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Investigating Components of Pranayama for Effects on Heart Rate Variability

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Abstract

Objective: Traditional Indian breath control practices of Pranayama have been shown to increase indices of heart rate variability (HRV) that are generally held to reflect parasympathetic nervous system (PNS) tone. To our knowledge, individual components of pranayama have not been separately evaluated for impact on HRV. The objective of this study was to isolate five components of a pranayama practice and evaluate their impact on HRV.

Methods: In a crossover clinical trial, 46 healthy adults were allocated to complete five activities in random order, over five separate visits: 1) sitting quietly; 2) self-paced deep breathing; 3) externally-paced deep breathing; 4) self-paced Sheetali/Sheetkari pranayama; and 5) externally paced Sheetali/Sheetkari pranayama.

Results: Our final sample included 25 participants. There was a significant increase in a time-domain index of HRV, the root mean square successive differences between RR intervals (RMSSD), during the five interventions. The change in logRMSSD ranged from 0.2 to 0.5 ($p<.01$ in all conditions by paired t-test). Greater increases were evident during externally-paced breathing

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than during self-paced breathing (mean pre-during logRMSSD change of 0.50 vs. 0.36, p=.02) or sitting quietly (mean, 0.17 ms; p=.005 and 0.02 when comparing Activities 3 and 5 to Activity 1 by random intercept model with Tukey correction for multiple comparisons). Lastly, pre-during increase in RMSSD was greater for Sheetali/Sheetkari vs. deep breathing, when controlling for respiration rate, though not significantly different (p=.07 in random intercept model).

Conclusions: RMSSD increased with paced breathing, deep breathing, and Sheetali/Sheetkari pranayama, reinforcing evidence of a physiologic mechanism of pranayama.

Trial Registration: NCT03280589 https://www.clinicaltrials.gov/ct2/show/NCT03280589?term=sheetali&draw=2&rank=1

Keywords

Autonomic Nervous System (ANS); Blood Pressure; Heart Rate Variability (HRV); Pranayama; Respiratory Rate; Sheetali

BACKGROUND

In recent years, breathing exercises, yoga, and meditation have become popular in the United States [1]. Beyond their perceived social and spiritual benefits, these practices have been shown to provide at least short-term benefit in patients with a broad spectrum of chronic conditions such as hypertension [2] and type 2 diabetes [3]. The health benefits of these traditional Eastern practices have been posited to stem from regulating the autonomic nervous system [4], baroreceptor sensitivity [5-7] and the hypothalamic-pituitary adrenal axis [8].

Among different indices of the parasympathetic nervous system, beat to beat variability in heart rate, hereafter referred to as heart rate variability (HRV), has attracted much systematic investigation [7, 9-14]. For example, several studies have provided evidence that breathing techniques, [15-18] yoga, [15] tai chi, [19] meditation, [20] and other mind-body practices [21] impact HRV. Specifically, most evidence of breathing and meditation practices have measured acute HRV change (pre/post or during a single practice [15, 16], or pre/post the intervention period (weeks or months)[17, 19].

Indices of HRV are mathematically calculated in frequency and time domains [22] and are increasingly seen as predictors of health and life expectancy. For example, an index of HRV over 24 hours, the standard deviation of heart beat N-N intervals (SDNN), has been shown to predict mortality in individuals with sepsis [23], congestive heart failure [24] and cirrhosis [25]. Pranayama (a distinct limb of Yoga defined as “controlled breathing” [26, 27]) and other deep breathing practices [28, 29] have been shown to lower elevated blood pressure, [6, 29-31] and to increase the HRV indices known to reflect PNS tone [15, 30]. Enhancing PNS tone has been demonstrated to enhance baroreflex-mediated reductions in blood pressure [6, 32].

Importantly, however, breathing practices such as pranayama are complex and passed down from generation to generation. To our knowledge, no systematic study has tried to deconstruct the impact of individual components of pranayama on HRV. Identifying which

components of a pranayama practice produce the greatest changes in PNS activity (possibly resulting in greater effects on blood pressure) could offer several advantages. Researchers can use these findings to design future mechanistic studies on pranayama, which in turn aid creation of targeted interventions for chronic diseases. These findings may also help clinicians tailor interventions in the context of unique circumstances for their patients, providing at least short-term improvements to an individual's autonomic nervous system.

The present study builds on a previous work conducted by the authors of this study [30], which evaluated a pair of pranayama practices called Sheetali and Sheetkari [27, 33] (Figure 1), traditionally refered to as "cooling breaths", and used for treatment of high blood pressure. In this randomized trial, participants with hypertension completed 20 minutes of Sheetali/Sheetkari per day for one month. Results demonstrated a 16 mm Hg decrease in systolic blood pressure, decreased respiration rate, and an increase in high frequency HRV (HF-HRV).

The 16 mm Hg decrease in systolic blood pressure demonstrated in our previous work is particularly noteworthy since systolic blood pressure has been shown to be a greater predictor of cardiovascular risk than diastolic blood pressure [34]. For instance, in the Systolic Blood Pressure Intervention Trial (SPRINT), a mean systolic blood pressure difference of ~15 mmHg predicted a significant reduction in cardiovascular events and improved mortality. This trial randomized 9,361 individuals with a baseline systolic BP of 130-180 mmHg to standard treatment (target systolic BP of <140 mmHg) or intensive treatment (target systolic BP of <120 mmHg), and used pharmacologicals alongside lifestyle interventions in their treatment protocols [35]. Thus, a non-pharmacologic intervention that provides a comparable treatment effect in blood pressure reductions, if confirmed in larger phase 3 trials, will have high public health importance.

The present study investigated, within a healthy population, the impact of five distinct components of pranayama on HRV (sitting quietly, depth of breath, pace of breath, mouth shape, and duration of practice). We hypothesized that an index of HRV, which is known to reflect PNS tone at respiratory rates less than 9 breaths per minute (b/m) [14, 36] the root mean square of successive differences in adjacent heartbeats (RMSSD), will increase during the four active components of the practice, compared to sitting quietly, and the greatest increase would be evident during the Sheetali and Sheetkari practices. The choice of RMSSD rather than blood pressure as the primary outcome stems from the fact that we were investigating the impact of pranayama in real time, and the breathing rates during some of the components of pranayama would vary, and be less than 9 b/m, i.e. at rates in which RMSSD is a the better index of cardiac parasympathetic tone [14, 37, 38].

METHODS

A crossover clinical trial with the order of experimental conditions assigned by randomization was conducted at the Helfgott Research Institute at the National University of Natural Medicine (NUNM) in Portland, Oregon. The NUNM Institutional Review Board approved this trial (approval number: AL2222017) and it was registered through ClinicalTrials.gov (NCT03280589). All participants provided written informed consent.

Aims

The study aimed to (1) evaluate a proposed mechanism of pranayama by measuring HRV during pranayama vs. sitting quietly; (2) evaluate which component(s) of Sheetali/Sheetkari pranayama most strongly contribute to physiological changes, by comparing breathing interventions that include strategic combinations (Table 1) of sitting quietly, deep breathing, pacing, and facial modifying components of this pranayama practice; and (3) to investigate the relationship between respiratory rate and HRV.

Recruitment, Enrollment, and Randomization

Participants were recruited from the general population in the Portland metro area using flyers, newspaper, radio, and web-based advertisements. Healthy adults ages 25 - 55, naïve or only moderately exposed to yoga and relaxation practices at the time of recruitment were invited to participate in the trial. Moderate exposure to yoga/relaxation practices was defined as having no formal certification in mind/body practices, and not practicing yoga more than once per week. Participants were additionally excluded if they were unable to roll their tongue into a tube, as this skill is an integral part of Sheetali breathing. Participants were asked to avoid extreme physical exercise (heart beats over 120 beats/minute), and to refrain from alcohol, over the counter medications, and any recreational drugs 24-hours prior to their scheduled visit. Additionally, participants had to agree to not begin a new diet or dietary supplement regime during the course of participation in the trial.

Participants were excluded if they had conditions that may be adversely impacted by deep breathing, e.g. asthma, chronic obstructive pulmonary disease, restrictive lung disease, etc; were currently taking medications that are known to impact HRV measurements or respiratory sinus arrythmia, e.g. beta-blockers, beta-agonists, calcium channel blockers, analgesics, antidepressants, anticholinergics, or anxiolytics; had smoked tobacco products in the last 6 months; or had a current diagnosis of diseases (e.g. significant heart disease, hypertension, hypotension) that could impact baseline HRV indices and cause HRV indices to be less responsive to our experimental procedures than they would be in healthy adults. [31]

Interested participants were screened over the phone with the help of a standardized script, which included the Nijmegen Questionnaire to screen potential participants for hyperventilation. [39] Those who were eligible after the phone screen were invited for a screening visit when anthropometrics, blood pressure, current medication use, and current exercise practices were recorded. Those who met the inclusion/exclusion were offered recruitment after providing informed consent through signature. Participants were then assigned a unique alpha-numeric ID, and allocated to all of five activities in random order, determined through a random sequence generator, (R software®, R foundation for Statistical Computing, Vienna, Austria [40]) set for 5x5 crossover design with a Williams Latin Square. [41]

Intervention Procedure

Each intervention activity was completed in the morning during one of five separate study visits; morning was selected to reduce dirunal variation in HRV. The five activies were: (1)

sitting quietly with no external instructions on breath rate; (2) self-paced deep breathing; (3) externally-paced deep breathing at a rate of 6 b/m, (4) self-paced Sheetali and Sheetkari pranayama for five minutes each; and (5) externally-paced Sheetali and Sheetkari pranayama intervention at a rate of 6 b/m, for five minutes each. Activity 1, i.e. sitting quietly served as the passive control, and Activity 3 was considered a positive control, based on evidence that deep breathing at 6 b/m increases HRV. [5] Measurement periods during each visit consisted of four ten-minute sessions with a one-minute break every five minutes to allow the participant to rest, drink water, adjust posture, and ask questions: ten minutes of baseline before the intervention, ten minutes of breathing intervention (normal, deep, or Sheetali), a second ten minutes of breathing intervention (normal, deep, or Sheetkari), and ten minutes post-intervention.

Participants were compensated a modest amount for their time and travel expenses.

Description of Interventions—Intervention Activities 1-5, and the components of pranayama they are used to assess, are outlined in Table 1, and described in detail below.

Activity 1: Sitting quietly (control).: In this control activity, participants watched a video demonstrating a seated posture in a chair with both feet flat on the floor, hands face down on thighs, eyes closed or slightly open, and resting the back on the chair. They were then instructed to release tension in the body, remain still, allow the chair to support them, and breathe at their own natural pace.

Activity 2: Deep breathing (self-paced).: Participants began with the instructions detailed in the control activity. Deep breathing instructions were added with video-guidance to breathe through the nostrils, fully filling and emptying the lungs with each breath. Participants were instructed to bring attention to each breath.

Activity 3: Deep breathing (externally-paced, positive control).: Participants began with the instructions detailed in the deep breathing activity. Pacing is added through audio cues (sound of a bell) indicating when to inhale and exhale, following a pace of 6 b/m.

Activity 4: Sheetali and Sheetkari (self-paced).: Participants began with the instructions detailed in the deep breathing activity. Facial manipulation was added through video guidance on how to perform Sheetali and Sheetkari (Figure 1). As in our previous work, Sheetali and Sheetkari were considered complementary parts of the same intervention, and were each performed consecutively in two five-minute segments (totaling twenty minutes), with video instruction before each breathing style. In Sheetali, the participants are instructed to follow a series of five steps, i.e. protrude their tongue a comfortable distance; roll into a tube; inhale through the tube; retract the tube back into the mouth; and exhale through the nose. In Sheetkari, lips are kept apart, and inhalation takes place through clenched teeth and a relaxed tongue. Exhalation occurs through the nose.

Activity 5: Sheetali and Sheetkari (externally-paced).: Participants began with the entire instructions outlined in Activity 4. Pacing was added through audio cues indicating when to inhale and exhale, following a pace of 6 b/m.

Description of Measures

The Nijmegen Questionnaire.: This validated screening tool has been shown to have a sensitivity of 91% and specificity of 95% for hyperventilation [39] and give a broad view of symptoms associated with dysfunctional breathing patterns. Participants are asked to record their responses regarding 16 conditions on a five-point scale, ranging from 'never' recorded as 0 to 'very often' counted as 4; thereby yielding a total score ranging between 0 and 64. Those with scores greater than 20 were excluded at screening.

Heart Rate Variability.: The ProComp8 Infiniti Encoder® (Thought Technology Ltd., Montreal, Canada) was used to measure HRV, using a sampling rate of DC 512 *Hz* at 2048 samples/second, [42] which provides adequate sensitivity for our outcomes. [43] Electrocardiogram (EKG) (used to measure R-R intervals for calculation of HRV) was measured via electrodes placed on both wrists using adjustable bands. HRV data generally includes (1) the distance between adjacent “R” waves in an EKG, and (2) the power density spectrum of the EKG waves. [44] HRV data was analyzed in terms of “time domain” i.e. SDNN and RMSSD and “frequency domain” i.e. HF-HRV (0.15 – 0.4 *Hz*) and LF-HRV (0.04 – 0.15 *Hz*). [22] Our primary HRV outcome was RMSSD because it is the most appropriate HRV index for our intended respiratory rate range (< 9 b/m), while HF-HRV, and standard deviation of N-N intervals (SDNN) were tested as secondary outcome variables because they are also associated with PNS response, yet HF-HRV is best for respiratory rates between 9-24 b/m and SDNN is the suggested index for 24-hour heart rate recordings [14, 37].

Respiratory Rate.: The ProComp8 Infiniti Encoder® (Thought Technology Ltd., Montreal, Canada) was also used to measure respiratory rate. Respiratory measurements (breaths per minute or b/m) were acquired via thoracic and abdominal straps secured across the chest and abdomen. HRV and respiratory rate data was aquired and processed from this device using Biograph Infiniti® software (Thought Technologies Ltd., Montreal).

Data Collection and Management

All physiological data acquisition was accomplished within the instrument software and then exported to an encrypted computer for analysis and uploaded to REDCap®. HRV data was processed within the Cardio-Pro software (Thought Technology Ltd., Montreal, Canada), [45] wherein each five-minute segment was subdivided into one-minute epochs for examination and manual artifact correction by two study investigators (AS, RH), blinded to the activity being tested.

Error across R-R interval time series was measured and flagged digitally and confirmed manually. Potential outliers were identified by the software, and then visually examined by AS and RH for confirmation and removal. Ultimately, participants that needed to be excluded from analysis due to error in their HRV measurements were determined using this process. Artifacts created by movement, coughing, or other interferences were manually identified in the EKG spectra and normalized using the Thought Technology software. There were three additional participants that were removed before final data analysis due to incomplete data (i.e., two completed only one visit, and the other only two visits).

Statistical Analysis

Prior to analysis of effects, we summarized respiratory rate and HRV measures into three summary averages: “pre”, defined as the average across the two 5-minute segments occurring *before* the breathing exercise (steps 1 and 2); “during”, defined as the average across the four 5-minute segments occurring *during* the breathing exercise (steps 3-6); and “post”, defined as the average across the two 5-minute segments occurring *after* the breathing exercise (steps 7 and 8).

The main analyses comparing five conditions used random-intercept models adjusted for the condition-dependent pre-intervention measure of the outcome, using either *pre-during* change or *pre-post* change as the dependent variable and condition as the main predictor. Significance of contrasts between pairs of conditions was made using a Tukey’s HSD correction. For comparisons of different times within the same condition, we used paired t-tests. We separately tested two-way random-intercept models for each outcome, with pacing (self- or externally-regulated) and breathing style (deep breathing vs pranayama) as factors, using only data from Conditions 2-5.

Missing data were not imputed, as the random effects model is able to use all available outcomes, without imputation. In order to test sensitivity to missing data, we reran all analyses using only data from participants who completed all activities. All analysis were completed using R® version 3.6.0 (Vienna, Austria) and a threshold of $P < 0.05$ was considered indicative of statistical significance.

Sample Size Calculation—Applying estimates of baseline HRV parameters from our previous research, [30] and utilizing a crossover design, we calculated that a sample size of n=30 would provide 80% power with an alpha of 0.05 to detect a 50% increase in HRV parameters. Therefore, our targeted enrollment was 45 participants, to allow for up to 12.5% attrition to account for incomplete follow-up, data artifacts, and/or inability of the participant to complete study visits. We note that, with the resultant n=25 participants obtained, our calculation would have indicated only 72% power to detect the same effect relative to control.

RESULTS

Two hundred and two participants were screened for this study, 46 were eligible and enrolled after applying inclusion/exclusion criteria, and 80% (37 participants) completed all five visits, as shown in Figure 2.

Although 37 participants finished the study, after artifact processing by two blinded assessors, and removal of three participants who were extreme biological outliers (with excessive HRV throughout all activities), data for 25 participants were finally used in our analyses. Participant demographics are detailed in Table 2.

Physiological Changes

Respiratory Rate—The relationships between respiratory rate, activity, and time course of the intervention period are diagramed in Figures 3, 4, and 5. Respiration rate significantly

decreased from pre-intervention to during the intervention for both types of pranayama (deep breathing and Sheetali/Sheetkari, Activities 2-5) while there was very little change in the control activity of sitting quietly (Activity 1, Figures 3 and 4). The decrease in respiratory rate for Activities 3-5 was significantly greater than observed in Activity 1 ($p<.05$ for all comparisons of Activities 3-5 to Activity 1, when using a Tukey HSD correction for five comparisons), with a pre-during change of −4.29 ± 2.63 b/m for Activity 3 (pre: 11.17 vs. during: 6.88), −3.83 ± 3.15 b/m for Activity 4 (pre: 11.17 vs. during: 7.35), and −4.16 ± 2.3 for Activity 5 (pre: 11.57 vs. during: 7.41).

Figure 5 shows variability in breath rate, giving us insight into the effects of self pacing vs. external pacing. At baseline (**5A**), breath rates in all conditions were similarly variable and in the same range (interquartile range, ~10-13 b/m). During the intervention period (**5B**), as designed, there was less variability in breath rates during externally-paced pranayama (Activities 3 and 5) than self-paced versions of pranayama (Activites 2 and 4), indicating adherence to pacing prompts. Although average breath rate was lowest for externally-paced breathing (closer to the targeted 6 b/m, with an interquartile range of ~6-8), average breath rates produced during Activities 2-5 were not statistically different from one another.

Heart Rate Variability (RMSSD)—When comparing pre-during changes between all interventions (Activities 1-5), the externally-paced pranayamas, Activities 3 (deep breathing) and 5 (Sheetali/Sheetkari), resulted in greater RMSSD than the control Activity 1 when adjusting for “pre” activity values for each intervention ($p=.005$ and $p=.02$, respectively, using a Tukey HSD correction for comparison of five estimates), with an average logRMSSD of 4.05 ± 0.4 ms during Activity 3, 4.01 ± 0.4 ms during Activity 5, and 3.82 ± 0.49 ms during Activity 1.

When comparing pre-during changes between pranayama interventions (Activities 2-5) in a two-way model using breathing style (normal vs. Sheetali/Sheetkari) and pacing (self- vs. externally-paced) as factors, we found no significant interaction. Pacing was associated with a significant main effect on HRV, with external pacing (Activities 3 and 5) resulting in a greater increase in HRV from pre-intervention to during the intervention than self pacing used in Activities 2 and 4 (effect on pre-during logRMSSD change; $b=−0.17$, $p=.02$), with a pre-during RMSSD increase of 0.5 ± 0.42 ms and 0.49 ± 0.41 ms for Activities 3 and 5, compared to a pre-during RMSSD increase of 0.3 ± 0.43 ms and 0.41 ± 0.48 ms for Activities 2 and 4. Within pacing types (self-pacing and external-pacing), breathing styles (i.e. Deep Breathing vs. Sheetali/Sheetkari) were not associated with different magnitudes of HRV change. Specifically, average pre-during change in RMSSD for Activities 2 vs. 4 were not significantly different from one another ($p = 0.54$), nor were those values for externally-paced practices, Activities 3 vs. 5: ($p =.98$). Yet, when controlling for respiratory rate, Activity 4 (Sheetali/Sheetkari) showed a greater pre-during increase compared to Activity 2 (Deep Breathing) yet this difference was not significant ($p=.07$).

Changes in RMSSD over the duration of each visit (Steps 1-8 during Activities 1-5) can be seen in Figure 6. Baseline values taken during the first 10-minutes of each visit (steps 1 and 2) were similar in all activities, but there are notable differences between baseline and the intervention period (steps 3-6), as well as the intervention period and the

post-practice period (steps 7 and 8) for all activities, especially Activities 3-5. Summary means of RMSSD during the “pre”-, “during”-, and “post”- intervention steps are shown in Figure 7A. Increases in RMSSD from “pre” to “during” were seen across all five activities (all p-values <0.05). Activities 3 and 5 (externally-paced deep breathing and Sheetali/Sheetkari, respectively) showed significant differences in RMSSD between all steps (pre-during, during-post, and pre-post, as shown in Fig. 7A), with the largest change seen from pre to during these activities (logRMSSD change of 0.50 ms for Activity 3 and 0.49 ms for Activity 5), while during-post change was 0.32 ms for both externally-paced activities, and pre-post change was 0.19 and 0.17 ms for Activities 3 and 5, respectively.

HF-HRV and SDNN (Secondary Outcomes)—When comparing pre-during changes between all interventions (Activities 1-5), Activities 3, 4 and 5 showed a significantly greater pre-during increase in logSDNN (0.57 ± 0.36 ms, 0.48 ± 0.42 ms, and 0.50 ± 0.33 ms) than Activity 1 (0.2 ± 0.31 ms) using the same model (p-values: <0.001, 0.005, and 0.02, respectively, when comparing Activities 3-5 to Activity 1).

Supporting the effect of pacing on PNS response, similar results were seen for SDNN outcomes as were shown for RMSSD, indicating externally-paced Activities 3 and 5 produced a stronger change in PNS response than self-paced Activities 2 and 4, with an effect on pre-during logSDNN change of b=−0.12, and p=.04.

Changes in SDNN and HF-HRV throughout the visit (pre-during-post intervention) are shown in Figure 7B and 7C. Significant increases in SDNN from “pre” to “during” were seen across all five interventions (Activities 1-5), with all p-values <.05 (**7B**). There were no significant changes in HF across any activity or time comparison (**7C**), yet a strong, positive correlation existed between HF and RMSSD (r > 0.80 for all activities and time points; data not shown), which was hypothesized. [36, 46, 47]

DISCUSSION

To our knowledge, this is the first study to separately evaluate the impact of the specific components of pranayama practice on HRV. Our results demonstrate an increase in an index of cardiac parasympathetic tone during pranayama (both deep breathing and Sheetali/Sheetkari), as expected, and that sitting quietly, deep breathing, and pacing were all associated with that response. We found that externally-paced breathing practices resulted in a greater pre-during increase in parasympathetic tone than self-paced activities. Our results suggest that Sheetali/Sheetkari pranayama merits further investigation in larger controlled trials. We also see merit in investigating whether distinct oral morphology used in some practices may increase physiologial benefits [28, 48]. Although our study was conducted in a healthy population, our results are congruent with a recent randomized controlled trial of Sheetali pranayama in participants with essential hypertension, that demonstrated both relatively large increases in RMSSD and blood pressure lowering effects compared to those not performing pranayama [53]. However, unlike our study, this trial did not assess if one specific component of pranayama was able to demonstrate greater benefit over others, and is limited by a lack of an active comparator.

The primary importance of this work lies in understanding the mechanism by which various pranayama and other breathing practices may differentially affect the autonomic nervous system (i.e. through the simple act of sitting quietly, the pace of breathing, and/or the modifications made to the face and airways during practice). Of these components, only pace of breathing has been isolated and investigated in prior work, with physiologic mechanisms explaining the relationship of slow-breathing pranayama and it's effects on autonomic modulation proposed by Jerath et al [54]. They suggest that pranayama breathing, and inhalation specifically, enhances both the actions of stretch-induced inhibitory neural signals via activation of slowly adapting stretch receptors, as well as, fibroblast-mediated hyperpolarization. These initial responses led to downstream inhibitory effects within the hypothalamus and brainstem, lowering metabolic activity which upregulates parasympathetic activity relative to sympathetic activity.

With targeted investigation of components beyond pace of breath, we can begin to understand the most important components of a practice. These results can lead to more specific and targeted breathing practices being tested for adoption to routine clinical conditions such as hypertension, type 2 diabetes and anxiety disorders. Moreover, the findings can be extended to examine the impacts of components of other pranayama practices.

The strengths of this trial include the novel comparison of several individual components of a pranayama practice, the randomized crossover design, which enabled each case to act as their own control. Our study demonstrates a technique through which components of a proven traditional practice can be isolated and tested alongside one another, within a relatively small sample size using a crossover design, in order to elucidate which aspects of the practice may be most responsible for its physiological, psychological or other measurable effects.

The study results needs to be viewed in light of its limitations. We had an attrition rate of 20% (losing nine participants from our starting sample size of 46 at Visit 1 to 37 at Visit 5), indicating a larger drop-out rate than we predicted. We also did not expect such a high sample loss due to HRV artifacts and extreme outliers, leaving us with 25 participants, rather our intended 30, and we did not evaluate for inter-reader reliability between two blinded HRV assessors. The sample included healthy individuals without a history of hypertension or cardiovascular diseases, thus, it is unknown if the same mechanisms or magnitude of changes observed here would be consistent in a diseased population. And lastly, each person only completed each breathing practice one time. It is possible there was some stress involved in learning a new skill (or being observed), and with more practice (in a longer duration study), greater benefits could be experienced.

Future studies involving larger sample sizes, among individuals who suffer from hypertension are likely to offer more definitive results. It would be of interest to investigate whether other components contribute to the effectiveness of pranayama, including: varied pacing (slow, medium, fast, using external pacing); lung volume used (as a marker for depth of breath in order to predict a possible increase in tidal capacity, which is one goal of pranayama [49]); inhalation to exhalation ratio (a 1:2 breathing ratio is used in

most pranayama practices [33, 49, 50] as well as in pursed-lip breathing, which has been proven to increase oxygenation of the blood, triggering a PNS response[4] and slowing respiration [48]); opening the mouth (during inhale to radiate moisture and heat, or during exhale to excrete more CO_2 from the lungs as is done in Pursed-lip Breathing [28]); Sheetali vs. Sheetkari (comparison to one another [33]). Additionally, variations on the practice that could be explored include: pausing between breaths (allowing time for gas exchange, increasing breathing efficiency, [50] and increasing HRV [11, 51-53]); and adding buzzing bee breath/ bhramari (humming) during exhalation (a traditionally recommended augmentation to be used with Sheetali/Sheetkari for enhanced relaxation [33]).

CONCLUSIONS

In this preliminary study, we completed the first investigation into how several components of a pranayama practice can impact HRV. We found that all five practices increased HRV as compared to the pre-intervention period. When using external pacing (at 6 b/m), both deep breathing and Sheetali/Sheetkari increased HRV (from pre- to during the practice) more than sitting quietly while breathing normally. We found external pacing (6 b/m) resulted in greater cardiac parasympathetic tone compared to self-pacing. Additionally, both self-paced and externally-paced practices were associated with descreased respiratory rate, compared to sitting quietly. These findings suggest that sitting quietly, deep breathing, and slow pace of breath need to be targeted for investigation as active components of the practice. Lastly, there was preliminary evidence from this study that the oral morphology used in Sheetali/ Sheetkari may play a role in HRV changes, but this question merits more investigation with a larger sample size.

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Acronymns:

HRV	heart rate variability
PNS	parasympathetic nervous system
HF-HRV	high frequency heart rate variability
RMSSD	root mean square of successive differences between R-R intervals
SDNN	standard deviation of heart beat N-N intervals
b/m	breaths per minute

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- Five elements of pranayama were compared for effects on HRV using a crossover design.
- Sitting quietly, depth of breath and breath pacing were associated with an increase in an HRV index considered reflective of cardiac parasympathetic tone
- Pranayama was associated with a decreased breath rate (vs. sitting quietly) independent of external pacing.

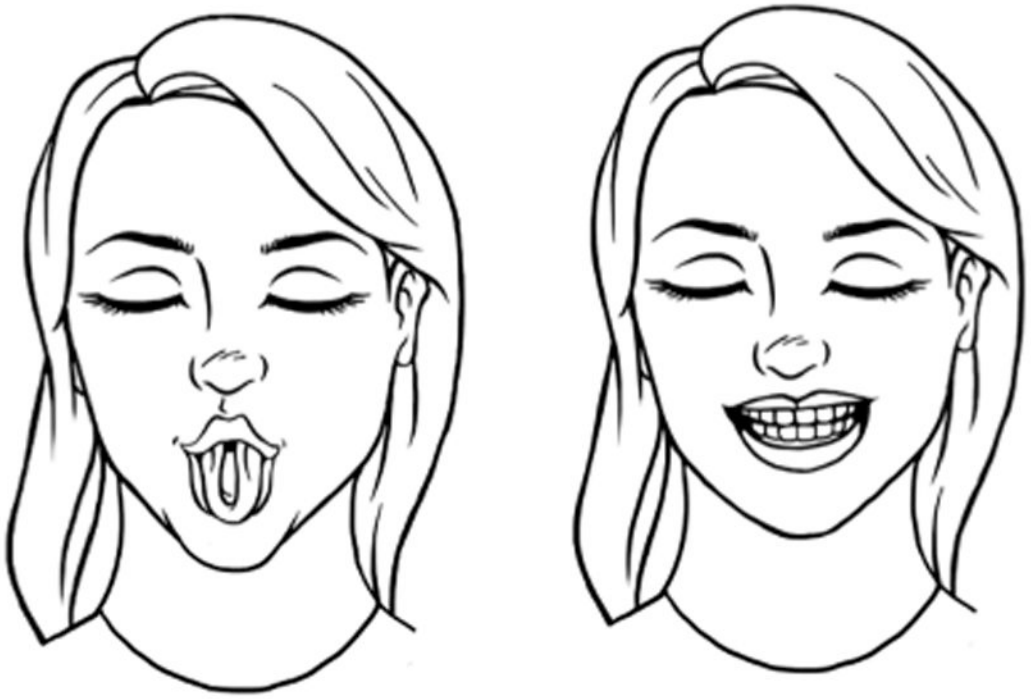

Figure 1.
Illustration of Sheetali and Sheetkari pranayamas, involving inhaling through an open mouth (rolled tongue, Sheetali; exposed teeth, Sheetkari) and exhaling through the nose.

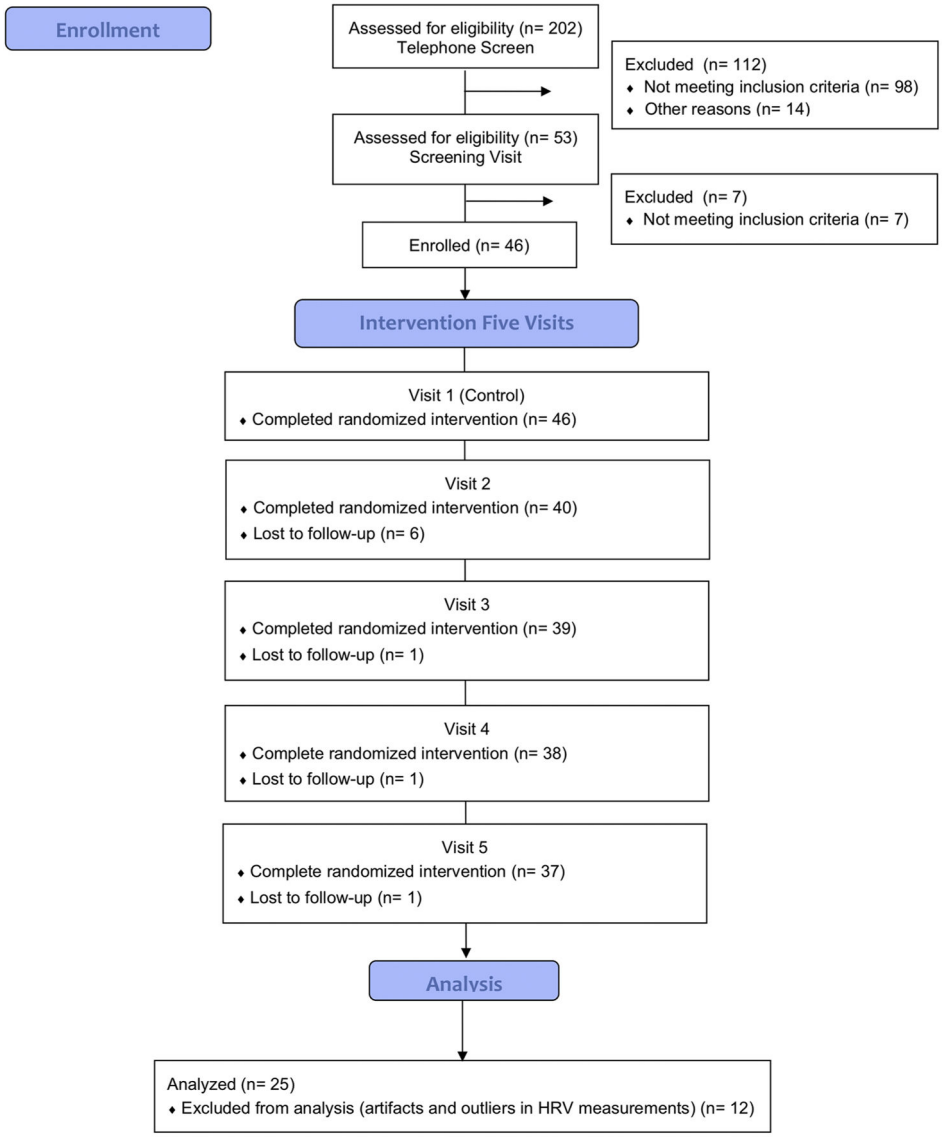

Figure 2.

Recruitment for our study, detailing numbers assessed for eligibility and enrolled, as well as those who completed visits and were analyzed.

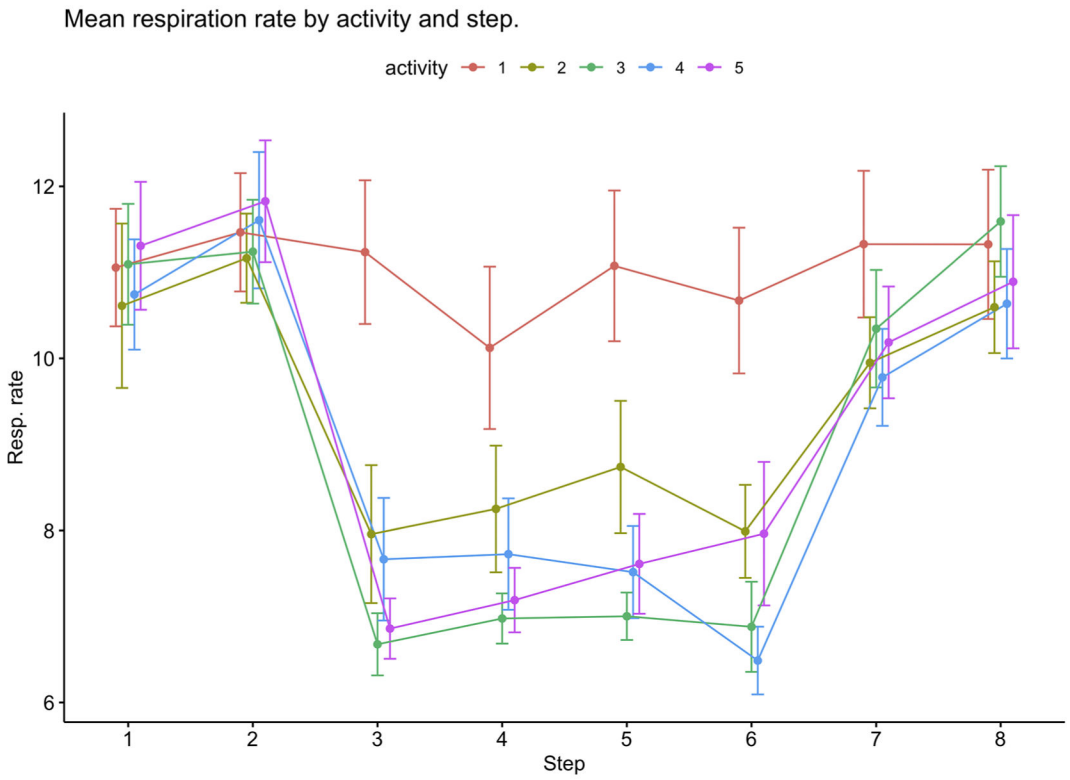

Figure 3.

Respiratory rate (y-axis) during eight 5-minute steps (x-axis) at each of five visits/breathing activities (Activities 1-5, indicated by colored lines). Activity 1 was Sitting Quietly, Activity 2 was Deep Breathing (self-paced), Activity 3 was Deep Breathing (externally-paced), Activity 4 was Sheetali and Sheetkari (self-paced), and Activity 5 was Sheetali and Sheetkari (externally paced). Steps 1 & 2 are baseline/ pre-intervention measurements, in Steps 3-6 the breathing activity for the visit is administered per the randomization sequence, and Steps 7-8 are post-intervention measurements. For Sheetali and Sheetkari interventions, Sheetali took place during Steps 3-4 and Sheetkari took place during Steps 5-6. Error bars represent ± 1 SE.

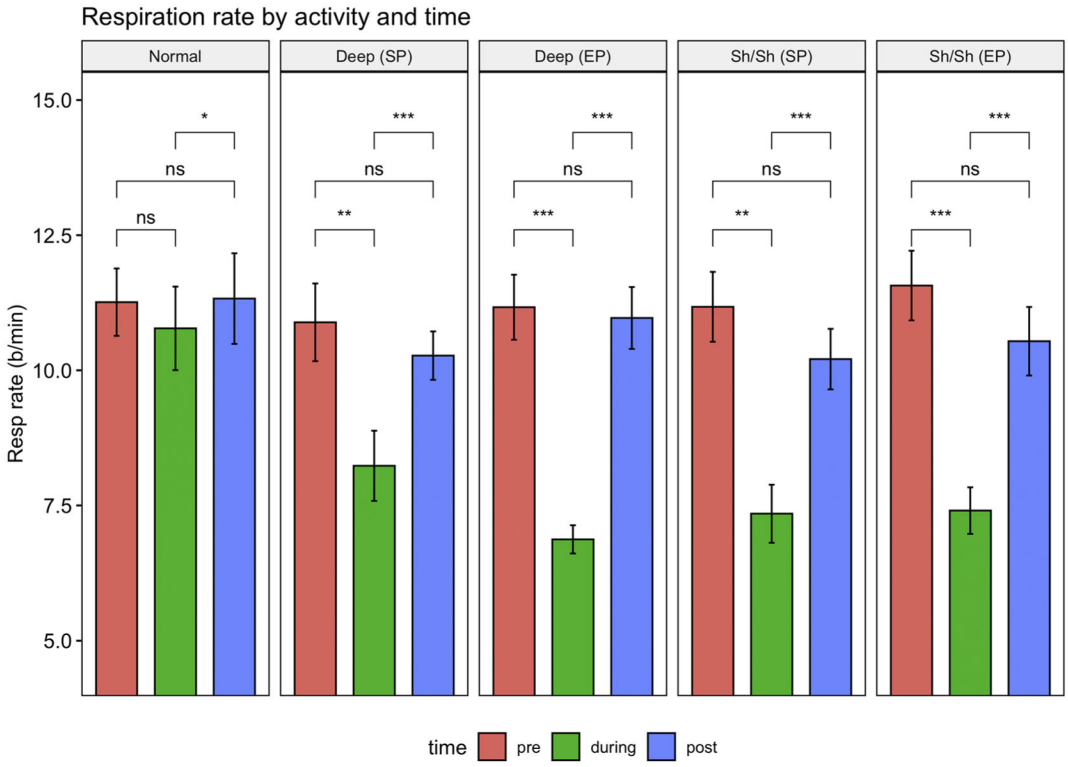

Figure 4:

Mean respiratory rate (breaths per minute, b/min) over time (pre, during, and post breathing activities) for each intervention (Activities 1-5). Activity 1 was Sitting Quietly, Activity 2 was Deep Breathing (self-paced), Activity 3 was Deep Breathing (externally-paced), Activity 4 was Sheetali and Sheetkari (self-paced), and Activity 5 was Sheetali and Sheetkari (externally paced). *P<0.05, ** P<0.01, ***P<0.001, NS P>0.05 (not significant). Error bars represent standard error.

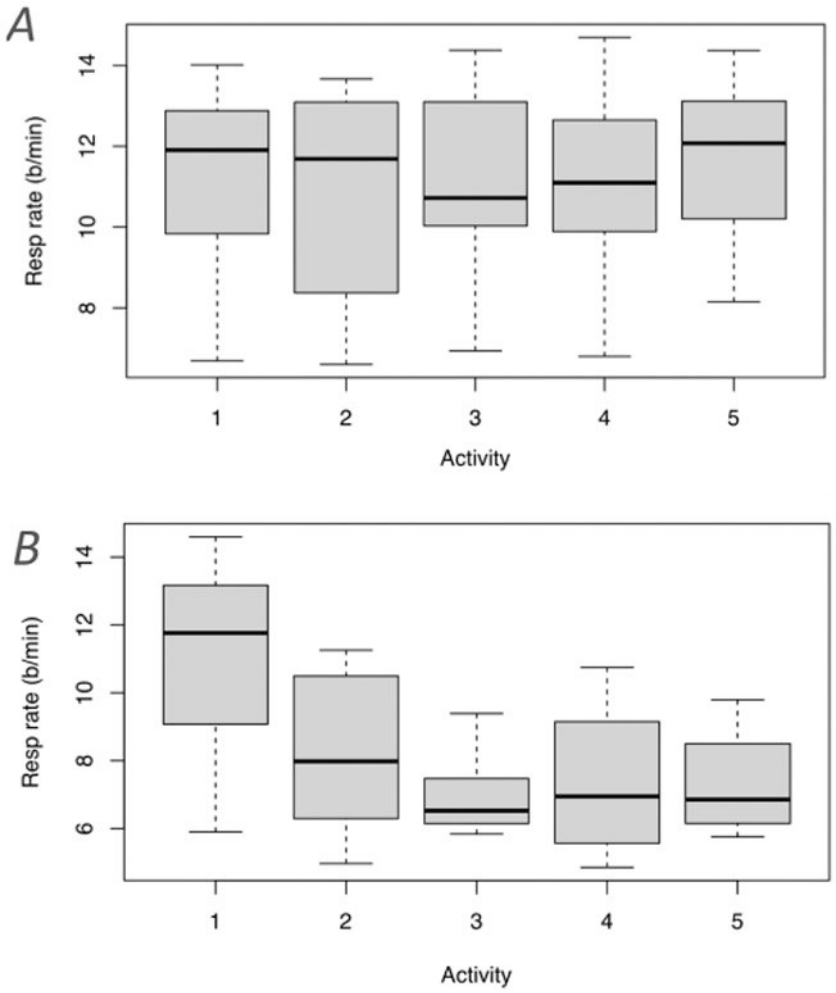

Figure 5.

Box and Whisker plots of variability in mean respiratory rate (breaths per minute, b/min) at baseline (**A**); and during each intervention, Activity 1-5 (**B**). The grey box represents interquartile range. The dark, solid line represents the median. The lowest and tallest lines represent minimum and maximum values, respectively.

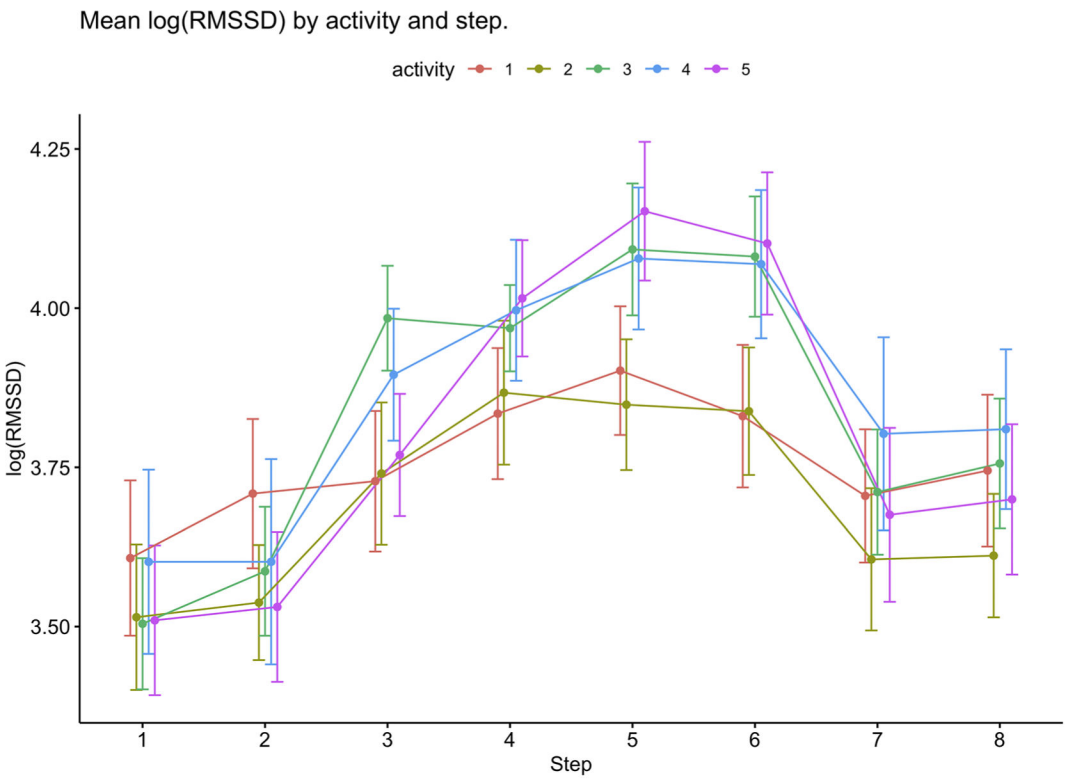

Figure 6.

Log of the root mean square of successive normal RR intervals (RMSSSD, y-axis) during eight 5-minute steps (x-axis) at each of five visits/breathing activities (indicated by colored lines). Steps 1 & 2 are baseline/ pre-intervention measurements, in Steps 3-6 the breathing activity for the visit is administered per the randomization sequence, and Steps 7-8 are post-intervention measurements. Breathing activities are defined as follows: Activity 1: Control/ Sitting quietly/ Normal breathing; Activity 2: Deep Breathing (self-paced); Activity 3: Deep Breathing (externally-paced); Activity 4: *Sheetali/Sheetkari* Breathing (self-paced); Activity 5: *Sheetali/ Sheetkari* Breathing (externally-paced). Error bars represent ± 1 standar error (SE).

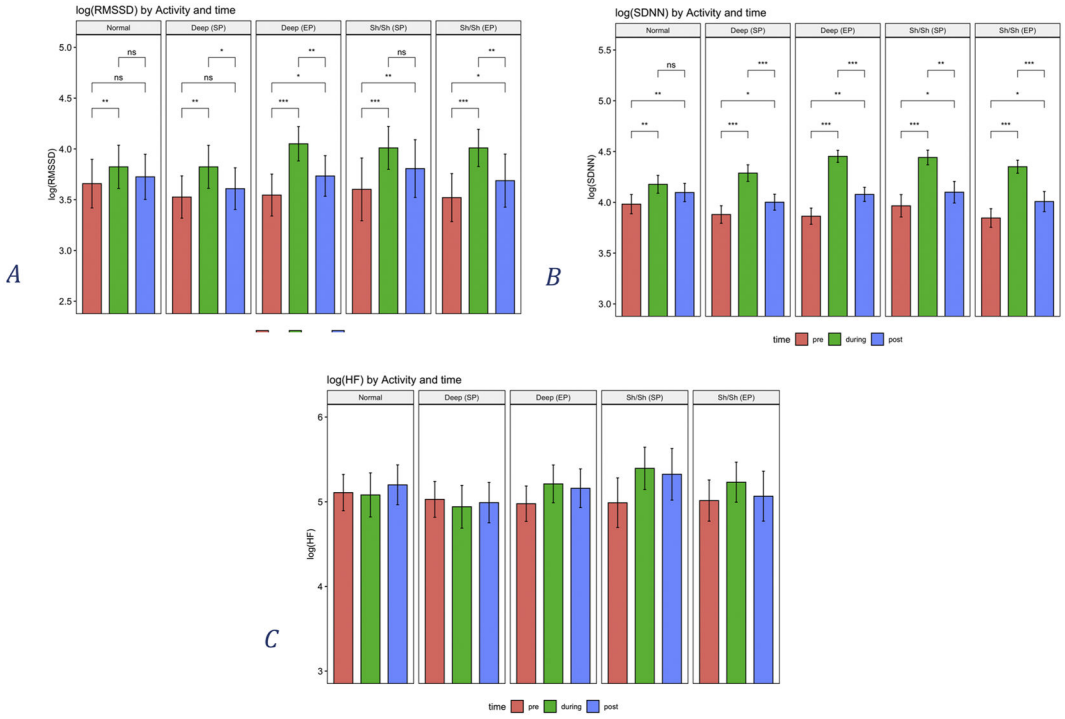

Figure 7.

HRV parameters over time (pre, during, post intervention) during each intervention (Activities 1-5) represented in terms of: (**A**) log of the root mean square of successive normal RR intervals (RMSSD); (**B**) log of the standard deviation of all normal RR intervals (SDNN); and (**C**) log of high-frequency (HF) heart rate variability. *P<0.05, ** P<0.01, ***P<0.001, NS P>0.05 (not significant). Error bars represent confidence intervals.

Table 1.

Combinations of modifiable components that form the activities tested

Condition/ Component	Activity 1: Sitting quietly	Activity 2: Deep breathing self-paced	Activity 3: Deep breathing externally-paced	Activity 4: Sheetali and Sheetkari self-paced	Activity 5: Sheetali and Sheetkari externally-paced
Component 1: Sitting quietly	X	X	X	X	X
Component 2: Depth of Breath		X	X	X	X
Component 3: Pace of Breath			X		X
Component 4: Mouth Shape				X	X
Component 5: Duration of Practice	X	X	X	X	X

Table 2.

Demographic and intake information for the 25 participants in our final data set

	Total Sample (N=25)
Age	
Mean (SD)	34 ± 8
Range	22 - 55
Gender	
Male	6 (24%)
Female	18 (72%)
Other	1 (4%)
Ethnicity	
Hispanic or Latino	1 (4%)
Not Hispanic or Latino	23 (92%)
Not Reported	1 (4%)
Race	
Black or African American	1 (4%)
White, Caucasian, or European-American	21 (84%)
Asian or Asian-American	2 (8%)
More than one Race	1 (4%)
Education	
Some University	1 (4%)
2-Year University	5 (20%)
4-Year University	10 (40%)
Some Graduate School	2 (8%)
Graduate Degree or Higher	7 (28%)
Major Changes to Exercise Habits [a]	
Yes	-
No	25 (100%)
Strenuous Exercise (frequency) [a]	
Never	11 (44%)
1 time/ week	3 (12%)
2 times/ week	4 (16%)
3 times/ week	5 (20%)
4 times/ week	2 (8%)
Mind-Body Practice [a]	
Yoga	9 (36%)
Meditation	4 (12%)
Qi Gong, Tai Chi, Martial Arts	1 (4%)
Breathing	3 (12%)
Major Diet Changes [a]	

	Total Sample (N=25)
Yes	-
No	25 (100%)
Servings of Caffeine per week [a]	
Coffee- drip	2.8 ± 3.3
Coffee- pressed	0.3 ± 1.6
Espresso	0.4 ± 1.2
Black Tea	1.2 ± 1.6
Chai	0.2 ± 0.5
Green or White Tea	1.4 ± 2.1
Energy Drink	0.04 ± 0.2
Soda Pop (caffeinated)	0.2 ± 0.4
Caffeine Pill	0.04 ± 0.2
Caffeinated Candy or Gum	0.04 ± 0.2
Body Mass Index (BMI, kg/m^2)	
Average	24 ± 3
Range	19 - 29

[a]During the last four weeks.