratory gains 3 days and 1week (primary outcome) after 5-day interventions will be assessed as an absolute change from pre-intervention, and as a fraction of the gains observed 1 day post-intervention.

Study Design: A double-blind, placebo-controlled, randomized, cross-over design is proposed. Participants will include 53 adults with chronic, incomplete SCI with >20% respiratory impairment based on maximal inspiratory or expiratory pressure generation (average annual enrollment is 14).

Clinical Impact: Innovative strategies are needed to reduce the impact of respiratory dysfunction in Veterans and civilians with SCI, particularly since the incidence of SCI has increased in the recent military conflicts, and recovery rates for battle-induced SCIs are lower than for non-combat SCIs.

Study Status: All local and federal regulatory approvals have been maintained. Research procedures have been reinstated following Covid-19 pandemic shutdown and remain ongoing in Year 4. Recruitment strategies are ongoing. A total of 21 individuals have enrolled and 17 have completed procedures. Study staff and procedures are established with ongoing concordance training and procedures updates.

15. SUBJECT TERMS

None listed.

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1. INTRODUCTION:

RESPONSE: The object of this research project is to test the hypothesis that combined acute intermittent hypoxia (AIH) and respiratory strength training will elicit great and more sustained gains in respiratory function than either treatment alone in people with chronic spinal cord injury (SCI). Aim 1 will test the hypothesis that combined AIH and respiratory strength training improves respiratory function more than AIH or respiratory strength training alone. Aim 2 will test the hypothesis that functional improvements from combined AIH and respiratory strength training elicits more sustained effects than either treatment alone. Functional respiratory gains 3 days and 1week (primary outcome) after 5-day interventions will be assessed. A double-blind, placebo-controlled, randomized, cross-over design will be used. Participants will include 53 adults with chronic, incomplete SCI with >20% respiratory impairment. Innovative strategies are needed to reduce the impact of respiratory dysfunction in Veterans and civilians with SCI, particularly since the incidence of SCI has increased in the recent military conflicts and respiratory impairment is the major cause of illness and death after SCI.

2. KEYWORDS:

RESPONSE: Respiratory function, breathing impairment, human SCI, chronic SCI, acute intermittent hypoxia, respiratory strength training, spinal plasticity

3. ACCOMPLISHMENTS:

What were the major goals of the project?

RESPONSE:
The Major Tasks for the entire study period, as stated on the approved SOW are:
Major Task 1: Coordinate and establish study operations which includes:

-Subtask 1-Prepare regulatory documents and study protocols
Milestone: IRB Approval
Milestone: HRPO approval
-Subtask 2-Complete training of personnel
-Subtasks 3-Establish study operations and assess adherence
Milestone: Site and personnel operations established

Major Task 2: Conduct a randomized, double-blind, repeated measures crossover study
-Subtask 1-Initiate and continue recruitment
Milestone: Subject enrollment and testing underway
-Subtask 2-Conduct testing and intervention protocols
Milestone: Complete data collection on 42 of 53 enrolled subjects

Major Task 3: Draft manuscript on the effect of combined dAIH and respiratory muscle strength training in humans with chronic incomplete SCI
-Subtask 1-Analyze study results
Milestone: Submit manuscript

What was accomplished under these goals?

RESPONSE:

The following activities and objectives have been addressed:

Major Task 1: Coordinate and establish study operations was largely achieved in Year 2 (2019 to 2020) with ongoing aspects and continual updates as necessary.

Subtask 1-Prepare regulatory documents and study protocols:

In Year 2 (2019 to 2020), milestones for IRB approval and HRPO approval were achieved.

Subtask 2 - Complete training of personnel

Study personnel are established and personnel have been cross-trained in all aspects of this research protocol. Training has been completed for all aspects such as equipment operations and cleaning, completion of respiratory tests, trouble shooting of tests and equipment, implementation of respiratory strength training, recording of surface electromyograms for respiratory function tests, administration of spinal cord injury (SCI) assessments, administration of SCI questionnaires, data storage, file organization, data security, communication and scheduling. Regular meetings are ongoing with study personnel and study leadership, PIs Fox and Mitchell. Initial training and achievement of Subtask 2 was completed in Year 2 (2019 to 2020). Ongoing training and ensuring concordance in all procedures is ongoing in years 3 and 4. Post-doctoral Fellow, Dr. Michela Mir, CCC-SLP, PhD joined the study team and has been trained in all study aspects.

Aligned with the training of early career scientists, post-doctoral fellows Joseph Welch and Dr. Jay Nair accepted faculty positions and completed their roles on this project.

Subtask 3 - Establish study operations and assess adherence

Operations are established and documented in the study procedures manual (Year 2, 2019 to 2020). In Years 3 and 4, Regular review and training sessions were attended to reinforce study operations, continue troubleshooting and to ensure adherence. Personnel were also cross-trained to help ensure sufficient coverage of study procedures.

A major aspect of operations is subject recruitment. We work closely with our study coordinators and Brooks Rehabilitation Research Recruitment specialists to maintain robust recruitment procedures. Recruitment includes both active and passive strategies with Brooks Rehabilitation and in the community. Recruitment was expanded to include involvement of the Brooks Rehabilitation SCI Day Treatment Program and the Inpatient Hospital. Although persons in the inpatient hospital are not eligible for participation, they have the opportunity to register with an IRB approved Brooks research registry which allows them to be contacted in the future regarding research participation.

Major Task 2: Conduct a randomized, double-blind, repeated measures crossover study

Subtask 1-Initiate and continue recruitment

Following the Covid-19 research shutdowns, approvals for Research Resumption from the University of Florida (August 2020) recruitment was initiated in August, 2020. At the completion of Year 4 (2021 to 2022) 25 participants have signed a screening informed consent form and 21 participants were enrolled. Seventeen individuals have completed study procedures at the conclusion of Year 4. Recruitment is ongoing.

Subtask 2-Conduct testing and intervention protocols

As described above and previously, research procedures for testing and intervention got underway in October 2020. At the completion of Year 4, 25 participants have signed a screening informed consent form and 21 participants have been enrolled in study procedures.

Major Task 3: Draft manuscript on the effect of combined dAIH and respiratory muscle strength training in humans with chronic incomplete SCI

Subtask 1-Analyze study results

Major task 3 has not been initiated since we are currently conducting testing and intervention protocols.

What opportunities for training and professional development has the project provided?

RESPONSE: This project is not specifically intended to provide training and professional development opportunities. However, the team members have been engaged in several training opportunities. Training and professional development aspects in this third year and ongoing include:

a) Engagement of the postdoctoral research associates and study team members in monthly scientific seminars, many seminars given by international experts in topics focused on control of breathing, effects of neurologic injury, central nervous system regulation of motor functions, and rehabilitation. Specifically, team members are engaged with the UF BREATHE Center (Breathing Research and Therapeutics) and the Control of Breathing and Airway Defense seminar series.
b) Engagement of study personnel, including postdoctoral research associates in weekly journal club discussions focused on rehabilitation, control of breathing, intermittent hypoxia, neurologic injuries such as SCI, rehabilitation and nervous system plasticity.
c) Team members are engaged with study leadership, one on one mentorship, specific training in aspects such SCI participant safety monitoring, test procedures, respiratory training procedures, as well as research regulations and data security.
d) Post-doctoral fellows, Drs. Joe Welch, Alicia Vose, and Jay Nair, were mentored by Drs. Fox and Mitchell to develop and submit research funding proposals and manuscripts related to the advancement of therapeutic intermittent hypoxia and responses in people with SCI. Each have presented scientific abstracts.

How were the results disseminated to communities of interest?

RESPONSE: Results from this study were not disseminated, as the study is still underway. However, there have been many activities undertaken to reach members of the scientific and clinical communities, as well as to advance the study of therapeutic intermittent hypoxia and interventions for breathing recovery after SCI. Drs. Fox and Mitchell were awarded a follow-up DoD SCI Clinical Trial Award to test new protocols of intermittent hypoxia, as well as genetic biomarkers associated with AIH-induced responses.

What do you plan to do during the next reporting period to accomplish the goals?

RESPONSE:
The primary goals are recruitment and enrollment of subjects, as well as testing and intervention procedures to complete the study goals (Major Tasks 1 and 2). We will continue to focus on recruitment strategies to meet enrollment goals. The study team is working collaboratively with other ongoing SCI research trials at Brooks to ensure recruitment databases are updated and individuals (Brooks' patients) who wish to be contacted for research may be informed of this clinical trial. PIs Fox and Mitchell will continue to work closely with team members to elevate methods, provide training, communication and mentorship to advance the team and create learning opportunities that will benefit this clinical trial and our long-term SCI research objectives. Data analysis procedures will be undertaken to review study outcomes and prepare for final steps in data analysis and results reporting.

4. **IMPACT:**

RESPONSE: Nothing to Report.

What was the impact on other disciplines?

RESPONSE: Nothing to Report.

What was the impact on technology transfer?

RESPONSE: Nothing to Report.

What was the impact on society beyond science and technology?

RESPONSE: Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

RESPONSE: Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

RESPONSE: Delayed approval of the study no cost extension has caused some confusion as to the future of the study and to operations such as replacing equipment and replenishing supplies.

Changes that had a significant impact on expenditures

RESPONSE: Delayed approval of the study no cost extension has caused some confusion as to the future of the study and to operations such as replacing equipment and replenishing supplies.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects-
RESPONSE: Nothing to Report.

Significant changes in use or care of vertebrate animals-
RESPONSE: Not applicable to this human subject clinical trial.

Significant changes in use of biohazards and/or select agents-
RESPONSE: Nothing to Report.

6. PRODUCTS:

• **Publications, conference papers, and presentations**

Journal publications.
RESPONSE: Nothing to Report

Books or other non-periodical, one-time publications.
RESPONSE: Nothing to Report.

Other publications, conference papers and presentations.

• **Website(s) or other Internet site(s)**
RESPONSE: The following publications/abstract/presentations were submitted or published. Study goals, methods and status were reported.

1. Mir MJ, Vose AK, Brunetti G, Cavka K, DeMark L, Hannah S, Wauneka CN, Welch, J, Nair J, Mitchell GS, Fox EJ. Effects of acute intermittent hypoxia on respiratory drive in humans with chronic spinal cord injury. American Physiology Summit. Long Beach, CA. April 20-23, 2023. Abstract accepted.
2. Vose AK, Welck J, Cavka K, Brunetti G, DeMark LA, Wauneka CN, Mir M, Nair J, Snyder H, Mitchell GS, Fox EJ. Acute Intermittent Hypoxia and Respiratory Strength Training to Improve Respiratory Function in Individuals with Chronic Spinal Cord Injury: Preliminary Outcomes. American Physiology Summit. Long Beach, CA. April 20-23, 2023. Abstract accepted.
3. Nair J, Welch J, Argento P, Lurk C, Hou T, Lu Q, Mitchell G, Fox EJ. Genetic biomarkers of therapeutic acute intermittent hypoxia-induced respiratory motor plasticity in humans. American Physical Therapy Association Combined Sections Meeting. San Antonio, Tx. February 2022. Poster presented.
4. Welch JF, Nair J, Argento PJ, Mitchell GS, Fox EJ. Acute intermittent hypercapnic-hypoxia elicits central neural respiratory motor plasticity in humans. J Physiol. 2022 Apr 28.

• **Technologies or techniques**

RESPONSE: Nothing to Report.

• **Inventions, patent applications, and/or licenses**

RESPONSE: Nothing to Report.

• **Other Products**

RESPONSE: Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

RESPONSE:

Name: Emily Fox, DPT, PhD, NCS
Project Role: Principal Investigator
Researcher Identifier: ORCID ID - 0000-0003-0142-3497
Nearest person month worked: 5
Contribution to project: Dr. Fox provided oversight of all activities including procedures, personnel training, refinement of study protocol, compliance with regulations and documentation, leadership of investigative team, regular communications, data storage and secure data sharing, as well as oversight of recruitment and data analysis.

Name: Gordon Mitchell, PhD
Project Role: Co-Principal Investigator
Researcher Identifier: ORCID ID- 0000-0002-8489-1861
Nearest person month worked: 1
Contribution to project: Dr. Mitchell provided oversight of study activities including refinement of procedures, compliance with regulations, leadership and training of investigative team, data analysis and communication strategies.

Name: Joseph Welch, PhD
Project Role: Post-doctoral scientist
Researcher Identifier: NA
Nearest person month worked: 5
Contribution to project: Dr. Welch contributed to the refinement of respiratory testing and ongoing advancement of the study manual of procedures. He also participated in study testing procedures, study communications, and development of data analysis.
Funding Support: Craig H. Neilsen Foundation Post-doctoral Fellowship

Name: Jay Nair, PT, PhD
Project Role: Post-doctoral fellow
Researcher Identifier: NA
Nearest person month worked: 5
Contribution to project: Dr. Nair contributed to study procedures, testing participants, and study communications.

Name: Alicia Vose, PhD, CCC-SLP
Project Role: Post-doctoral scientist
Researcher Identifier: NA
Nearest person month worked: 10
Contribution to project: Dr. Vose has contributed to the refinement of respiratory testing and ongoing updates to the study manual of procedures. She assisted conducting intervention procedures for respiratory training, participant testing procedures, study communications and data management as well as data analysis.

Name: Michela Mir, PhD, CCC-SLP
Project Role: Post-doctoral scientist
Researcher Identifier: NA
Nearest person month worked: 10
Contribution to project: Dr. Mir has trained in study procedures, contributed to study assessments. She also participated in study communications and data management as well as data analysis.

Name: Lou DeMark, DPT, NCS
Project Role: Study Coordinator
Researcher Identifier: NA
Nearest person month worked: 4
Contribution to project: Lou DeMark assisted with study procedures, particularly recruitment, and participant screening. Lou has focused on recruitment planning and ensuring communication, data storage and sharing, recruitment materials, communications, and oversight of regulatory aspects.

Name: Kathryn Cavka, DPT, NCS
Project Role: Research Physical therapist
Researcher Identifier: NA
Nearest person month worked: 6
Contribution to project: Kathryn Cavka conducted study procedures including respiratory testing, oversight of intervention procedures, conduct of clinical tests, study communication, documentation, data organization and training of team members.

Name: Clayton Wauneka, PhD
Project Role: Clinical Research Engineer
Researcher Identifier: NA
Nearest person month worked: 5
Contribution to project: Clay Wauneka has provided oversight of lab operations, set up of equipment, equipment maintenance, computer support, assist with testing procedures and data acquisition, data storage and security, equipment and software oversight.

Name: Gina Brunetti, DPT, NCS
Project Role: Research Physical therapist
Researcher Identifier: NA
Nearest person month worked: 5
Contribution to project: Gina Brunetti conducted study procedures including respiratory testing, conduct of clinical tests, study communication, documentation, data organization and training of team members.

Name: Hannah Snyder, MS
Project Role: Research Assistant and Coordinator
Researcher Identifier: NA
Nearest person month worked: 5
Contribution to project: Hannah contributed to procedures, coordinating and oversight of recruitment efforts, documentation, data organization and assist with data storage and regulatory documentation.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

RESPONSE: Overall, there is no significant change that impacts the level of effort of Key Personnel

New Award
***Title:** Transcutaneous spinal direct current stimulation to enhance locomotor rehabilitation after SCI
*Major Goals: Specific aim 1 will determine the effect size and variance of response to combined tsDCS and LT to enable power analysis for a larger clinical trial.
Specific aim 2 will test the hypothesis that tsDCS+LT increases spinal motor excitability and reflex modulation.
*Status of Support: Active
Project Number: 890032
Name of PD/PI: **Fox**
*Source of Support: Craig H. Neilsen Foundation
*Primary Place of Performance: Brooks Rehabilitation/ University of FL
Project/Proposal Start and End Date: 9/30/2022-9/29/2024
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period:
2022: 0.3
2023: 1.0

New Award
***Title:** Genetic biomarkers of intermittent hypoxia-induced respiratory motor plasticity in chronic SCI
*Major Goals: This clinical trial will examine genetic biomarkers in the saliva as a predictor of acute intermittent hypercapnic hypoxia-induced respiratory responses in people living with chronic spinal cord injury
*Status of Support: Active
Project Number: W81XWH-21-SCIRP-CTA
Name of PD/PI: **Fox** (Co-PI **Mitchell**)
*Source of Support: Department of Defense CDMRP
*Primary Place of Performance: Brooks Rehabilitation/University of FL
Project/Proposal Start and End Date: 9/30/2022-9/29/2026
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period:
2022: 0.9 (Fox); 0.4 (Mitchell)
2023: 1.5 (Fox); 0.8 (Mitchell)

New Award
***Title:** Breathing Research and Therapeutics
Major Goals: This grant is an institutional pre- and post-doctoral training grant devoted to breathing research and therapeutics in the Breathing Research and Therapeutics Center at the University of Florida.

* Status of Support: Active
* Project Number: T32HL134621
* Name of PD/PI: **Mitchell, G.S.**
* Source of Support: NIH/NHLBI
* Primary Place of Performance: University of Florida, Gainesville
* Project/Proposal Start and End Date: 8/2022 - 7/2027
* Project/Proposal Start and End Date: 8/1/2022 - 7/31/2027
* Total Award Requested (including Indirect Costs):
* Person Months (Calendar/Academic/Summer) per budget period.
2022: 0.45
2023: 0.6

What other organizations were involved as partners?

RESPONSE:

As detailed in the Statement of Work, Brooks Rehabilitation is a partner organization on this funded project.

-Organization Name: Brooks Rehabilitation, Brooks Clinical Research Center
-Location of Organization: Jacksonville, FL (main offices/facilities) and surrounding areas (associated clinical centers)
-Partner's contribution to the project: As detailed in our study description, IRB-approved protocol and regulatory documentation, Brooks Rehabilitation is involved in providing facilities for research activities and collaborating personnel. The University of Florida College of Public Health & Health Professions has a research collaboration with Brooks Rehabilitation (the Brooks-PHHP Research Collaboration) and PI Fox is a leading faculty member in this collaboration. She and her projects are supported by the resources, facilities and personnel of both organizations.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

RESPONSE: *Not applicable*

QUAD CHARTS:

RESPONSE: *See attached*

10. ***APPENDICES:***

RESPONSE: *Nothing to report*