BREATHING INTERVENTIONS FOR RELAXATION: DOSING THROUGH EXTENDED EXHALE AMONG HEALTHYADULTS

Principal Investigator:

Alfredo Gamboa, M.D.
Associate Professor, Clinical Pharmacology
Vanderbilt University Medical Center
Nashville, TN 37232-8300

Supported by:
National Center for Complementary and Integrative Health

TABLE OF CONTENTS

Page

STUDY TEAM ROSTER **1**
PARTICIPATING STUDY SITES 1
PRÉCIS 2

1. STUDY OBJECTIVES **3**

2. BACKGROUND AND RATIONALE **4**
2.1 Background 4
2.2 Study Rationale 4

3. STUDY DESIGN **5**

4. SELECTION AND ENROLLMENT OF PARTICIPANTS **5**
4.1 Study Sample Selection 5
4.2 Study Enrollment Procedures 6

5. STUDY INTERVENTIONS **8**
5.1 Interventions, Administration, and Duration 8
5.2 Concomitant Interventions 9
5.2.1 Allowed Interventions 9
5.2.2 Required Interventions 9
5.2.3 Prohibited Interventions 9
5.3 Adherence Assessment 9

6. STUDY PROCEDURES **11**
6.1 Schedule of Evaluations 11
6.2 Description of Evaluations 12
6.2.1 Screening Evaluation 12
6.2.2 Enrollment, Baseline, and/or Randomization 12
6.2.3 Blinding 13
6.2.4 Followup Visits 13
6.2.5 Completion/Final Evaluation 13

7. SAFETY ASSESSMENTS **14**
7.1 Specification of Safety Parameters 14
7.2 Adverse Events and Serious Adverse Events 14
7.3 Reporting Procedures 15
7.4 Followup for Adverse Events 16

7.5 Safety Monitoring 16

8. INTERVENTION DISCONTINUATION 16

9. STATISTICAL CONSIDERATIONS 17

9.1 General Design Issues 17

9.2 Sample Size and Randomization 18

9.3 Definition of Populations 18

9.4 Interim Analyses and Stopping Rules 18

9.5 Outcomes 19

9.5.1 Primary Outcome 19

9.5.2 Secondary Outcomes 20

9.6 Data Analyses 21

10. DATA COLLECTION AND QUALITY ASSURANCE 22

10.1 Data Collection Forms 22

10.2 Data Management 22

10.3 Quality Assurance 22

10.3.1 Training 22

10.3.2 Metrics 22

10.3.3 Protocol Deviations 23

10.3.4 Monitoring 23

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY 23

11.1 Institutional Review Board (IRB) Review 23

11.2 Informed Consent Forms 23

11.3 Participant Confidentiality 23

11.4 Study Discontinuation 23

12. REFERENCES 24

13. SUPPLEMENTS/APPENDICES 27

I. Consent Form

II. Questionnaires

a. Demographics

b. Medications

c. Perceived Stress Scale

d. Positive and Negative Affect Schedule-X (PANAS-X)

e. PROMIS Anxiety CAT Data Item Bank

f. PROMIS Depression CAT Data Item Bank

g. PROMIS Sleep Disturbance CAT Data Item Bank

h. Epworth Sleepiness Scale

i. Concomitant interventions

III. Breathing visit fidelity documentation

IV. Recruitment

a. Flyer for recruitment

b. Email and electronic message for recruitment

STUDY TEAM ROSTER

Alfredo Gamboa, MD, Principal Investigator
Vanderbilt University Medical Center
Campus zip 6602
Nashville, TN 37235-0002
Tele: 615-343-3649
Fax: 615-343-8649
alfredo.gamboa@vanderbilt.edu

Hui Nian, PhD, Co-investigator
Vanderbilt University Medical center
2525 West End, Ste. 110000
Nashville, TN 37203
Tele: 615.875.3004
Fax: 615.343.4924
hui.nian@vanderbilt.edu

Kenneth Wallston, PhD, Co-investigator
Vanderbilt University School of Nursing
421 Godchaux Hall
Nashville, TN 37235-0002
Tele: 615-343-3317
Fax: 615-343-5898
kenneth.wallston@vanderbilt.edu

Gurjeet Birdee, MD MPH, Former Principal Investigator
Vanderbilt University Medical Center
Campus zip 8300
Nashville, TN 37235-0002
Tele: 615-936-0584
Fax: 615-936-1269
gurjeet.birdee@vanderbilt.edu

PARTICIPATING STUDY SITES

Vanderbilt University Medical Center
Campus zip 8300
Nashville, TN 37235-0002
Tele: 615-936-0584
Fax: 615-936-1269

PRÉCIS

Breathing Interventions for Relaxation: Dosing Through Extended Exhale among Healthy Adults

Objectives

The objectives of this study are:

Specific Aims R61 (n=100)

1. Compare 12 weeks of slow breathing exhale>inhale vs. exhale=inhale on changes in physiological stress (***primary outcome: heart rate variability***; secondary outcomes: baroreflex sensitivity, catecholamines).
2. Compare 12 weeks of slow breathing exhale>inhale vs. exhale=inhale on changes in psychological stress **(*primary outcome: anxiety*;** secondary outcomes: affect, arousal).
3. Compute the correlations between changes in physiological and psychological outcomes (e.g., changes in heart rate variability correlated with changes in perceived stress).

Design and Outcomes

We propose a randomized study among 100 healthy adults comparing the effects on stress of two standardized slow breathing protocols derived from therapeutic breathing techniques from yoga (pranayama are yoga techniques related to breathing). In a 12 week study we will measure if exhale>inhale vs. exhale=inhale produces measurable and meaningful differences in stress among healthy adults. Participants will meet with mind-body therapists weekly to learn slow breathing, and asked to perform exercises daily for 12 weeks. Data will be collected at baseline, 6 and 12 weeks. There will be an optional questionnaire follow-up at 26 weeks.

Interventions and Duration

A. **Intervention:** Participants will be randomized to 12 weeks of slow breathing with either exhale>inhale versus exhale=inhale. Each week for 12 weeks, the participant will meet with the mind-body therapist for a 45 minute visit. Participants will be taught the breathing interventions in a private office on the Vanderbilt University campus.

B. **Duration:** Participants will be enrolled into 12 weeks of the interventions and have outcome measures assessed at baseline, 6 weeks, and 12 weeks. Participants may elect to be contacted for a 26 week follow-up questionnaire.

Sample Size and Population

A total of **100** healthy adults, age 30-60 years will be enrolled in this study. Each participant will be randomized using a 1:1 ratio stratified by gender.

1. STUDY OBJECTIVES

Specific Aim 1: Compare 12 weeks of slow breathing exhale>inhale versus exhale=inhale on changes in physiological stress (***primary outcome: heart rate variability***, secondary outcomes: baroreflex sensitivity, catecholamines).

Primary hypotheses: *We hypothesize that participants with exhale>inhale versus exhale=inhale will have higher increases in parasympathetic tone as measured by the high frequency component of heart rate variability (HF HRV).*

Secondary hypotheses:

1. *We hypothesize that participants with exhale>inhale versus exhale=inhale will have higher increases in parasympathetic tone as measured by baroreflex sensitivity alpha index.*
2. *We hypothesize that baseline parasympathetic tone will modify response to breathing interventions. Specifically, we hypothesize that participants with lower parasympathetic tone at baseline will have the greater increases in parasympathetic tone as measured by HF HRV and BRS alpha index.*

Specific Aim 2: Compare 12 weeks of slow breathing exhale>inhale versus exhale=inhale on changes in psychological stress **(*primary outcome- anxiety*,** secondary outcomes: affect, arousal).

Primary hypotheses: *We hypothesize that participants with exhale>inhale versus exhale=inhale will have further decreases in psychological stress as measured by the PROMIS Anxiety.*

Secondary hypotheses:

1. *We hypothesize that participants with exhale>inhale versus exhale=inhale will have further decreases in stress as measured by* Anxiety Sensitivity Index-3 (ASI-3), Beck Anxiety Inventory, Body Vigilance Scale, Panic Disorder Severity Scale, and Perceived Stress Scale-10 (PSS) and decreases in **negative** affect *as measured by PROMIS Depression scale.and Positive and Negative Affect Schedule-X (PANAS-X).*
2. *We hypothesize that baseline psychological stress will modify response to breathing interventions. Specifically, we hypothesize that participants with higher stress at baseline will have the further decreases in stress as measured by the PROMIS Anxiety scale.*

Exploratory research questions: *We consider arousal as an exploratory measure in this proposal. In our pilot, exhale>inhale versus exhale=inhale had improvements in daytime arousal as measured by the Epworth Sleepiness Scale. For the present proposal, we will examine differences in arousal from baseline, 6, to 12 weeks as measured by the Epworth Sleepiness Scale. We will also explore the relationship between baseline sleep disturbance as measured by PROMIS Sleep Disturbance and changes in arousal.*

Specific Aim 3: Compute the correlation between physiological and psychological changes (e.g. changes in heart rate variability correlated with changes in PROMIS Anxiety).

Primary hypotheses: *We hypothesize that changes in physiological and psychological measures will be moderately correlated.*

2. BACKGROUND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

Deep and slow breathing exercises are commonly used by individuals as a means to relax. According to the National Health Interview Survey, 12.5% of adults in the U.S. report using deep breathing exercises for health.[1] As it relates to health, breathing exercises are categorized as a mind-body practice.[2] There have been various clinical applications of slow breathing including treatment of stress-related disorders (anxiety, depression, acute or chronic pain), cardiovascular diseases (hypertension, heart failure), and pulmonary diseases (asthma, chronic obstructive lung disease).[3] Slow breathing is an integral part of most mind-body practices including yoga, t'ai chi, qi gong, meditation techniques (Zen, Transcendental Meditation, Vipassana, Mindfulness-Based Stress Reduction), and other relaxation techniques (relaxation response, biofeedback). The clinical efficacy of slow breathing for these conditions is largely unproven. Since slow breathing is a common component to many mind-body practices, it is important to understand the potential role of slow breathing exercises on human health.

Research has demonstrated that slow breathing produces changes in the autonomic nervous system.[4] In most studies, slow breathing has been defined as a respiratory rate of <10 breaths/min. Slower respiratory rates have been shown to decrease sympathetic and increase parasympathetic tone. This may be partially mediated through alteration of intra-thoracic pressures,[5-7] stimulation of arterial and cardiopulmonary baroreceptors[8,9] and afferent pulmonary stretch receptors.[6,10] There has been a particular focus on a respiratory rate of 6 breaths/min (0.10 Hz) where cardiopulmonary entrainment occurs.[11,12] Deep breathing exercises performed at 0.10 Hz produce increased baroreflex sensitivity and decreased sympathetic nerve activity as compared to spontaneous or regulated breathing at rest respiratory rates (~0.15 Hz).[13,14]

To date, researchers have assumed that a low respiratory rate is chiefly responsible for inducing physiological and psychological relaxation. However, based on wide variety of mind-body practices, respiratory rate is not the single factor that produces relaxation during slow breathing. Practitioners modify the ratio of inhalation and exhalation to enhance relaxation. Specifically, extension of exhalation relative to inhale is believed to augment physiological and psychological relaxation.

2.2 Study Rationale

There have been few studies examining if exhale>inhale versus exhale=inhale while breathing slowly augments physiological and psychological relaxation. All of these studies have examined acute effects from slow breathing. Most have focused on slow breathing at a respiratory rate of 6 breaths a minute.[15 16 17] Results from these studies are mixed. Some have reported exhale>inhale versus inhale=exhale produces more physiological relaxation,[15,17] whereas others report no significant differences between groups.[16] Only one study examined the acute effect of different breath ratios on physiological and psychological stress with exhale>inhale decreasing both more than exhale=inhale.[17] We collected preliminary data in a small randomized 6 week study among 21 healthy adults
comparing the effects of two standardized slow breathing protocols on stress.

We randomized participants to a daily practice of: (1) exhale greater than inhale in length

(exhale>inhale, goal ratio 2:1) versus (2) exhale equal to inhale in length (exhale=inhale). That pilot demonstrated our ability to recruit and retain participants and to have them complete physiological and psychological stress measures at designated time points. Although the exhale>inhale group showed trends towards more relaxation compared to the exhale=inhale group, the study had insufficient power to measure definitive differences between the two groups. The rationale of the present study is to build on our preliminary data with by conducting a larger (n=100) and longer (12 week) randomized study to assess if exhale>inhale vs. exhale=inhale produces measurable and meaningful differences in stress among healthy adults.

3. STUDY DESIGN

We propose a prospective, randomized, double-blinded, study among healthy patients to compare the effects of exhale>inhale versus exhale=inhale on physiological and psychological stress. In a sample of 100 participants we will compare the clinical effectiveness of a 12-week program of slow breathing exercises, consisting of exhale>inhale, to a 12-week program of slow breathing exercises, consisting of exhale=inhale. Outcome assessment will occur at baseline, 6 and 12- weeks for: physiological stress (autonomic tone: heart rate variability, baroreflex sensitivity), and psychological anxiety (PROMIS Anxiety scale, Anxiety Sensitivity Index-3, Beck Anxiety Inventory, Body Vigilance Scale, Panic Disorder Severity Scale, and Perceived Stress Scale-10), affect (PROMIS depression Scale, PANAS-X), and arousal (Epworth Sleepiness Scale, PROMIS Sleep Disturbance Scale]. Psychological measures will be repeated at 26 weeks after study completion as follow-up. **The primary outcomes of the clinical trial will be heart rate variability measured by spectral analysis and psychological stress measured by the PROMIS Anxiety.**

Randomization will occur after baseline testing and assessment of initial compliance to the intervention. Treatment assignments will be generated in a 1:1 ratio stratified by gender.Participants will be randomized to 12 weeks of slow breathing with either exhale>inhale versus exhale=inhale. Each week for 12 weeks, the participant will meet with the mind-body therapist for a 45 minute visit. Participants will be taught the breathing interventions in a private office on the Vanderbilt University campus.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Study Sample Selection

All participants enrolled will meet the inclusion and exclusion criteria of the study as stated in Table 1. PROMIS Anxiety scale will be administered during the screening phase. Only participants with a PROMIS Anxiety score of 45 or higher will be eligible (half a stand deviation below the mean or higher for the general population). We will also evaluate compliance during the screening phase. We will only include those who demonstrate high compliance to the intervention during the screening phase by practicing at least 3 times a three weeks. Secondly, participants must be able to achieve a respiratory rate of ≤8 breaths/minute but not ≤3 breaths/minute.

Efforts will be made to reduce the length of time between the screening phase and enrollment. However, if baseline measures show that, in the time between these events, the

participant no longer meets the inclusion criteria (as listed in Table 1) then participant eligibility may be reassessed. If the participant is removed from study for this reason it will be before the first intervention occurs.

Subjects must have the ability to understand study procedures and comply with them for the entire length of the study. For men and women of reproductive capability, contraception will not be necessary or required.

We will not exclude patients on the basis of gender, race, ethnicity, or religious preferences and practices.

Table 1. Sample Selection Criteria

Inclusion
• Age 30 to 60 years
• After two weeks of intervention:
  • Breathe 8 or less a minute while practicing
  • Not breathe 3 or less breaths a minute while practicing
  • Practiced 3 or more times a week
• English speaking
• PROMIS Anxiety scale ≥ 45

Exclusion • Hypertension • Heart disease: history of coronary artery disease, myocardial infarction, significant valvular disease, or congestive heart failure • Diabetes • Renal disease • Anxiety disorder • Depression • Other psychiatric conditions including schizophrenia or bipolar disorder • Attention-deficit-disorder or Attention-deficit-hyperactivity disorder • Musculoskeletal condition limiting capacity to perform simple movements such as chronic lower back pain or neck pain • Pulmonary disorder (asthma, chronic obstructive lung disease, obstructive sleep apnea) • Smoker • Currently taking blood pressure medications, oral diabetic medication or insulin • Current participation in a mind-body practice/program • Current cancer other than non-melanoma skin cancer • Regular swimmer • Plays wind or brass musical instruments • Pregnant •

4.2 Study Enrollment Procedures

Recruitment and Referral Sources: Participants will be recruited through flyers and research email lists.

(1) Flyers: Flyers will be posted in common areas of Vanderbilt University and public places in Nashville (groceries stores, retail stores, or restaurants on public notice boards). See flyers in Appendix.

(2) Research email lists:
   a. MyResearch: We will use MyResearch at Vanderbilt (MRAV) to email potential volunteers. MRAV is a repository of over 17,000 Vanderbilt patients that have opted in to be contacted directly by e-mail to participate in research or to provide input on research ideas. We will send an IRB approved study description through MRAV at 3 month intervals for recruitment during the enrollment period. See Appendix for sample email.

VICTR Research Notification Distribution List: VICTR Research Notification Distribution List is a recruitment tool, available to Vanderbilt researchers that reaches over 18,500 Vanderbilt faculty and staff, as well as members of the Middle Tennessee community. The email tool provides investigators a forum for advertising for volunteers for a specific study and the ever-growing distribution list allows all individuals interested in learning about research opportunities to subscribe to receive the email notifications. Email notifications are limited to
IRB approved language, describe study specifics and provide contact information. Supported by VICTR, an ongoing marketing plan exists for retaining and increasing distribution list subscribers. To submit an ad, investigators complete an Email Notification Request Form. Ads are emailed to the distribution list within the next 5 business days. We will send an IRB approved study description through the list at 3 month intervals during the enrollment period. See Appendix for sample email.

(3) Online websites: We will use ResearchMatch, an online recruitment tool hosted by Vanderbilt. ResearchMatch is a disease neutral online research recruitment and engagement platform that aims to remove the barriers to patient participation in research by partnering with researchers and advocacy groups to increase patient engagement and access to information across the research enterprise. The site is specifically designed to match individuals interested in research participation with researchers in their area and is funded in part by the National Center for the Advancement of Translational Science (NCATS), an office of the National Institute of Health (NIH). The system allows people from anywhere in the country to self-register and express an interest in participating in research studies. ResearchMatch then provides information about those volunteers to researchers. Researchers may register to use ResearchMatch with either feasibility or recruitment access. Feasibility access is limited in that it only allows researchers to view aggregate de-identified data. Researchers with recruitment access, however, are able to conduct a more targeted search using their study's inclusion/exclusion criteria.

We have applied for recruitment access to identify a list of potential volunteers. We will send an IRB-approved recruitment message through the secure ResearchMatch clearinghouse to each volunteer on the list. See Appendix for sample recruitment message. If a Volunteer responds, "Yes, I am interested!" ResearchMatch will release the volunteer's contact information to the researcher who is then able to directly contact the volunteer and follows normal study protocol. The registry includes all ages, healthy and with health conditions.

ResearchMatch was developed in 2009 by institutions affiliated with the Clinical and Translational Science Awards Consortium (www.ctsacentral.org) and is maintained at Vanderbilt University. The Vanderbilt Institutional Review Board (IRB) provides oversight of the project as a recruitment tool.

Enrollment Procedures:

Individuals interested in participation will go through screening through REDCap to determine initial eligibility based on criteria listed above. If potentially eligible, research staff will contact the individual to explain the research protocol, answer questions, and discuss informed consents. Potential subjects interested in participation will be scheduled for a research visit with research staff for primary screening. Primary screening will consist of a direct interview by research staff to confirm eligibility. If potentially eligible, participants will complete an informed consent to proceed with secondary screening (see Appendix I. Informed Consent for Screening).

Secondary screening will consist of baseline outcomes assessments including administration of PROMIS Anxiety Scale, and 2-weeks of intervention to assess compliance.

After secondary screening, eligible and willing individuals will complete a second informed consent to participate in the randomized clinical trial (see Appendix I. Informed Consent for clinical trial).

For enrolled participants, randomization will occur after 2 weeks of basic breathing and assessment of breathing by mind- body teacher. Treatment assignments will be generated in a 1:1 ratio stratified by gender.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Development: We designed breathing protocols to examine the effect of slow breathing on stress measures among healthy adults. The breathing interventions for this study were developed by Dr. Birdee in conjunction with an expert panel of 3 mind-body therapists. The breathing protocol uses a well-developed progression of breathing taught in the Krishnamacharya tradition of yoga (Viniyoga).[18,19, 20]

Breathing instruction: Participants will receive 12 weekly private classes taught by a certified mind-body teacher (yoga teachers) specifically trained to deliver the slow breathing protocols. Teachers are specifically instructed not to reveal the goal of the breathing exercises (i.e. relaxation). Each class will be 45 minutes long. The first two weeks of the protocol teaches same basic slow and deep breathing to both treatment arms. The purpose of the first two weeks is to allow all participants an opportunity to become familiar with basic components of deep breathing. Participants will be asked to perform breathing exercises daily in the evening.

Participants will be given written instructions and drawings to guide home practice. During the two weeks for secondary screening, the teacher will assess the subjects' respiratory rate while breathing deeply. Subjects will be eligible only if they achieve a respiratory rate of 8 breaths a minute or less. This is based on principles of pranayama that breath ratios above this respiratory rate are not useful. On the other end of the spectrum, subjects who are already able to breathe very slowly, defined as 3 breaths per minute or less, will not be included. Subjects will only be included who perform the practice 3 or more times a week, based on self-report, during the first two weeks.

To facilitate breathing regulation and standardization between participants, participants will be given an MP3 player with a recorded track that made an audible sound every second (60 Hz). Subjects will be asked to count the length of each breath based on the recording. Subjects demonstrating eligible respiratory rates will proceed to the randomized breathing interventions described below.

Breathing rates are assigned based on the assessment of deep breathing performed at 2 weeks. This provides an opportunity to identify a comfortable, starting, slow breathing rate for each participant. After randomization, participants will follow a week to week progression based on a pre-specified protocol for 10 weeks. This allowed breathing rates to be matched week by week between treatment groups.

5.1.1 Slow breathing with exhale=inhale:

Participants randomized to slow breathing with equal inspiration and expiration received progressive increases in both inspiration and expiration over 4 weeks until they reach a goal breath length or longest comfortable breath. Goal breaths were assigned based on initial breath assessment. Prior to performing the breathing practice, participants performed a few standardized yoga movements. The purpose of movements was to prepare the subject to sit and focus on breathing. Subjects randomized to exhale=inhale will receive progressive increases in both inspiration and expiration until they reach a goal breath length. Goal breaths will be assigned based on initial breath assessment. Prior to performing the breathing practice, subjects will perform a set of standardized breathing practices. The purpose of these few breathing practices is to prepare the subject to sit and focus on breathing.

5.1.2 Slow breathing with exhale>inhale:

Participants randomized to slow breathing with prolonged exhale to inhale ratio received progressive increases in expiration relative to inspiration based on initial assessment until they reached a goal breath length or longest comfortable breath. Prior to performing the breathing practice, subjects in this exhale>inhale condition performed the same yoga movements as the exhale-inhale group.

See Appendix for detailed breathing intervention protocol.

5.2 Concomitant Interventions

5.2.1 Allowed Interventions

All subjects will continue with routine treatment of medical conditions including all medications. Changes in medications will be at the discretion of usual healthcare providers. Any changes in medications or other concomitant interventions initiated during the study period will be documented (See Appendix for Concomitant Intervention data collection).

5.2.2 Required Interventions

No additional interventions are required except for those proposed for in study protocol.

5.2.3 Prohibited Interventions

Subjects receiving will be asked to not participate in other mind-body programs for the duration of the study.

5.3 Adherence Assessment

Fidelity measures: We will assess fidelity to breathing interventions through direct observation by the mind-body teacher, self-report, and biometric garments-HEXOSKIN.

(1) Observation Each week, mind-body teachers observe participants performing the practice along with an audible metronome to measure breath rate and length of exhale and

inhale. Mind-body teachers evaluate participate engagement, quality of technique, and breathe rate and exhale/inhale length (See Appendix for mind-body fidelity tracking form).
(2) Self-Report Each week, participants are asked how frequently they practiced at home by the mind-body teacher.
(3) Remote biometric garment: Lastly, we will use biometric garments, HEXOSKIN, to ascertain remote adherence to home breathing practice. As described previously, HEXOSKIN accurately records respiratory parameters including breathing rate, length of exhale/inhale through thoracic and abdominal strain gauges embedded in the shirt. Data for is stored in a small unit worn in the shirt. Each participant will be asked to wear a HEXOSKIN shirt during practice for one week for two time periods between weeks 3 to 8 and weeks 9 to 12. For 2-3 nights during each of these weeks the participant will be asked to keep the shirt on overnight to monitor sleep. Participants will return the shirt after one week, and data from the device will be downloaded for analyses. We will use VivoSense software with the Complex Respiratory Module to analyze breathing intervention fidelity (Vivonoetics, San Diego, CA).

6. STUDY PROCEDURES

6.1 Schedule of Evaluations (Tx, Treatment, Wk, Week)

Activity	Primary screening	Secondary screening			Clinical Trial											
	(Day-21 to Day -1)	(Day 0)	Wk1	Wk2	Wk3	Wk4	Wk 5	Wk6		Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 13
Assessment/Intervention		Research visit 1	Tx Visit 1	Tx Visit 2	Tx Visit 3	Tx Visit 4	Tx Visit 5	Tx Visit 6	Research visit 2	Tx Visit 7	Tx Visit 8	Tx Visit 9	Tx Visit 10	Tx Visit 11	Tx Visit 12	Research visit 3
Inclusion/Exclusion Criteria	X	X	X	X												
Enrollment/informed Consent • Consent for screening • Consent for clinical		X		X												
Data collection Physiological stress Psychological stress		X							X							X
Commitment interventions/medications		X							X							X
Randomization					X											X
Breathing intervention visits			X	X	X	X	X	X		X	X	X	X	X	X	
Breathing intervention assessment					X											
Adherence: self-reported frequency of breathing practice at home			X	X	X	X	X	X		X	X	X	X	X	X	
Adherence: Remote monitoring (1 week period)						X				X						
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

6.2 Description of Evaluations

6.2.1 Screening Evaluation

Consenting Procedure

Subjects eligible and willing to participate in will be given a written informed consents by the research staff. There are two consents for this research project. The first consent is to participate in the secondary screening. The informed consent for screening asks participants to participate in a 2 week study consisting of baseline assessment and 2 intervention visits. The purpose of the secondary screening is to determine if the individual is eligible for the clinical trial.

The second consent is to participate in a clinical trial consisting of attending intervention sessions once a week for 10 weeks and 2 research visits.

Details of all study procedures will be provided, including purpose of the studies, content of classes and details of tests to be done, frequency of classes and time commitment, risks and benefits, and voluntary nature of the program. Any changes to research procedures or intervention will prompt revision of the consent forms, with subsequent review and approval from Vanderbilt IRB. A copy of the signed consents will be stored in a locked file cabinet located in the secured office of the principal investigator.

Screening

Individuals interested in participation will go through screening through REDCap to determine initial eligibility based on criteria listed above. If potentially eligible, research staff will contact the individual to explain the research protocol, answer questions, and discuss informed consents. Potential subjects interested in participation will be scheduled for a first research visit with research staff. Initial eligibility will be confirmed with direct interview. If eligible, partipants will be consented for secondary screening. Secondary screening will consist of baseline assessments and 2 weeks of intervention to assess compliance.

6.2.2 Enrollment, Baseline, and/or Randomization Enrollment

Enrollment into the clinical trial will be defined by patient having met all screening criteria and signed the informed consent for participation in the clinical trial

Baseline Assessments

For participants who have completed primary screening for eligibility and aenrolled for secondary screening, baseline assessments will be performed to measure study outcomes.

- Autonomic tone: We will estimate autonomic tone of participants by standard autonomic testing of cardiovagal and sympathoneural functioning including heart rate variability and baroreflex sensitivity. Subjects will have fasted the night before testing, and testing will occur at the same time of the day for each subject to account for temporal variations of autonomic tone.
- Questionnaires: PROMIS Anxiety Scale, Anxiety Sensitivity Index-3 (ASI-3), Beck Anxiety Inventory, Body Vigilance Scale, Panic Disorder Severity Scale, and Perceived Stress Scale-10 (PSS), PROMIS Depression scale, and PANAS-X.
- Catecholamine Profile: Plasma norepinephrine and epinephrine levels will be

measured at baseline in supine and standing positions.
- Current Medications
- Concomitant Interventions

Randomization

Randomization will occur after secondary screening is complete. Treatment assignments will be generated in a 1:1 ratio stratified by gender.

6.2.3 Blinding

Blinding is summarized in the table below, and described in detail as follows:

1. Participants: Participants will not be able to be blinded to the treatment interventions as this is a behavioral intervention. However, during recruitment, screening, and consent, research staff and mind-body therapists will not state the specific hypothesized function/effect of the treatment interventions- physiological or psychological relaxation. We will describe the intervention as "focused breathing" that is being researched in regards to effects on healthy, and that focused breathing has been shown to improve general well-being. Mind-body therapists have been trained to deliver the protocol without specifying the function of the treatment interventions.
2. Mind-body therapists: Therapists delivering the treatment intervention will not be blinded to the treatment interventions. Therapists will be blinded to primary and secondary outcome measures of stress. Therapists will have access to biometric data regarding fidelity of breathing interventions at home to ensure compliance to the intervention during the study period.
3. Outcome assessors: Research staff collecting physiological and psychological stress data will be blinded to treatment intervention assignments. A research staff member separate from those collecting stress measures will collect data regarding fidelity to breathing interventions.
4. Data analysts/statistician: Data analysts including the statistician will be blinded to treatment assignments until data analyses are complete.
5. Principal investigator: Principal investigator will be blinded to treatment assignments until data analyses are complete.

BLINDING TABLE:

Stake holder	Intervention group assignment	Primary Mechanistic Outcome Measure (Physiological stress)	Secondary Mechanistic outcome measure (Psychological stress)
Participants	*No*	*Yes*	*Yes*
Instructors	*No*	*Yes*	*Yes*
Outcome Assessors	*Yes*	*No*	*No*
Statistician	*Yes*	*No*	*No*

Principal Investigators	*Yes*	*No*	*No*

6.2.4 Follow-up Visits

- Once a week in a private office on the Vanderbilt University campus
  - Slow breathing with either exhale > inhale or exhale = inhale
  - Adherence to intervention: Measure subject adherence to study protocol including quality, duration, and breathing goals
  - Adverse events: Document frequency and severity of adverse events during the study
  - Fidelity of breathing by self-report and direct observation by mind-body teachers
- At 6 weeks
  - Autonomic tone
  - Catecholamine Profile
  - Questionnaires: PROMIS Anxiety Scale, Anxiety Sensitivity Index-3 (ASI-3), Beck Anxiety Inventory, Body Vigilance Scale, Panic Disorder Severity Scale, and Perceived Stress Scale-10, PROMIS Depression scale, and PANAS-X.
  - Current Medications
  - Concomitant Interventions
- Two one week periods from weeks 3-6 and 7-12:
  - Fidelity measure using biometric garment- HEXOSKIN

6.2.5 Completion/Final Evaluation

- At 12 weeks
  - Autonomic tone
  - Catecholamine Profile
  - Questionnaires: PROMIS Anxiety Scale, Anxiety Sensitivity Index-3 (ASI-3), Beck Anxiety Inventory, Body Vigilance Scale, Panic Disorder Severity Scale, and Perceived Stress Scale-10, PROMIS Depression scale and PANAS-X.
  - Current Medications
  - Concomitant Interventions

Participants who discontinue the study intervention early will require no specific evaluations.

The reason for discontinuation will be documented. If research participants no longer want to participate in the research study, we will terminate themfrom the study. Participants terminated from the study will undergo no further monitoring or follow-up once they have stopped the study intervention.

7. SAFETY ASSESSMENTS

This research protocol employs breathing techniques among healthy adults that involves low risk to human subjects. Subjects will be frequently asked about symptoms relating to adverse events or discomfort. While expected to be very

rare, possible serious adverse events related to breathing practices may include:

- cerebrovascular accident
- life threatening arrhythmias (e.g. ventricular tachycardia)
- myocardial infarction
- sudden death

Non-serious adverse events from breathing techniques may include

- acute symptomatic episodes from problems with blood pressure,
- anxiety
- lightheadedness/dizziness
- muscle cramps,
- musculoskeletal injury,
- respiration (shortness of breath or respiratory discomfort),

Other theoretical risks from study tests include:

- local risks associated with venipuncture blood draws

There may be risks and side effects that are currently unknown and/or unanticipated.

Because these risks are unlikely given previous behavioral studies mind-body therapy practices, and most are inherent risks within the general population and not solely due to study procedures, the potential benefits of this study to the individual and society reasonably outweigh the risks.

7.1 Specification of Safety Parameters

We will monitor for all adverse events during the study. The Data Safety and Monitoring Board (DSMB) will monitor and review adverse events, and toxicities and events that may be related to the intervention. This will include verification that, when indicated, these events have been reported to the appropriate agencies (e.g. IRB) and that such reports have been made in a timely manner. The DSMB (TBN) will consist of a biostatistician, medical physician, clinical trials researcher, and clinical psychologist. Dr. Birdee will also review information on the completeness of data and the rate of patient accrual relative to the protocol timeliness.

7.2 Adverse Events and Serious Adverse Events

Definition - An adverse event (AE) is any untoward medical occurrence in a subject temporally associated with participation in the clinical study or with use of the experimental agent being studied. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.) or any combination of these.

A Serious Adverse Event (SAE) is any adverse event that results in one or more of the following outcomes:

- Death
- A life-threatening event

- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly or birth defect
- Important medical event based upon appropriate medical judgment

Research staff and mind-body therapists will document and report solicited AEs including physical or psychological discomfort related to anxiety, blood pressure changes, depression, lightheadedness/dizziness, muscle cramps, musculoskeletal injury, or respiration. Unsolicited events will be captured through observation by research staff. Research staff will document unsolicited events and report AEs according to data safety monitoring plan. Events will be documented in the RedCap data entry system including date and time of event which will avoid double capture.

Adverse Reporting: The Independent Monitoring Committee (IMC) will monitor and review adverse events, and toxicities and events that may be related to the intervention.

a) Non-serious adverse events will be reviewed on a monthly basis by the IMC. The IMC will be notified of serious adverse events within 24 hours of occurrence and reviewed within 48 hours.

b) Unexpected fatal or life-threatening AEs related to the intervention will be reported to the NCCIH Program Officer within 7 days. Other serious and unexpected AEs related to the intervention will be reported to the NCCIH Program Official within 15 days.

c) Anticipated or unrelated SAEs will be handled in a less urgent manner but will be reported to the Independent Monitor(s), IRB, NCCIH, and other oversight organizations in accordance with their requirements. In the annual AE summary, the Independent Monitor(s) Report will state that they have reviewed all AE reports.

7.3 Reporting Procedures

Adverse events will be documented by research staff in RedCap as shown in Appendix for adverse event documentation. Research staff will notify Dr. Birdee of non-serious adverse events within 24 hours. Dr. Birdee will present all non-serious adverse events to the IMC for monthly review. Research staff will notify Dr. Birdee of serious adverse events immediately. Dr. Birdee will notify the IMC within 24 hours of occurrence, who will review the event within 48 hours. If upon review the SAE is determined to be related to the intervention, Dr. Birdee will report the occurrence to the NCCIH program officer in 7 days for unexpected fatal or life threatening AEs and 15 days for other serious and unexpected AEs. After being informed, Dr. Birdee will verify the severity of all AEs. Dr. Birdee will initially assess if the AE is related to the study interventions as definitely, probably, possibly or unrelated. The IMC will review and confirm the relatedness and severity of AEs. AEs reports will be distributed to the IMC electronically via email.

7.4 Follow-up for Adverse Events

AEs will be monitored by the IMC and Dr. Birdee for recurrences, resolution, and ongoing toxicities that may be related to the intervention. Documentation and reporting for follow-up AEs will occur according to reporting procedures described in Section 7.3. The duration for follow-up will be for the length of the study period while patients are enrolled in the study. If non-serious adverse events are related to the breathing practice, then the protocol will be modified to avoid adverse events. If the frequency of non-serious adverse events is higher than anticipated or alters the benefit risk ratio, the study investigators after conferring with IMC, will also modify the breathing protocol. Two or more specific adverse events among subjects will prompt modification of the breathing protocol.

7.5 Safety Monitoring

This Human Subjects research study will undergo independent monitoring from an IMC, which is described in detail in the Data Safety Monitoring Plan (DSMP) Monitoring Committee under the guidelines provided by NCCIH. The IMC will meet prior to enrollment of subjects into studies.

8. INTERVENTION DISCONTINUATION

The intervention will be discontinued for a participant if: 1) the intervention is associated with an adverse effect for a specific subject i.e. subject does not tolerate the intervention, 2) the subject no longer is interested or willing to receive the intervention or participate in the study, or
3) the subject's healthcare provider no longer recommends that the patient receive the intervention for medical reasons. The IMC and the principal investigator will review cases upon occurrence for discontinuation of intervention.

This study will be stopped prior to its completion if: (1) the interventions are associated with adverse effects that call into question the safety of the intervention, or (2) difficulty in study recruitment or retention will significantly impact the ability to evaluate the study endpoints, or
(3) any new information becomes available during the trial that necessitates stopping the trial; or
(4) other situations occur that might warrant stopping the trial.

Participants will be followed with their permission if the study is discontinued. The duration of follow-up will be length of the proposed study. We will continue to document adverse events, medications, and vital signs during the follow-up period.

Temporary discontinuation of the treatment may occur if the participants are absent due to travel/vacation, illness including hospitalization, non-compliance, or difficulties with transportation to sessions. Treatment will continue upon return to regularly scheduled sessions. We will continue to document adverse events and medications during temporary discontinuation.

Subjects may withdraw from the study at any time. Investigators may end the participation of the subject if they no longer qualify, have a serious adverse event, or upon request of the DSMB. To withdraw from the study, subjects will notify Dr. Alfredo. At that time, patients will no longer take part in any interventions and no further research data will be collected.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

All analyses will be performed as intention-to-treat. Our intent-to-treat population will include all participants who are randomized with baseline assessment and at least one post-randomization assessment.

Specific Aim 1: Compare 12 weeks of slow breathing exhale>inhale vs. exhale=inhale on changes in physiological stress (***primary outcome: heart rate variability***, secondary outcomes: baroreflex sensitivity, catecholamines).

Primary hypotheses: *We hypothesize that participants with exhale>inhale vs. exhale=inhale will have higher increases in parasympathetic tone as measured by the HF HRV.*

Secondary hypotheses:

3. *We hypothesize that participants with exhale>inhale vs. exhale=inhale will have higher increases in parasympathetic tone as measured by baroreflex sensitivity alpha index.*
4. *We hypothesize that baseline parasympathetic tone will modify response to breathing interventions. Specifically, we hypothesize that participants with lower parasympathetic tone at baseline will have the greater increases in parasympathetic tone as measured by HF HRV and BRS alpha index.*

Specific Aim 2: Compare 12 weeks of slow breathing exhale>inhale vs. exhale=inhale on changes in psychological stress **(*primary outcome- anxiety,*** secondary outcomes: perceived stress, arousal, and affect).

Primary hypotheses: We hypothesize that participants with exhale>inhale vs. exhale=inhale will have further decreases in psychological stress as measured by the PROMIS Anxiety scale.

Secondary hypotheses:

We hypothesize that participants with exhale>inhale vs. exhale=inhale will have further decreases negative affect as measured by PROMIS Depression and PANAS-X and stress as measured by Perceived Stress Scale, Anxiety Sensitivivity Index-3, Beck Anxiety Inventory, Body Vigilance Scale, and the Panic Disorder Severity Scale

We hypothesize that baseline psychological stress will modify response to breathing interventions. Specifically, we hypothesize that participants with higher anxiety at baseline will have the further decreases in anxiety as measured by the PROMIS anxiety instrument.

Exploratory research questions: We consider arousal as an exploratory measure in this proposal. In our pilot, exhale>inhale had improvements in daytime arousal vs. exhale=inhale as measured by the Epworth Sleepiness Scale. For the present proposal, we will examine differences in arousal from

baseline, 6, to 12 weeks as measured by the Epworth Sleepiness Scale. We will also explore the relationship between baseline sleep disturbance as measured by the PROMIS Sleep Disturbance scale and changes in arousal. We will also explore if there are any sustained changes at 26 week in psychological measures and if participants continue to breathe after the intervention.

Specific Aim 3: Compute the correlation between physiological and psychological changes (e.g. changes in heart rate variability correlated with changes in anxiety).

Main hypotheses: *We hypothesize that changes in physiological and psychological measures will be moderately correlated.*

Analyses in preparation for second phase of research project (R33): A After reaching 100% enrollment and baseline data collection, we will perform analyses of baseline data to identify a targeted population for the second phase. The purpose of these analyses is to examine the distribution of selected physiological and psychological stress measures in the general population. We will compare baseline data collected with other collected data or published studies. To identify a study population for R33 phase, we will choose stress thresholds (lower limit) based on the median of the general population or the lower quartile of previously-studied known high-stress population, whichever is higher.

Alternative: We will investigate the effect of baseline physiological and psychological stress level on the intervention-induced stress change using smoothing splines with adequate knots, and identify a starting point of baseline stress level that is associated with desirably large change. Subjects with baseline stress level higher than this point will be enrolled for R33 phase study.

Anticipated Results: We expect that both exhale=inhale and exhale>inhale slow breathing will improve physiological and psychological relaxation, and that extending the length of exhale will show greater benefits.

Compared to exhale=inhale, exhale>inhale slow breathing will have greater increase in HF HRV and baroreflex sensitivity (Aim 1). Exhale>inhale slow breathing will also have further decrease in the PROMIS Anxiety instrument (Aim 2). In addition, we expect that the changes in HF HRV will correlate with changes in Perceived Stress Scale in a moderate way (Aim 3).

9.2 Sample Size and Randomization

Our pilot study demonstrates an increase in HF HRV after 6-week exhale=inhale slowing breathing (N=9, mean 58 ± SD 248) or after 6-week exhale>inhale slow breathing (N=9, 173 ± 531). The pooled SD from the two groups is 406, which is similar to published values among health adults. By 12 weeks, we expect that the difference in HF HRV between the two groups will be 265, and a sample size of 38 per arm will give us 80% power to detect this effect at a 0.05 two-sided significance level. Accounting

for 20% drop out, we will enroll 50 participants per arm. If the difference detected is 280 and 300, the power will be 84% and 89%, respectively.

With 50 participants per arm, we will have 80% power to detect a 5.2-point difference in 12-week PROMIS Anxiety Scale reduction assuming a SD of 8 and a type I error of 0.05. With a total N of 76, the study is able to detect a correlation coefficient of 0.7 between changes in HF HRV and changes in PROMIS Anxiety with a margin of error of 0.14.

9.3 Definition of Populations

All analyses will be performed as intention-to-treat. Our intent-to-treat population will include all participants who are randomized with baseline assessment and at least one post-randomization assessment.

9.4 Interim Analyses and Stopping Rules

We will not conduct interim analyses of 6 and 12-week outcomes in this clinical trial. After reaching 100% enrollment, baseline data will be analyzed to submit reports to NCCIH and prepare for second phase of grant award (R33). We will monitor subject accrual, adherence to interventions, and safety during the study period. Dr. Birdee will present data on subject accrual with research staff and IMC. The enrollment period will be 15 months with an anticipated enrollment rate of 7 participants per month.

SAE's related to the intervention will suspend enrollment and/or the study intervention until a safety review is convened (either routine or ad hoc) by IMC to determine whether the study intervention should continue per protocol, proceed with caution, be further investigated, discontinued, or be modified and then proceed. If non-serious adverse events are related to breathing practice, then the breathing protocol will be modified to avoid adverse events. If the frequency of non-serious adverse events is higher than anticipated or alters the benefit risk ratio as determined by the IMC and the principal investigator, the study investigators will also modify the breathing protocol. Two or more specific adverse events among subjects will prompt modification of the breathing protocol.

9.5 Outcomes

9.5.1 Primary Outcome (Study Aim 1)

Assessment will occur at the Vanderbilt Autonomic Dysfunction Center as per standardized protocol. Blood will be analyzed in the Vanderbilt University Medical Center laboratory according to standard procedures. Assessments will be performed at baseline, 6, and 12 weeks.

Physiological stress: We will estimate autonomic tone of participants by standard autonomic testing of cardiovagal and sympathoneural functioning including heart rate variability and baroreflex sensitivity. Subjects will have fasted the night before testing, and testing will occur at the same time of the day for each subject to account for temporal variations of autonomic tone.

(1) Heart rate variability: Heart rate variability determines the cardiac autonomic tone of the subject, measuring instantaneous heart rate fluctuations in beat-to-beat intervals during continuous electrocardiographic monitoring with power spectral analysis. Spectral analysis detects beat-to-beat values of R-R intervals[21] and blood pressure values will be interpolated, low pass filtered (cutoff 2 Hz) and resampled at 4 Hz. Data segments of at least 300 seconds during 10 minutes of undisturbed recordings, will be used for spectral analysis.

Subjects will be asked to maintain a regular breathing frequency paced to an auditory signal at 12 cycles per minute during recordings to control for confounding of respiratory sinus arrhythmia from respiration frequency. Linear trend will be removed and power spectral density will be estimated with the fast Fourier transform-based Welch algorithm using segments of 256 data points with 50% overlapping and Hanning window. The power in the frequency range of low frequencies (LF: 0.04 to <0.15 Hz), and high frequencies (HF: 0.15 to <0.40 Hz) will be calculated according to the Task Force recommendations.[22] We will use the HF component of heart rate variability as an assessment of vagal control to the heart,[23,24] and the LF component of the systolic blood pressure oscillation as an

indicator of sympathetic modulation.[25]

(2) Baroreflex sensitivity: Baroreflex sensitivity will be measured through changes in heart rate and blood pressure while deep breathing (6 breaths per minute) for one minute, Valsalva maneuver, and 10 minute standing. Testing will occur in a relaxing environment, with adequate time for heart rate response to normalize tests. Heart rate will be monitored with a 3-lead electrocardiogram (EKG) and continuous non-invasive finger blood pressure measurements. Baroreflex sensitivity will be determined based on these measurements through spectral analysis using a transfer function analysis.

For heart rate response to deep breathing (E:I ratio), the patient will be asked to breathe deeply and slowly (about six breaths per minute) for at least one minute while heart rate is recorded. The ratio of maximum to minimum R-R interval will be calculated and compared to normative data.

- For the Valsalva maneuver, subjects will be asked to breathe into a closed tube, generating at least 40 mm Hg of pressure for at least 10 seconds. Heart rate and beat-to-beat blood pressure variability (BPV) will be measured. A Valsalva ratio will be calculated and compared to normative data for gender and age. The blood pressure response will be inspected for any evidence of sympathetic dysfunction (for example, significant blood pressure dip during phase II of Valsalva, or failure for blood pressure to increase during phase IV).
- For standing tests, subjects will be placed on an examination table, and then asked to stand-up for 10 minutes. BP and EKG recording will occur throughout the tilt.

(3) Catecholamines: Plasma norepinephrine and epinephrine levels will be measured at baseline, 6 and 12 weeks in supine and standing positions. Catecholamine samples will be drawn after 20 minutes of rest with an intravenous catheter in place. After centrifugation, plasma will be separated and stored at -70 °C until analyzed. Catecholamines analyses will be performed using high-performance liquid chromatography with electrochemical detection.[26]

9.5.2 Secondary Outcomes (Specific Aims 2 and 3)

Specific Aim 2

Psychological stress: Psychological effects related to relaxation will also be assessed at baseline, 6, and 12 weeks. Questionnaires will be administered online through the RedCap data capture system. Participants unable to access the questionnaires online will be given printed copies at scheduled research visits.

(l) Anxiety:

- Anxiety will be measured with the PROMIS Anxiety computerized adaptive test (CAT)

(http://www.nihpromis.org/measures/domainframework1). This 29 item instrument assesses symptoms of self-reported fear, anxious misery, hyperarousal, and somatic symptoms related to arousal. The CAT allows for participant responses to guide the system's choice of subsequent items from the full item bank. PROMIS Anxiety correlates with PANAS Negative Affect which we used in our pilot study.[29] PROsetta Stone® provides a conversion of PANAS scores to PROMIS Anxiety (http://www.prosettastone.org). We used this conversation table to estimate the baseline and change in PROMIS Anxiety used in our statistical plan below. PROMIS Anxiety has been validated among health adults.

We will administer other instruments of anxiety and stress as secondary measurements.

This will provide validation of results and inform translation to other clinical populations in the future

- Anxiety Sensitivity Index-3 (ASI-3) This 18-item instrument measures an individuals beliefs about feared consequences of symptoms associated with anxious arousal.[1] This instrument measures three components: physical, social, and cognitive concerns.
- Beck Anxiety Inventory: This 21 item instrument measures anxiety in adults[2] and has been shown to be valid and reliable.[3]
- Body Vigilance Scale: This is a validated scale to measure conscious attendance to internal cues and is validated in healthy and anxiety disorder adults. [4,5]
- Panic Disorder Severity Scale: This is a 7 item scale that measures panic disorder and agoraphobia symptom severity among adults that has shown to be valid and reliable.[6,7]
- Perceived Stress Scale-10 (PSS): This 10 item instrument measures an individuals perceived stress.[27,28]

(2) Affect: Negative affect will be measured with the PROMIS Depression CAT and PANAS-X. This 28 item instrument assesses negative mood, negative views of the self, negative social cognition, and decreased positive affect and engagement. PROMIS Depression has been validated among healthy adults and chronic disease populations.[30,31] The PANAS-X will be used to assess affect over the past week. This is a 60 item instrument that has been shown to be valid and reliable among healthy adults.[8]

(3) Arousal: Relaxation is associated with decreased arousal and increased sleepiness. To account for changes in arousal we will measure daytime sleepiness with the Epworth Sleepiness Scale. [32] We will also administer the PROMIS Sleep Disturbance instrument to assess sleep quality.

Fidelity measures: We will assess fidelity to breathing interventions through direction observation of the mind-body teacher, self-report, and biometric garments-HEXOSKIN.

(1) Self-report: Each week, mind-body teachers observe participants performing the practice along with an audible metronome to measure breath rate and length of exhale and inhale. Mind-body teachers evaluate participate engagement, quality of technique, and breathe rate and exhale/inhale length (See Appendix for mind-body fidelity tracking form). Also each week, participants are asked how frequently they practiced at home by the mind-body teacher.

(2) Remote biometric garment: Lastly, we will use biometric garments, HEXOSKIN, to ascertain remote adherence to home breathing practice. As described previously, HEXOSKIN accurately records respiratory parameters including breathing rate, length of exhale/inhale through thoracic and abdominal strain gauges embedded in the shirt. Data for is stored in a small unit worn in the shirt. Each participant will be asked to wear a HEXOSKIN shirt during practice for one week for two time periods between weeks 3 to 8 and weeks 9 to 12. For 2-3 nights during each of these weeks the participant will be asked to keep the shirt on overnight to monitor sleep. Participants will return the shirt after one week, and data from the device will be downloaded for analyses. We will use VivoSense software with the Complex Respiratory Module to analyze breathing intervention fidelity (Vivonoetics, San Diego, CA).

Specific Aim 3

We will compute the correlation between physiological and psychological changes (e.g. changes in heart rate variability correlated with changes in perceived stress).

9.6 Data Analyses

Standard graphing and screening techniques will be used to detect outliers and to ensure data accuracy. Distributions of continuous outcomes will be assessed for normality. If normality is violated, proper data transformation will be applied or non-parametric analysis methods will be considered. Summary statistics for both continuous and categorical variables will be provided by randomization groups to describe the study sample.

This is a two-arm randomized study with repeated measures. The effects of either of the slow breathing interventions on HF HRV, PROMIS Anxiety and other endpoints will be estimated as within-subject mean change from baseline to 12 week, along with their 95% confidence intervals. A paired t-test will be performed to compare the endpoints within the group. The difference between the two treatment groups will be estimated by the mean difference in the within-subject changes, along with their 95% confidence intervals, and a t-test will be performed. If normality is violated, proper data transformation will be applied.

Mixed-effect models will also be used to analyze the data with a random subject effect and with intervention (exhale>inhale vs. exhale=inhale), time points (6 week and 12 week), baseline measurements and interaction between intervention and time points as fixed effects. Change in the outcomes from baseline to 6/12 weeks will be used as responses. We will include covariates age, gender, and baseline psychological stress in the models to adjust for their effects.

Specific inferences on effects of interest will be made by reporting a point estimate along with a 95% confidence interval and the p-value. Hypotheses will be tested at the level of α=0.05.

Based on our pilot study, we anticipate 20% missingness on post-randomization assessment. Mixed-effects models are robust in the sense that they can include subjects with missing data at one time point (6 week or 12 week) to estimate the effects of interest. We will also perform multiple imputation for missing data to corroborate our findings. Specifically, all the baseline variables and outcome variables will be included in the imputation model, and flexible additive imputation models and predictive mean matching approach will be used to impute missing values. We will use Rubin's method to combine the estimated regression coefficients and variances from the models based on ten imputed complete data sets.A Pearson correlation coefficient between changes in HF HRV and changes in PROMIS Anxiety will be calculated at each post-randomization assessment, for each intervention group or for all the participants respectively, along with 95% confidence interval. In addition, linear regression analysis of HF HRV changes on PROMIS Anxiety changes, age and gender will be conducted.

Per-protocol analysis will be also performed as a sensitivity analysis, and only participants who practice breathing at home at least 3 times a week and achieve prescribed breathing rate and ratio for at least 2 time periods after randomization will be included.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Research staff blinded to treatment assignment will administer a laptop or tablet to collect data through REDCap. Subjects may opt to complete questionnaires online at home. Physiological measures will be assessed by a blinded lab technician blinded at the Autonomic Dysfunction Center with results entered into REDCap. Physiological measures will be performed by research staff and documented through data software programs with migration to REDCap.

To protect confidentiality, all data will be stored in a locked file cabinet located in the secured office of the research coordinator. All computers and data files will be password protected. All data processed will be in aggregate form and data collection forms will only be labeled with subject's unique identification number.

Data collection forms are presented in the appendices as outline in the Table of Contents.

10.2 Data Management

The principal investigator will be responsible for data management.

10.3 Quality Assurance

10.3.1 Training

All research staff will have completed the online Collaborative Institutional Training Initiative Training (CITI) prior to participation in research activities. Staff will participate in ongoing monthly workshops offered by the Vanderbilt Human Subjects Protection Program Responsible Conduct of Research-related principles and practices offered through the Human Research Protection Program. Dr. Birdee will directly oversee training of research staff in regards to conducting to the planned research.

10.3.2 Metrics

Dr. Birdee will monitor data collection to assure quality control. Data will be entered into REDCAP, which is a web-based application to support data capture for research studies, allowing assessment of data collection quality including timely and complete data entry. Dr. Birdee will provide monthly updates to the IMC and research mentors regarding patient recruitment and enrollment.

10.3.3 Protocol Deviations

Protocol deviations will be captured through direct observation by research staff regarding patient recruitment and enrollment, intervention administration, adherence, and safety monitoring. Protocol deviations will be reviewed by the principal investigator and discussed with research mentors and as needed with the IMC.

10.3.4 Monitoring

Dr. Birdee will monitor for protocol compliance, data quality, and review of documentation to assure protocol compliance. This will include review of inclusion/exclusion criteria to insure eligibility (during recruitment), informed consents (upon enrollment, adverse event reporting (upon occurrence), and data

collection in REDCap (monthly basis).

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent documents and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study.

11.2 Informed Consent Forms

A signed consent form will be obtained from each participant. For participants who cannot consent for themselves, such as those with a legal guardian (e.g. person with power of attorney), this individual must sign the consent form. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant or legal guardian and this fact will be documented in the participant's record.

11.3 Participant Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NCCIH, and the OHRP.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NCCIH, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

12. REFERENCES

1. Taylor S, Zvolensky MJ, Cox BJ, et al. Robust dimensions of anxiety sensitivity: development and initial validation of the Anxiety Sensitivity Index-3. *Psychol Assess.* Jun 2007;19(2):176-188.
2. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. *Journal of Consulting and Clinical Psychology.* 1988;56(6):893.
3. Fydrich T, Dowdall D, Chambless DL. Reliability and validity of the Beck Anxiety Inventory. *Journal of Anxiety Disorders.* 1992;6(1):55-61.
4. Schmidt NB, Lerew DR, Trakowski JH. Body vigilance in panic disorder: evaluating attention to bodily perturbations. *J Consult Clin Psychol.* Apr 1997;65(2):214-220.
5. Olatunji BO, Deacon BJ, Abramowitz JS, Valentiner DP. Body vigilance in nonclinical and anxiety disorder samples: structure, correlates, and prediction of health concerns. *Behav Ther.* Dec 2007;38(4):392-401.
6. Wuyek LA, Antony MM, McCabe RE. Psychometric properties of the panic disorder severity scale: clinician-administered and self-report versions. *Clin Psychol Psychother.* May-Jun 2011;18(3):234-243.
7. Shear MK, Rucci P, Williams J, et al. Reliability and validity of the Panic Disorder Severity Scale: replication and extension. *J Psychiatr Res.* Sep-Oct 2001;35(5):293-296.
8. Watson D, & Clark, L. A. *The PANAS-X: Manual for the positive and negative affect schedule: Expanded form*. Iowa City: Univeristy of Iowa; 1994.