AWARD NUMBER: W81XWH-22-1-0478

TITLE: Genetic Biomarkers of Intermittent Hypoxia-Induced Respiratory Motor Plasticity in Chronic SCI

PRINCIPAL INVESTIGATOR: Dr. Emily Fox, PhD

CONTRACTING ORGANIZATION: University of Florida

REPORT DATE: OCTOBER 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
OCTOBER 2023	Annual	30SEPT2022 - 29SEPT2023

4. TITLE AND SUBTITLE
Genetic Biomarkers of Intermittent Hypoxia-Induced Respiratory Motor Plasticity in Chronic SCI

5a. CONTRACT NUMBER
W81XWH-22-1-0478

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Emily Fox, PhD

E-Mail: ejfox@phhp.ufl.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Florida
Division of Sponsored Research
1523 Union Rd. Rm 2017
Gainesville, FL 32611-1941

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Background: Spinal cord injury (SCI) disrupts neural pathways to respiratory motor neurons, diminishing breathing capacity and airway defense (e.g., cough). Indeed, respiratory impairment is a leading cause of lung infection, re-hospitalization and death after SCI. There is a critical need for new strategies to restore breathing ability and airway defense in people living with chronic SCI. Acute intermittent hypoxia (AIH) – repetitive exposure to brief episodes of low inspired oxygen is a promising new strategy to restore breathing capacity by promoting spinal neuroplasticity. Exciting outcomes in nine SCI trials demonstrate that AIH improves human respiratory and limb function. Unfortunately, ~40% of individuals exhibit minimal response to AIH, making it crucial to optimize AIH protocols to maximize functional benefits and identify genetic biomarkers distinguishing those most/least likely to benefit from AIH-based treatments. An essential goal of our proposed study is to optimize AIH-based treatments by 1) combining hypoxia with hypercapnia (5% inspired CO2) during each episode (AIHH) to augment respiratory motor plasticity; and 2) combining AIHH with an established form of task-specific training (respiratory strength training). Genetic biomarkers for the efficacy of AIH have not yet been established but are vital to advance AIH-based treatment towards phase 3 clinical trials. Since we have a strong understanding of cellular mechanisms underlying AIH-induced respiratory motor plasticity, single nucleotide polymorphisms (SNPs) in gene coding for molecules necessary for plasticity may differentiate "responders" from "non-responders." Thus, the fundamental objective of this proposed project is to improve the therapeutic potential of AIH via protocol optimization and identification of genetic biomarkers for AIH efficacy. We propose three specific aims:

Aim 1: *Test the hypothesis that acute intermittent hypercapnic hypoxia (AIHH) is a more potent stimulus to respiratory motor plasticity than AIH alone (without hypercapnia) in individuals with chronic SCI.* We predict a single AIHH session (15, 1-min episodes of 9% O2 with 5% CO2; 1.5-min intervals) will elicit greater respiratory motor plasticity than AIH alone (15, 1-min episodes of 9% O2) as it does in uninjured individuals. The primary outcome will be the amplitude of diaphragm motor-evoked potentials elicited by transcranial magnetic stimulation (TMS).

Aim 2: *Test the hypothesis that combined daily AIHH and respiratory strength training enhance respiratory function more than AIH plus respiratory strength training in individuals with chronic SCI.* We predict that functional gains after 5 days of AIHH and respiratory strength training will be greater than 5 AIH sessions (without CO2) and strength training. Primary outcomes will be maximal inspiratory and expiratory pressure generation 1 day post-intervention. Secondary outcomes will be sustained effects (3 & 7 days) post- intervention.

Aim 3: *Test the hypothesis that dysfunctional genetic variants linked with molecules known to be necessary for AIH-induced respiratory motor plasticity characterize individuals with minimal respiratory motor plasticity in response to AIH or AIHH in individuals with chronic SCI.* We predict individuals with dysfunctional SNPs associated with pro-plasticity genes have blunted responses to AIH or AIHH treatments. Salivary genomic DNA will be assessed for a panel of genes associated with chemosensitivity, serotonergic function, and key signaling molecules necessary for respiratory motor plasticity (or impaired plasticity). These biomarkers will be correlated with outcomes following single AIH and AIHH sessions (Aim 1 outcomes) and 5 successive days of AIH and AIHH plus respiratory strength training (Aim 2 outcomes).

Study Design: Double-blind, placebo-controlled, randomized, 3-way cross-over design studies will be performed in 62 adults with chronic SCI (1 year post-injury). Enrollment is based on our least powerful comparisons to detect differences at power >0.8 (α=.05), and accounts for 15% attrition. Participants will complete 3, 1-day sessions with a 1-week washout (Aim 1), followed by 3, 5-day intervention blocks with a 3-week washout between blocks (Aim 2).

Clinical Impact: Our proposal is in direct alignment with FY21 SCIRP areas of encouragement since we propose to maximize function in residual neural circuitry by optimizing AIH (*via* AIHH & combination with respiratory strength training), and identify/validate genetic biomarkers for prognosis of treatment efficacies (guided by our understanding of cellular mechanisms giving rise to AIH-induced respiratory motor plasticity). Given a growing body of evidence concerning safety and efficacy, AIH-based treatments have real potential to be adopted in clinical practice to advance recovery and improve respiratory (and non-respiratory) motor function in people living with chronic SCI.

Study Status:

15. SUBJECT TERMS NONE LISTED					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	UU	17	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. INTRODUCTION:

An essential goal of our proposed study is to optimize AIH-based treatments by 1) combining hypoxia with hypercapnia (5% inspired CO_2) during each episode (AIHH) to augment respiratory motor plasticity; and 2) combining AIHH with an established form of task-specific training (respiratory strength training). Genetic biomarkers for the efficacy of AIH have not yet been established but are vital to advance AIH-based treatment towards phase 3 clinical trials. Since we have a strong understanding of cellular mechanisms underlying AIH-induced respiratory motor plasticity, single nucleotide polymorphisms (SNPs) in gene coding for molecules necessary for plasticity may differentiate "responders" from "non-responders." Thus, the fundamental objective of this proposed project is to improve the therapeutic potential of AIH via protocol optimization and identification of genetic biomarkers for AIH efficacy. A double-blind, placebo-controlled, randomized, 3-way cross-over design studies will be performed in 62 adults with chronic SCI (1 year post-injury). Enrollment is based on our least powerful comparisons to detect differences at power >0.8 (α=.05), and accounts for 15% attrition. Participants will complete 3, 1-day sessions with a 1-week washout (Aim 1), followed by 3, 5-day intervention blocks with a 3-week washout between blocks (Aim 2).

2. KEYWORDS:

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Coordinate and establish study operations which includes
Subtask 1-Prepare regulatory documents and study protocols
Subtask 2-Complete training of personnel
Subtasks 3-Establish study operations and assess adherence
Major Task 2: Conduct randomized, double-blind, repeated measures crossover study
Subtask 4-Initiate and continue recruitment
Subtask 5-Conduct testing and intervention protocols
Major Task 3: Draft manuscript on the effect of AIH-based treatments with CO2, respiratory strength training, and genetic biomarkers associated with respiratory motor plasticity
Subtask 6-Analyze study results

What was accomplished under these goals?

The major activities and objectives are included under "Major Task 1: Coordinate and establish study operations". The associated subtasks and achievements are outlined below:

Subtask 1: Prepare regulatory documents and study protocols – We have obtained all regulatory approvals, including the University of Florida Institutional Review Board and the DoD Office of Human Research Oversight (9/7/23). We have also obtained a license exemption from the Florida Department of Business and Regulations (Drugs, Devices, and Cosmetics) for Dr. Fox to obtain and store medical grade gases (required for study interventions and equipment calibration) at the Brooks Rehabilitation site. This license exemption was approved on 9/27/2023.

Subtask 2: Complete training of personnel - Clinical testing and intervention procedures have been finalized. Procedures for measurement of respiration and the intermittent hypoxia and acute intermittent hypercapnic hypoxia interventions are established and finalized. Procedures for respiratory strength training are established and final. Testing and intervention procedures have been documented and assembled in a procedures manual. Additional training of team members who are leading the transcranial magnetic stimulation portion of the protocol has been initiated and are in the final stages of training. Additionally, the overnight sleep monitoring device has arrived at Brooks Rehabilitation, and procedures have been reviewed and finalized by study team members. Study Lived Experience Consultants Chad Smith and Francois Fried have been consulted regarding feedback on study intervention and assessment procedures in order to limit participant burden.

Subtask 3: Establish study operations and assess adherence –
After receipt of gas use exemptions from state of FL, the gasses to be used in the study intervention sessions (AIH, AIHH, Sham) as well as for equipment calibration have been delivered to Brooks Rehabilitation. All data acquisition units, pneumotachographs, respiratory strength trainers, and face masks have arrived at Brooks Rehabilitation. The study recruitment plan has been established with applicable personnel at Brooks Rehabilitation and University of Florida to ensure successful recruitment once initiated.

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

This project is not specifically intended to provide training and professional development opportunities. However, the team members have been engaged in several training opportunities, which includes:

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.
b) Engagement of the postdoctoral research associates and study team members in a two-day training course on the background, safety, and procedures for transcranial magnetic stimulation.

How were the results disseminated to communities of interest?

Nothing to report.

The primary goal for the next reporting period will be to initiate participant recruitment and begin enrollment. We have completed major task 1 and will focus on accomplishing Major task 2. Focus areas will be on execution of recruitment plans, ensuring adherence with all activities, adherence to regulatory guidelines, reporting, safety monitoring, data collection and monitoring.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

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

We have experienced a delay in the ordering of medical gases required for this project. The license exemption process that is required to order and store these gases has been longer than anticipated, but the approval was granted on 9/27/23. We have also experienced a delay in initiation of study procedures due to certain requirements of safety devices and signage in the storage room where gases will be stored. A mechanical ventilation fan has been ordered as well as appropriate signage for the room. In addition, an oxygen depletion sensor has been ordered and will be installed this month. We anticipate these items will be resolved as of December 2023.

Changes that had a significant impact on expenditures

Regulatory approvals were delayed and therefore activities with human subjects were not initiated. Delays in establishing procedures with gasses also slowed expenditures. Expenses are focused on team member time in establishing operations, training, study set up, regulatory approvals, procedures in Major Task 1.Expenses were delayed and lower than budgeted but will increase and catch up as Major Task 2 is initiated and underway.

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

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

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

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Emily Fox, DPT, PhD, NCS
Project Role: Principal Investigator
Researcher Identifier: ORCID ID - 0000-0003-0142-3497
Nearest person month worked: 3
Contribution to project: Dr. Fox provided oversight of all activities including establishment of procedures, personnel recruitment and training, development of study protocol, compliance with regulations, and leadership of investigative team.

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 establishment of procedures, personnel recruitment and training, development of study protocol, leadership of investigative team.

Name: Lou DeMark, DPT, NCS
Project Role: Study Coordinator
Researcher Identifier: NA
Nearest person month worked: 1
Contribution to project: Lou DeMark provided assistance with development of procedures, assisting Drs. Fox and Mitchell with set up of operations, assistance with protocol development and preparation of documents, development of recruitment plan including work with the recruitment specialists.

Name: Gina Brunetti, DPT, NCS
Project Role: Blinded Assessor
Researcher Identifier: NA
Nearest person month worked: 1
Contribution to project: Gina Brunetti provided assistance with development of procedures, particularly study testing and interventions, assisting Drs. Fox and Mitchell with set up of operations, assistance with protocol development and preparation of documents, assistance with development of a recruitment plan and study procedures manual.

Name: Hannah Snyder, MS
Project Role: Study Coordinator
Researcher Identifier: NA
Nearest person month worked: 1
Contribution to project: Hannah contributed to development of recruitment plan, establishment of study communications and development of regulatory documentation.

Name: Michela Mir, CCC-SLP, PhD
Project Role: Post-doctoral scientist
Researcher Identifier: NA
Nearest person month worked: 4
Contribution to project: Michela contributed to development of procedures, establishment of a procedures manual, development of regulatory documents and communications.

Name: Tommy Sutor, PhD
Project Role: Post-doctoral scientist
Researcher Identifier: NA
Nearest person month worked: 7
Contribution to project: Tommy contributed to development of procedures, establishment of a procedures manual, development of regulatory documents and communications.

Name: Quing Lu, PhD
Project Role: Statistician
Researcher Identifier: NA
Nearest person month worked: 1
Contribution to project: Dr. Lu contributed review of statistical plan, randomization plan, review of biomarker pilot data to inform study procedures, study planning and establishment of procedures.

Name:	Quing Lu, PhD
Project Role:	Statistician
Researcher Identifier:	NA
Nearest person month worked:	1
Contribution to project:	Dr. Lu contributed review of statistical plan, randomization plan, review of biomarker pilot data to inform study procedures, study planning and establishment of procedures.

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

Nothing to report.

What other organizations were involved as partners?

Thomas Jefferson University
Jayakrishnan Nair, PT, PhD – study co-investigator
Dr. Nair contributes expertise in SCI, control of breathing and molecular genetics. Dr. Nair contributes to establishment and oversight of genetic biomarker collection, storage, analysis and interpretation. Dr. Nair has contributed in establishment of procedures and study design.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: ***N/A***

QUAD CHARTS: N/A

9. APPENDICES: N/A