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Slow breathing for reducing stress: The effect of extending exhale[★]

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Abstract

Introduction: Slow breathing techniques are commonly used to reduce stress. While it is believed by mind-body practitioners that extending the exhale time relative to inhale increases relaxation, this has not been demonstrated.

Methods: We conducted a 12-week randomized, single-blinded trial among 100 participants to compare if yoga-based slow breathing with an exhale greater inhale versus an exhale equals inhale produces measurable differences in physiological and psychological stress among healthy adults.

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Dr. Birdee participated in designing, implementing, and supervising the study, also in the analysis of the data and in writing the manuscript, Ms. Nelson participated in designing, and implementing the study, also in collecting the data and writing the manuscript, Dr. Wallston participated in designing, and implementing the study, also in the analysis of the data and in writing the manuscript, Dr. Naan participated in designing, and implementing the study, also in the analysis of the data and in writing the manuscript, Dr. Diedrich contributed to the ultimate design of the study, in the analysis of the data and in writing the manuscript, Mr. Paranjape contributed to the collection of the data and writing the manuscript, Dr. Abraham participated in designing, and implementing the study, also in the analysis of the data and in writing the manuscript, Dr. Gamboa participated in designing, implementing, and supervising the study, also in the analysis of the data and in writing the manuscript.

Conflicts of interests

None of the authors have any conflicts of interest with this work.

Results: Participants mean individual instruction attendance was 10.7 ± 1.5 sessions out of 12 offered sessions. The mean weekly home practice was 4.8 ± 1.2 practices per week. There was no statistical difference between treatment groups for frequency of class attendance, home practice, or achieved slow breathing respiratory rate. Participants demonstrated fidelity to assigned breath ratios with home practice as measured by remote biometric assessments through smart garments (HEXOSKIN). Regular slow breathing practice for 12 weeks significantly reduced psychological stress as measured by PROMIS Anxiety (-4.85 S.D. ± 5.53 , confidence interval $[-5.60, -3.00]$), but not physiological stress as measured by heart rate variability. Group comparisons showed small effect size differences ($d = 0.2$) with further reductions in psychological stress and physiological stress from baseline to 12 weeks for exhale greater than inhale versus exhale equals inhale, however these differences were not statistically significant.

Conclusion: While slow breathing significantly reduces psychological stress, breath ratios do not have a significant differential effect on stress reduction among healthy adults.

Keywords

Slow breathing; Yoga; Mind-body practices; Stress; Relaxation techniques

1. Introduction

Psychological and physiological stress have a significant impact on health. Higher psychological stress is associated with hypertension¹, coronary artery disease², and congestive heart failure³. Higher physiological stress as measured by the autonomic nervous system is associated with increased anxiety disorders^{4,5}. More research is needed to identify mechanisms and efficacy of specific stress reduction modalities.

Twelve percent of adults in the U.S. report using deep breathing practices for health⁶. Slow breathing is believed to reduce stress. 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)⁷. Slow breathing is an integral part of many mind-body practices including yoga, t'ai chi, qi gong, meditation techniques (Zen, Transcendental Meditation, Vipassana), and other relaxation techniques (relaxation response, biofeedback). Yoga, a common and ancient mind-body practice, has specific slow breathing techniques called *pranayama*. Yoga emphasizes that breathing be performed comfortably while trying to slow the breath without perceived dyspnea. Though, research is lacking to show that specific slow breathing techniques have different health effects.

Slow breathing in research has been defined as a respiratory rate less than 10 breaths a minute⁸. Breathing at slow rates reduces psychological stress as measured by self-report⁹⁻¹². A majority of psychological studies have focused on the acute rather than long-term effects of slow breathing. The reduction in psychological stress have been attributed to slow breathing rhythms entraining neuronal activity in networks that affect emotion, cognition, and memory¹³. Slow breathing also has been reported to reduce physiological stress as measured by changes in the autonomic nervous system with a decrease in sympathetic and increase parasympathetic tone^{14,15}.

A widely accepted method to measure sympathetic and parasympathetic tone is by the analysis of heart rate variability (HRV), which can be done using the power spectrum of HRV. In particular, high-frequency variability of HR (HF_{RRI}), has been proposed as a measure of cardiac parasympathetic tone^{16,17}. On the other hand, low frequency variability of HR (LF_{RRI}) reflects both sympathetic and parasympathetic modulation of heart rate, while the ratio of $LF_{RRI}:HF_{RRI}$ could may indicate sympathovagal balance in these special circumstances^{16,18}. These spectral measures of autonomic modulation need to be performed under standardized conditions. It is especially important to consider how different respiratory rates can influence HRV. We and others have looked at the importance of paying attention to different respiratory rates and different control breathing protocols when assessing autonomic cardiovascular rhythms¹⁹⁻²². This is of the utmost importance when slow breathing is involved^{15,23}, low breathing rates can interfere and “modulate” some of the measurements of HRV. It has been proposed that respiratory rates of 6 or more breaths per minute reflect exclusively vagal tone, while respiratory rates between 4 and 6 breaths per minute modulate vagal and sympathetic activity²⁴.

Yoga 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. Possible mechanisms for this may lie with expiration versus inhalation altering limbic brain areas related to emotions and fear^{13,25}. While this belief extends to many mind-body traditions and Western psychology, no study has been able to document a long-term difference in relaxation by breath ratio. A recent study has shown that acutely, paced breathing with longer exhalation without changing subject’s baseline breathing patterns, can increase vagal tone (increase HF_{RRI}) in healthy individuals²⁶. A prior study showed similar acute changes vagal tone and increased subjective symptoms of relaxation in healthy adults²⁷. There are no studies on the chronic effects of slow breathing with different exhale to inhale ratios on physiological or psychological relaxation. The objective of this study was to compare the effect of slow breathing with different exhale to inhale ratios among healthy adults on physiological and psychological relaxation. A standardized 12-week protocol for slow breathing was developed by expert yoga teachers. We hypothesized that regular slow breathing practice with a ratio of exhale greater than inhale ($E > I$) versus exhale equal to inhale ($E = I$) would produce more relaxation as measured by autonomic tone and self-reported stress scales.

2. Methods

2.1. Study design

We conducted a randomized, single-blinded study comparing $E > I$ versus $E = I$ on physiological and psychological stress. The first two weeks of the intervention consisted of basic slow breathing techniques without any specific breath ratios. After the two-week screening phase, eligible participants were randomized to one of two breathing interventions ($E > I$ versus $E = I$) for 10 weeks. We hypothesized that we would observe medium effect sizes between groups from baseline to 12 weeks in high frequency heart rate variability (HF_{HRV}) and PROMIS Anxiety Scores. We define a medium effect size ($d=0.5$) for changes in

stress measures between groups and within groups to be: (1) 203 increase in HF HRV ($d=81/406$) and (2) 4 decrease in PROMIS Anxiety scores ($d = 1.6/8$).

2.2. Participants

The study was approved by the Vanderbilt University Institutional Review Board. Written consent was obtained from each participant and the study was registered in [ClinicalTrials.gov](https://clinicaltrials.gov) prior to enrollment (NCT02870868).

We enrolled healthy adults that met the inclusion and exclusion criteria of the study as stated in Table 1. Participants were enrolled by study coordinator (KN). Participants initially went under a screening phase to determine eligibility. A PROMIS Anxiety scale was administered at the beginning of the screening phase to exclude individuals who were more relaxed than the general population. In the general population, the mean score of PROMIS Anxiety is 50 with a standard deviation of ± 10 (<http://www.nihpromis.org/measures/domainframework1>). Only participants with a PROMIS Anxiety score of 45 or higher (half a standard deviation below the mean or higher for the general population) to proceed with the screening phase. During the screening phase, participants were scheduled to attend 2 private classes over two weeks with a yoga teacher to learn basic slow breathing. Participants were asked to practice daily during this phase. Participants were included in the second phase if after the first two weeks: (1) they practiced 3 times or more a week, and (2) had a breath rate between 3 and 8 breaths per minute. Participants were recruited through flyers and research emails lists at Vanderbilt University Medical Center (VUMC).

2.3. Study settings

Participants were screened through surveys administered online through REDCap followed by an in-person screening interview. The slow breathing interventions were delivered individually in a private room at VUMC. Physiological outcome assessments were performed at the Vanderbilt Autonomic Dysfunction Center at VUMC. Questionnaires were administered online through REDCap at participant's discretion. Participants were asked to practice slow breathing at home in addition to weekly classes.

3. Slow breathing interventions

3.1. Development

Slow breathing protocols were developed by the principal investigator (GB) in conjunction with an expert panel of 3 mind-body therapists. The breathing protocol used a well-developed progression of breathing taught in the Krishnamacharya tradition of yoga (Viniyoga)^{28,29}.

3.2. Breathing instruction

Participants received 12 weekly private classes taught by a certified yoga teacher trained to deliver the slow breathing protocols. The teacher was instructed not to reveal the goal of the breathing exercises (i.e. relaxation). Each class was 45 min long. During the screening phase, the same basic slow and deep breathing was taught to all participants. The purpose of the screening phase was to familiarize participants with basic components of slow breathing

and assess adherence to the intervention. Participants were asked to perform breathing exercises daily for 12 weeks.

Participants were given printed instructions to guide home practice. During the two weeks for secondary screening, the teacher assessed participants' respiratory rate while practicing during classes. Participants were eligible for the trial only if they achieved a respiratory rate of 8 breaths a minute or less by the third class. This is based on principles of yoga slow breathing that breath ratios above this respiratory rate are not useful. On the other end of the spectrum, subjects who were already able to breathe very slowly, defined as 3 breaths per minute or less, were not included as the ability to further slow the breath or extend the exhale is limited.

To facilitate breathing regulation and standardization between participants, participants were provided a link to a website with a recorded track that made an audible sound every second (1 Hz). Participants were asked to count the length of each breath based on the recordings. Breathing rates were assigned based on the assessment of deep breathing performed at 2 weeks. This provided an opportunity to identify a comfortable, starting, slow breathing rate for each participant. After randomization, participants followed a week-to-week progression based on a pre-specified protocol for 10 weeks for slow breathing.

3.3. Slow breathing with exhale=inhale

Participants randomized to slow breathing with equal inspiration and expiration received progressive increases in both inspiration and expiration over 10 weeks until they reached a goal breath length or longest comfortable breath. Prior to performing the breathing practice, participants performed a few brief standardized yoga movements. Yoga movements are traditionally used to prepare the practitioner to sit focused while breathing.

3.4. Slow breathing with exhale>inhale

Participants randomized to slow breathing with prolonged exhale to inhale ratio received progressive increases in expiration relative to inspiration over 10 weeks until they reached a goal breath length or longest comfortable breath. Prior to performing the breathing practice, participants performed the same yoga movements as the E = I group.

3.5. Outcomes

Outcome assessments were performed at baseline, 6 weeks, and 12 weeks. The primary outcomes were pre-specified as physiological stress and psychological stress measured by spectral analyses of heart rate and PROMIS Anxiety, respectively.

3.6. Physiological stress

Assessments were performed at the Vanderbilt Autonomic Dysfunction Center as per standardized protocol at baseline, 6, and 12 weeks. Participants fasted 8 h before testing. Medications or supplements that interfere with autonomic tone were held prior to assessments. Heart rate variability (HRV) determines the cardiac autonomic modulation of the subject. HRV measures instantaneous heart rate fluctuations in beat-to-beat intervals during continuous electrocardiographic monitoring with power spectral analysis. Beat-to-

beat values of R-R intervals³⁰ and blood pressure values were interpolated, low pass filtered (cutoff 2 Hz) and resampled at 4 Hz. Data segments of at least 300 s during 10 min of undisturbed recordings were used for spectral analysis.

We used recommended guidelines and criteria for HRV assessment. Linear trend was removed, and power spectral density were 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) were calculated according to the Task Force recommendations³¹. We used the HF component of heart rate variability (HF_{RRI}) as an assessment of vagal control to the heart¹⁶.

3.7. Psychological Stress

Psychological stress assessments were administered at baseline, 6, and 12 weeks. Stress was 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 arousal³². PROMIS Anxiety has been validated among healthy adults. The CAT allows for participant responses to guide the system's choice of subsequent items from the full item bank.

3.8. Fidelity measures

We assessed fidelity to breathing interventions through direct observation by the yoga teacher, self-report, and remote biometric monitoring with smart garments (HEXOSKIN).

3.9. Direct observation

Each week, a yoga teacher observed participants performing the practice along with an audible metronome to measure breath rate and length of exhale and inhale. A yoga teacher evaluated participant engagement, quality of technique, breath rate and exhale/inhale length.

3.10. Self-report

The yoga teacher asked the participant every week how frequently they practiced at home.

3.11. Remote biometric garment

We used biometric garments, HEXOSKIN, to ascertain remote adherence to home breathing practice. HEXOSKIN accurately records respiratory parameters including breathing rate and length of exhale/inhale through thoracic and abdominal strain gauges embedded in the shirt. Data is stored in a small unit worn in the shirt. Each participant was asked to wear a HEXOSKIN shirt during home practice once at the beginning of the study (weeks 3–8) and again at the end of study (weeks 9–12) for one week. Participants returned the shirt after one week and data from the device will be downloaded for analyses. We used VivoSense software with the Complex Respiratory Module to analyze breathing intervention fidelity (Vivonoetics, San Diego, CA).

3.12. Sample size calculation

In a prior pilot study of slow breathing healthy adults, we observed an increase in HF HRV after 6 weeks of E > I (N = 9, 173 ± 531) and of E = I breathing (N = 9, 58 ± 248) (unpublished). The pooled SD from the two groups was 406, which is similar to published values among healthy adults. By 12 weeks, we expected 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 anticipated enrolling 50 participants per arm. If the difference detected was 280 and 300, the power will be 84 % and 89 %, respectively. With 50 participants per arm, we 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.

3.13. Randomization

Randomization by study statistician (HN) occurred after baseline testing and assessment of initial compliance to the intervention. Participants were randomized to 12 weeks of slow breathing with either exhale>inhale versus exhale=inhale with 1:1 ratio. The treatment assignment were stratified by gender. Within each stratum, a block randomization algorithm with a block size of 2 was used.

3.14. Blinding

Blinding is summarized in Table 2 for the study. In addition, recruitment materials and informed consent did not use the term or connotation of relaxation. All study materials described the intervention as “focused breathing”. The yoga teacher was asked to not describe the intervention or cue participants regarding relaxation, stress reduction, or other similar terminology.

3.15. Statistical methods

Descriptive statistics of participant characteristics were presented as quartiles, mean ± SD for continuous variables and frequency for categorical variables, and Wilcoxon test and chi-square test were used to compare between intervention groups. The effects of either of the slow breathing interventions on HF HRV and PROMIS Anxiety was estimated as within-subject mean change from baseline to 12 week, along with their 95 % confidence intervals. A paired t-test was performed to compare the endpoints within the group. The difference between the two treatment groups was estimated by the mean difference in the within-subject changes, along with their 95% confidence intervals, and a t-test was performed. Generalized least square linear models with compound symmetry correlation structure were fitted for HF HRV and PROMIS Anxiety on intervention, time points (6, 12 week), baseline measurements as well as interaction between intervention and time points. Gender and age were also included as covariates. Specific inferences on effects of interest were made by reporting a point estimate along with a 95 % confidence interval and the p-value. Per-protocol analysis was 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.

4. Results

We screened 679 adults enrolling a total of 99 healthy participants from October 2016 to March 2018. See Fig. 1. Study Flow Diagram. Among all participants enrolled after screening, 95 participants had completed the study with 94 participants providing completing both psychological and physiological data assessments for baseline, 6 weeks, and 12 weeks by end of July 2018. ($E > I$ $n = 48$, $E = I$ $n = 46$). Our intention to treat analysis included 97 participants. Table 3 displays study participant characteristics without significant differences for baseline measures in sociodemographic variables and stress measures. The mean age for the study population was 41 ± 8.9 years with 79 % being females.

Among participants enrolled and randomized, the mean class attendance was 10.7 ± 1.5 sessions out of 12 offered sessions. The mean weekly home practice was 4.8 ± 1.2 practices per week. There was not statistical difference between treatment groups for frequency of class attendance or home practice. A total of 30 adverse events were observed during the study period including 12 during a yoga class, 14 outside of yoga classes, and 4 during physiological outcome assessments. 24 of these events were categorized as mild and 6 were categorized as moderate. Events were categorized as definitely related ($n = 9$), probably related ($n = 4$), possibly related ($n = 1$), and not related ($n = 16$). All 12 adverse events occurring during yoga classes were categorized as mild, but related to slow breathing including lightheadedness ($n = 9$) and musculoskeletal strain from inhaling ($n = 1$), tingling sensation in extremities ($n = 1$), negative emotional feelings ($n = 1$), and pain ($n = 1$).

In Fig. 2 we display the observed breathing ratio to the assigned breathing ratio for two study time periods (See Fig. 2. Breath ratio (Inhale:Exhale) of home practice versus assigned practice during two study time periods). Patients wore biometric shirts to measure home breathing practices. Assigned breathing practices were based on breathing prescribed during that specific time point of the study. All participants in the exhale>inhale treatment group had achieved an exhale longer than inhale during both time periods. Very few participants in the exhale=inhale group had not achieved the correct ratio. No significant differences were observed between treatment groups for frequency of home practice, achieved respiratory rate week by week, mean respiratory rate over the 12-week period, or percentage of participants breathing at assigned respiratory rate and breath ratio. Table 4. displays slowest and comfortable respiratory rate achieved during slow breathing practice week-by-week directly observed by yoga teacher during class. There was no statistical significance in achieved respiratory rate between treatment groups and participants were breathing on average slightly less than 3 breaths per minute. Table 5 shows baseline and 12-week measures of psychological and physiological stress measures for all participants and by treatment group. Baseline measures for PROMIS Anxiety and physiological stress measures did not differ by treatment group as measured by a Wilcoxon test. Both groups had significant changes from baseline and 12 weeks in PROMIS Anxiety based on Wilcoxon signed-rank test. The between group differences for Exhale=Inhale and Exhale>Inhale treatment groups was 1.07 ± 5.53 [CI - 1.19, 3.32]. This represented a non-significant ($p = 0.350$) further reduction in psychological stress in the Exhale>Inhale group as compared to the Exhale=Inhale with a small effect size ($d=0.2$). For physiological stress from baseline

to 12 weeks, high frequency heart rate variability (HF_{RRI}) increased in both groups, not reaching statistical significance in either group. For Exhale>Inhale group, the between group differences for the Exhale=Inhale and Exhale>Inhale treatment groups was $-111.892 \pm 905.65 \text{ ms}^2$ [CI $-494.73, 270.94$]. This represented a non-significant ($p = 0.563$) further increase in high frequency heart rate variability in the Exhale>Inhale group as compared to the Exhale=Inhale group with a small effect ($d=0.12$) for between and within group differences. Other measures from spectral analyses including LF_{RRI} and LF/HF_{RRI} are shown in Table 5 without significant changes from baseline to 12 weeks. Changes in square root of mean squared successive differences (RMSSD) were also insignificant for $E = I$ (baseline = 42.5 ± 25.3 , 12 weeks = 40.8 ± 25.3) and $E > I$ (baseline = 42.3 ± 25.6 , 12-weeks = 49.5 ± 35.8). In our multivariable models there was no significant effect of Exhale>Inhale versus Exhale=Inhale on 12-week PROMIS Anxiety (Effect 0.89 CI $[-0.99, 2.76]$, $p = 0.354$) or HF_{RRI} (Effect 0.81 CI $[2.85, 0.44]$, $p = 0.440$).

5. Discussion

Regular slow breathing practice for 12 weeks, regardless of ratio, significantly reduced psychological stress measured by PROMIS Anxiety, but not physiological stress as measured by heart rate variability. Group comparisons showed small effect size differences with further reductions in psychological stress and physiological stress from baseline to 12 weeks for $E > I$ as compared to $E = I$. In our analyses for group differences, breath ratios do not have a significant effect on psychological and physiological stress measures among healthy adults.

We have found no prior prospective randomized clinical trials that have compared the effect of different breath ratios on stress measures. Several acute slow breathing studies have been conducted. One study among healthy adults showed acute increases in relaxation as measured by heart rate variability (increases in high frequency component) and self-reported stress with exhale greater than inhale (I/E ratio 0.42) compared to inhale greater than exhale (I/E ratio 2.33) for breathing at 6 breaths a minute²⁷. No changes were noted at 12 breaths per minute. Another acute study similar increases in vagal tone among participants performing slow breathing, but no significant difference by varying inhale to exhale ratios³³. A third study has tried to determine the ideal dose of slow breathing that would be most beneficial to increase cardiac vagal tone³⁴. In this acute study, 5, 10, 15 and 20 min of slow breathing at 6 bpm were compared. All 4 slow breathing durations increased vagal tone, with no differences between doses. All three studies had small sample sizes ($N = 36-59$) and were not prospective. Generally, slow breathing has been shown to reduce both physiological and psychological stress measures^{14,35}.

Emphasis on extending exhale relative to inhale is a common relaxation technique. Among a non-stressed population, $E > I$ did not have a more significant effect. Prior research suggests there may be differences in acute autonomic tone changes with prolonged exhalation but normal breathing patterns^{27,33,35}, or with different breath ratios, but long-term practice from yoga-based slow breathing has not been examined. Future studies need to be examined to see if slow breathing with different breath ratios have a significant effect among populations with higher psychological and/or physiological stress. Generally, the mechanism

of how slow breathing alters physiological stress is not completely understood. Possible explanations may changes are mediated through alteration of intra-thoracic pressures^{36,37}, stimulation of arterial and cardiopulmonary baroreceptors³⁸ and afferent pulmonary stretch receptors³⁹. Noble and Hochman have a more in-depth review of possible mechanisms by which slow breathing can contribute to physiological relaxation⁴⁰.

Limitations of our study are: We did not have a usual care group (attention control with no breathing techniques learned/practiced). However, our primary question was if breathing ratios had a significant difference, as it has already been established that slow breathing reduces stress. Also, our study provided a very frequent intervention with high adherence. It is unclear if similar affects would be seen outside of a study setting where adherence would be lower. Future studies on how much slow breathing is necessary to produce relaxation may help clarify dose effects. Our study population had more women than men, and treatment response may vary by sex. As a behavioral intervention, study participants could not be completed blinded to the intervention, however, we did blind to other treatment groups getting different breath ratios and the purpose of the slow breathing being for relaxation. The effects observed in this study may not be from breathing alone (e.g. instructor, prior perception of potential benefit, behavioral activation), but many other effects should be accounted for due to randomization. High frequency heart rate variability, measured with spectral analysis, has been shown to be a reliable determinant of vagal modulation of sinus node function, but it is not without limitations. The primary limitation being its wide range of interindividual variability. Another limitation is the fact that we did not fully control for the stage of menstrual cycle of female participants which could influence our results. Lastly, we based our sample size on preliminary studies, but our sample size may have been insufficient to observe differences by treatment group.

In summary, a 12-week slow breathing practice showed non-significant reductions in self-reported stress and autonomic tone among $E > I$ as compared to $E = I$. While medium effect sizes were observed among a healthy adult population, further research is necessary to see if breath ratios are relevant in populations with higher psychological and physiological stress.

Acknowledgments

Study data were collected and managed using REDCap electronic data capture tools hosted at Vanderbilt University Medical Center^{41,42}. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for data integration and interoperability with external sources.

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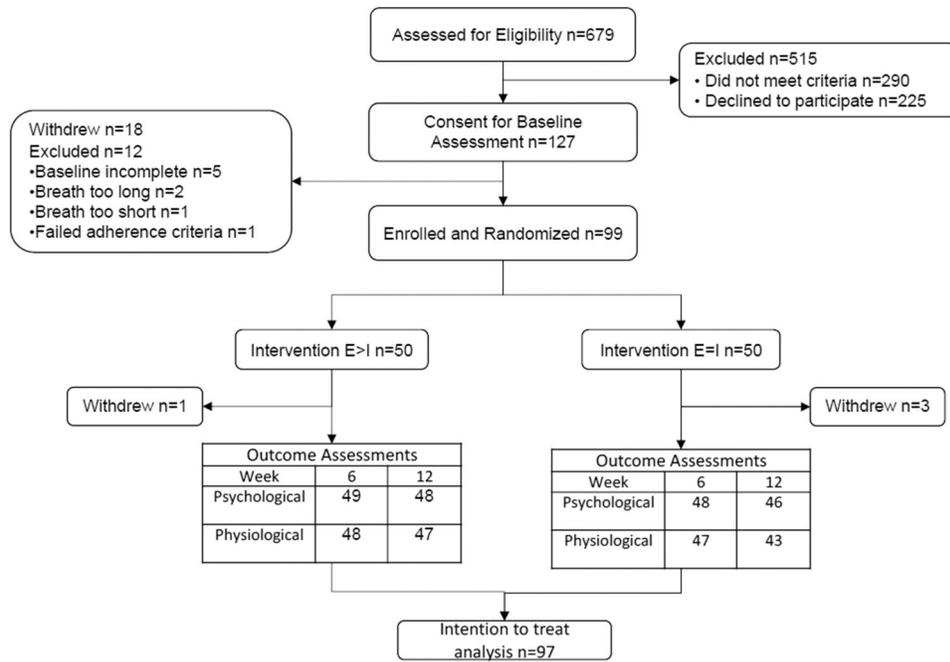


Fig. 1.
Study Flow Diagram.

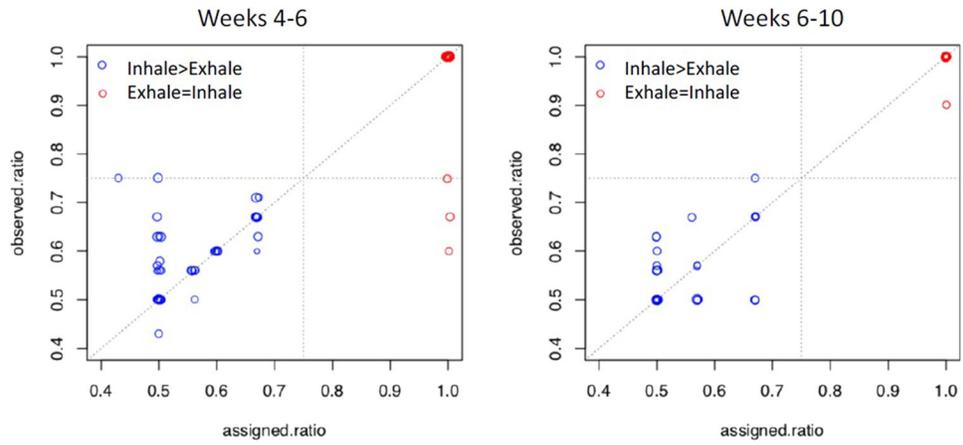


Fig. 2. Breath ratio (Inhale:Exhale) of home practice versus assigned practice during two study time periods.
^a Each participant was given a biometric device to record observed home practice. Assigned ratios were prescribed practices at that time point in study.

Table 1

Sample selection criteria.

<p>Inclusion</p> <ul style="list-style-type: none"> • Age 30–60 years • After two weeks of intervention: • Breathe 8 or less a minute while practicing • Not breathe 3 or less breaths a minute while practicing 	<ul style="list-style-type: none"> • Practiced less than 3 times a week • English speaking • PROMIS Anxiety scale 45
<p>Exclusion</p> <ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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

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Table 2

Blinding table.

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

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Table 3

Baseline Sociodemographic Characteristics.

Sociodemographic	Exhale = Inhale N = 50	Exhale > Inhale N = 49	Combined N = 99	P- value
Age	33.8 39.1 49.2 (41.2 ± 9.2)	33.8 33.4 48.1 (41.0 ± 8.7)	33.8 38.7 48.9 (41.2 ± 8.9)	0.928 ¹
Gender				0.846 ²
Female	78 % (39)	80 % (39)	79 % (78)	
Male	22 % (11)	20 % (10)	21 % (21)	
Race				0.075 ²
White	90 % (45)	69 % (34)	80 % (79)	
Black or African American	8 % (4)	12 % (6)	10 % (10)	
Asian	2 % (1)	12 % (6)	7 % (7)	
American Indian or Alaskan Native	0 %	0 %	0 %	
Native Hawaiian or Other Pacific Islander	0 %	2 % (1)	1 % (1)	
Decline to Identify	0 %	4 % (2)	2 % (2)	
Ethnicity				0.526 ²
Hispanic/Latino	12 % (6)	8 % (4)	10% 10	
Not Hispanic/Latino	88 % (44)	92 % (45)	90 % (89)	
Decline to identify	0 %	0 %	0 %	
Highest Level of Education				0.227 ²
Never in high school	0 %	0 %	0 %	
Some high school but did not graduate	0 %	0 %	0 %	
High school graduate or GED	2 % (1)	2 % (1)	2 % (2)	
Some college or 2 year degree	16 % (8)	4 % (1)	10 % (10)	
Graduated from 4 year college	24 % (12)	16 % (8)	20 % (20)	
More than 4 years of college	8 % (4)	12% (6)	10 % (10)	
Graduate degree	50 % 25)	65 % (32)	58 % (57)	
What category best describes your current primary living situation:				0.169 ²
Live alone	16 % (8)	6 % (3)	11 % (11)	
Live with partner or family member	80 % (40)	84 % (41)	82 % (81)	
Live with other	4 % (2)	10 % (5)	7 % (7)	
What best describes your current, primary marital status:				0.706 ²
Single (never married)	16 % (8)	14 % (7)	15 % (15)	
Married/significant relationship or partner	72 % (36)	71 % (35)	72 % (71)	
Separated	0 %	2 % (1)	1 % (1)	
Divorced	10 % (5)	12 % (6)	11 % (11)	
Widowed	2 % (1)	0 %	1 % (1)	
What is your most recent annual household income?				0.301 ²
Less than \$15,000	0 %	0 %	0 %	
15,000–24,999	2 % (1)	0 %	1 % (1)	

Sociodemographic	Exhale = Inhale N = 50	Exhale > Inhale N = 49	Combined N = 99	P- value
25,000–34,999	6 % (3)	2 % (1)	4 % (4)	
35,000–49,999	10 % (5)	12 % (6)	11 % (11)	
50,000–74,999	20 % (10)	33 % (16)	26 % (26)	
75,000–99,999	34 % (17)	22 % (11)	28 % (28)	
\$100,000 – or more	29 % (14)	29 % (14)	24 % (24)	
Decline to answer	8 % (4)	2 % (1)	5 % (5)	
What is your current employment status?				0.506 ²
Employed	90 % (45)	94 % (46)	92 % (91)	
Part-time	6 % (3)	2 % (1)	4 % (4)	
Student	4 % (2)	2 % (1)	3 % (3)	
Homemaker	0 %	0 %	0 %	
Volunteer	0 %	0 %	0 %	
Retired	0 %	0 %	0 %	
Disabled	0 %	0 %	0 %	
Unemployed	0 %	2 % (1)	1 % (1)	

a b c represents lower quartile a, the median b, and the upper quartile c for continuous variables. $x \pm s$ represents mean \pm SD. Numbers after proportions are frequencies. Tests used: 1 Wilcoxon test; 2 Pearson test.

Group A and Group B received slow breathing with exhale greater than inhale and exhale equal to inhale respectively. a b c represents lower quartile a, the median b, and the upper quartile c for continuous variables. $x \pm s$ represents mean \pm SD. Numbers after proportions are frequencies. Tests used:

¹ Wilcoxon test

² Pearson test.

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Table 4

Weekly slow breathing respiratory rate by treatment group.

	<u>Exhale>Inhale</u>		<u>Exhale=Inhale</u>		P-value
	N	mean±SD	N	mean±SD	
week 1	50	6.4 ± 2.0	49	6.6 ± 2.5	1.000
week 2	50	5.2 ± 1.5	48	5.4 ± 2.0	0.752
week 3	44	4.8 ± 1.1	44	5.0 ± 1.3	0.491
week 4	42	4.4 ± 1.0	41	4.3 ± 1.1	0.444
week 5	43	4.0 ± 1.1	45	4.1 ± 0.9	0.562
week 6	44	3.6 ± 0.9	41	3.9 ± 0.9	0.127
week 7	42	3.3 ± 0.8	42	3.7 ± 0.7	0.003
week 8	42	3.2 ± 0.7	43	3.4 ± 0.6	0.050
week 9	40	3.1 ± 0.7	36	3.4 ± 0.7	0.043
week 10	43	2.8 ± 0.6	41	3.2 ± 1.0	0.087
week 11	41	2.7 ± 0.6	35	2.8 ± 0.5	0.319
week 12	46	2.7 ± 0.6	41	2.8 ± 0.6	0.321

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Table 5

Baseline, 12 weeks, and delta () psychological measure by Promis Anxiety scale and Physiological Stress Measures by heart rate variability (LF, HF, and LF/HF).

	Baseline (mean ± SD)		12 weeks (mean ± SD)		12 weeks (mean ± SD) [CI]	
	Exhale>Inhale	Exhale=Inhale	Exhale>Inhale	Exhale=Inhale	Exhale>Inhale	Exhale=Inhale
Promis Anxiety	56.59 ± 5.39	56.27 ± 4.84	51.22 ± 5.61	51.97 ± 4.32	-5.37 ± 6.46 [- 7.24, - 3.49]	-4.30 ± 4.32 [- 5.60, - 3.00]
HF _{RRI} (ms ²)	420.69 ± 349.67	375.96 ± 271.65	487.38 ± 476.11	381.13 ± 392.62	66.70 ± 489.12 [- 101.33, 234.72]	5.17 ± 278.85 [- 87.80, 98.15]
LF _{RRI} (ms ²)	760.66 ± 778.62	750.62 ± 996.86	967.72 ± 840.64	765.51 ± 774.94	207.06 ± 861.24 [- 68.38, 482.50]	14.89 ± 547.48 [- 167.65, 197.43]
LF/HF RRI	1.83 ± 1.30	2.75 ± 3.37	3.20 ± 4.18	3.75 ± 5.00	1.37 ± 3.92 [0.12, 2.62]	1.006 ± 5.54 [- 0.84, 2.86]

Mean ± SD or [Confidence Interval] RRI, R-R interval; LF, low frequency; HF, high frequency.