Optimizing Chronic Slow Breathing Training to Cause a Therapeutic Effect on Heart Rate Variability

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OPTIMIZING CHRONIC SLOW BREATHING TRAINING TO CAUSE A THERAPEUTIC EFFECT ON HEART RATE VARIABILITY

by

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B.S. Exercise Science, May 2010, Old Dominion University

A Thesis Submitted to the Faculty of Old Dominion University in Partial Fulfillment of the Requirements for the Degree of

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Heart rate variability (HRV) is a valid and reliable tool that can be used to determine the basic state of an individual’s autonomic health. The current study attempted to establish the minimum frequency of breathing practice necessary to produce a therapeutic effect on HRV over the course of a four-week period with four different treatment groups, specifically, groups that practiced a slow breathing protocol for either 2, 3 or 5 times per week, and a control group. Forty-three subjects (14 males, 29 females), ages 18 – 50 years, were screened, pre-tested, matched for sex, age and HRV, assigned to a specific group for a four-week training period, and completed the study. All pre- and post-test measurements were made during a 10-minute period of supine rest, and included systolic and diastolic blood pressure (BP), heart rate (HR), and the following HRV measures: standard deviation of the normal to normal R waves (SDNN), root mean square of successive differences (RMSSD), high frequency power (HF), low frequency power (LF), and HF/LF ratio. During the 4-week training period, subjects kept a log of their breathing training and then returned for post-testing of the same variables. A two-way analysis of variance with repeated measures on one factor and post hoc T-tests were used to evaluate the data. Significance was set at the 0.05 level. Following the 4-week training period, the control group experienced an increase in systolic BP, while all three training groups experienced a decrease. For diastolic BP, the control group experienced an increase, while only the 2 times per week training group experienced a decrease. For HR, the 2 times per week and 5 times per week groups demonstrated a decrease, while no other groups changed. There were no differences in any HRV measures as a result of training in any group. In conclusion, although previous research had found that breathing training affected HRV measures, there was no effect in this study regardless
of the frequency of training. Breathing training did cause a reduction in resting BP and heart rate, but the frequency of training had little to no differential effect. The study was unable to demonstrate a minimum frequency of breathing training for affecting the autonomic nervous system.
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CHAPTER I
INTRODUCTION

Problem Description

Research within the past several decades has shown heart rate variability (HRV) to be a valid and reliable tool that can be used to determine the basic state of an individual's autonomic health (Acharya et al., 2006; Pinna et al., 2007). Surprisingly simple and accessible, HRV has been gaining credibility in recent years and has spread through multiple branches of scientific research. Current research shows that a depressed or lowered HRV correlates with various endocrine, pulmonary, psychiatric, and sleep related disorders (Anders et al., 2010; Balachandran et al., 2001; Cohen et al., 2003; Kardelen et al., 2006; Lehrer et al., 2006; Moldabek et al., 2011; Volterrani et al., 1994). Depressed autonomic activity and lowered HRV status also has been found to be correlated with ischemic activity and sudden cardiac death (Kochiadakis et al., 2000; Kudaiberdiera et al., 2007). Investigators have found evidence of depressed or lowered HRV in subjects with these diagnoses and have sought ways to increase autonomic health and subsequently raise HRV. Many things negatively affect HRV, including prolonged lack of sleep and mental stress (Anders et al., 2010; Keary et al., 2009; Owen et al., 2003; Thayer et al., 2012) or long-term smoking (Berhera et al., 2010). Positive changes can occur for the short term as well, with slow breathing making a significant impact on BP (Anderson et al., 2010; Elliot et al., 2004 Pramanik et al., 2009), reducing arrhythmias in the human heart (Dabhade et al., 2012), and reducing asthma symptoms (Lehrer et al., 2006) and chronic obstructive pulmonary disease (COPD) symptoms (Bartels et al., 2004; Gosselink et al., 2003).

When examining the literature available, research indicated that it would be beneficial to investigate further to determine the amount of breathing practice necessary to produce a therapeutic effect on autonomic function as measured by HRV. Previous research has largely focused on breathing or meditation training interventions that have involved 5 or 7 days per week
(Anderson et al., 2010; Ankad et al., 2011; Bhimani et al., 2011; Dhungel et al., 2008; Elliot et al., 2004; Pal et al., 2004; Phongsupap et al., 2008). These investigations have all found various significant effects on autonomic functions (reduced resting HR, reduced systolic and diastolic BP) or HRV measurements (reduced very low frequency and low frequency) with the implementation of very frequent practice. Acute interventions (1 or 2 day studies) have been executed to determine acute effects of breathing practice on autonomic functioning and have obtained significant positive results during the testing sessions; however, none have determined the lasting effect of the sessions performed (Dane et al., 2002; Driscoll et al., 2000; Grossman et al., 1990; Melville et al., 2012; Pramanik et al., 2009). While the research that is available has found significant positive results, some studies have lacked proper controls and clear repeatable methods (Bhimani et al., 2011, Wang et al., 2010). A need for additional foundational research to be performed with appropriate control groups to solidify the findings surrounding chronic breathing training and HRV in an orderly, reproducible manner is evident. Additionally, investigating the use of breathing protocols further could prove to be quite practical as an easy to access tool that the majority of the population could perform if they desired to do so, irrespective of insurance availability or access to special facilities. This research proposal attempted to scientifically establish the frequency of breathing practice necessary to produce a therapeutic effect on HRV over the course of a 4-week period.

**Statement of Purpose**

The purpose of the study was to measure what effects different frequencies of a 4-week standardized slow breathing protocol would have on autonomic nervous system function in healthy volunteers as determined by measurement of HRV, BP, and HR, specifically to determine if a lower frequency of practice than typically employed would be effective.
Significance of the Study

The significance of the study was to provide foundational research that may be used for preventative and therapeutic measures for healthy populations and non-pharmacological treatments for disease populations.

Research Hypotheses

1. Slow breathing practice performed 2, 3 or 5 times a week for thirty minutes will increase the HRV measures of SDNN, RMSSD, HF and the HF/LF ratio (see Operational Definitions below for definitions of these terms) when compared to baseline values.
2. Slow breathing practice performed 2, 3 or 5 times a week for thirty minutes will decrease LF HRV when compared to baseline values.
3. Slow breathing practice performed 2, 3 or 5 times a week for thirty minutes will decrease resting BP and HR values when compared to controls.

Variables

Independent Variables

The experimental variables in this study were the slow breathing protocol, and frequency of practice, either 2, 3 or 5 days per week.

Dependent Variables

The dependent variables in this study were resting HR, resting BP, and various measures of HRV, frequency domain measures [LF and HF in milliseconds, HF/LF ratio] and time domain measures [SDNN and RMSSD].
Limitations

Training sessions were not supervised and subjects were asked to record their training on provided breathing training journals. It is not known if the subjects actually performed the amount of training they recorded. Subjects were asked to refrain from caffeine and food for 3 hours prior to baseline testing and post testing. It wasn’t known if the subjects actually complied with these requests.

Delimitations

The study was restricted to subjects between the ages of 18 - 50 years that were in good health and lived in the Hampton Roads area. Based on previous research that has been performed involving HRV and breathing practice, individuals who smoked or suffered from known diabetes, deviated septum, major pulmonary, cardiac, autonomic disorders, or other diseases that may interfere with obtaining accurate measurements were not allowed to participate (Anderson et al., 2010; Behera et al., 2010; Kardelen et al., 2006; Volterrani et al., 1994).

Operational Definitions

- HRV – HRV or heart rate variability is a measure of beat-to-beat variation in heart rate, for example, if a person has a heart rate of 60 beats per minute (bpm), the average time between beats is 1 second, however, the time between any pair of beats may be more or less than 1 second. During normal breathing, the heart rate tends to increase during inhalation and decrease during exhalation, creating HRV. HRV is believed to provide insight regarding autonomic control, i.e., the balance of sympathetic and parasympathetic influence on heart rate.
- LF – low frequency, in some circles thought of exclusively as a sympathetic marker of the autonomic nervous system, in others it is a marker of both the parasympathetic and
sympathetic nervous system. In general, the higher an individual’s LF reading, the poorer their health or disease status.

- **HF** – high frequency, a marker of vagal (parasympathetic) activity. A higher reading of this component is believed to be a marker of greater autonomic health, while lower readings are associated with disease or poor health.

- **SDNN** – the standard deviation of normal R to R intervals; represents the standard deviation between the beat to beat measurements of regular or consecutive heart beats.

- **RMSSD** – Root mean square of successive differences of sequential R to R intervals.
CHAPTER II
LITERATURE REVIEW

Many processes contribute to management of HR dynamics. HR is essentially directed internally by the sinoatrial node (SA node) which is located inside the right atrium of the heart itself. Autonomic regulation contributes to the ongoing regulation of heart beat and related processes and without this input, as in cardiac transplant, the heart will essentially default to a heart rate of approximately 100 bpm. Manipulation or management of autonomic regulators that affect the heart have become of great interest, with varying degrees of success in research projects, resulting in the reduction of HR. HRV itself indicates the differences or changes in beat-to-beat intervals within sequential heart cycles and is more dependent on extrinsic factors (Acharya et al., 2006). Contributing modifiers of HRV include sympathetic and parasympathetic influences that act in opposition to produce balance within the autonomic nervous system (ANS). An increase in parasympathetic influence and a reduction in sympathetic influence cause a reduction in HR from a combination of the two factors.

Common measurements of HRV would include time domain measurements and frequency domain measurements. Time domain measurements would include the SDNN and RMSSD. The SDNN represents more of a total picture of HRV while RMSSD largely represents vagal input (parasympathetic) influences. Frequency domain measures involve the measurement of items such as LF and HF power. LF has most typically been associated with sympathetically dominant influences, although some modern researchers have viewed it as both a marker of sympathetic and parasympathetic influences (Burr, 2007). Lowering LF via an intervention is considered to be a positive outcome associated with better health. HF is associated with vagal or parasympathetic influences. Raising HF due to an intervention is considered a positive outcome and associated with better health. The HF/LF ratio serves as a representation of the balance of the
two forces in the body, literally a physiological representation of the balance of the parasympathetic and sympathetic branches of the ANS.

The purpose of this literature review is to examine evidence that is targeted towards breathing protocols that have the potential of not only positively affecting the ANS, but also those with the protocols to create beneficial breathing practices for both normal human volunteers as well as diseased populations. The aim will be to collect enough information to a strong link between specialized breathing protocols and autonomic function as evidenced by traditional measures (BP, and HR) and by HRV (Bhimani et al., 2011; Dhungel et al., 2008; Pal et al., 2004; Raguhuraj et al., 1998; 2008 Veerabhadrappa et al., 2011). In doing this research, we hope to lay a foundation for future work and to add to the available body of knowledge to form greater understanding in this area.

Effect of Chronic Breathing Training

Anderson et al. (2010) performed a 4-week training study with pre-hypertensive and mildly hypertensive patients who did not require medication for their condition. The purpose of their study was to determine the effectiveness of device-guided breathing on resting BP and on BP over a 24-hour period in individuals with stage 1 hypertension. This clinical research included 102 men and women who were screened for pulmonary, renal and cardiovascular diseases and given a physical examination. Diabetes, tobacco use, hormone use, angiotensin-converting enzyme inhibitors, beta-blockers and other drugs that might interfere with the central nervous system (CNS) were prohibited. Baseline testing was performed over the course of two visits which included monitoring breathing patterns and end tidal carbon dioxide (PetCO₂) throughout the two 25-minute testing periods and measuring BP every 6 minutes. BP was also measured for a full 24-hour period prior to the beginning of the study. Subjects were randomized into a device-guided breathing (DGB) condition or a control (CTL) condition. The DGB subjects received a microchip computer that was worn about the waist along with a set of earphones that allowed
them to listen to sounds that influenced breathing. Breathing patterns were monitored and melodic ascending and descending tones were created for the subject through the device to match their breathing pattern to while slowly lowering the rate down towards 10 breaths per minute or even as low as 6 breaths per minute. The device monitored and recorded the total amount of time it was used during the course of the study, with the target time for daily use being 15 minutes per day. Both the DGB and CTL subjects received BP cuffs to monitor their BP daily for the 4-week period. The CTL group is reported to have replaced the device-guided time with 15 minutes of a meditation relaxation exercise, but did not monitor breath rate. This element skews the results of the “controls” of this study since meditation itself is a form of intervention and has been reported to have an effect on autonomic function. After the 4-week period, the subjects returned for post testing. The results revealed that there was a significantly greater reduction in resting systolic and diastolic BP in the DGB group when compared to the CTL group. The breathing rate significantly decreased more in the DGB group and tidal volume of the DGB group significantly increased more than controls. The PetCO$_2$ was significantly lower in the DGB group following the intervention when compared to the first session of testing. The post-intervention daytime systolic and diastolic BP was significantly lower than the pre-intervention values in the DGB group.

Ankad et al. (2011) performed a training study for a period of two weeks with 50 healthy individuals ages 20 – 60 years. The aim of the research was to ascertain if the short term practice of pranayama and meditation would elicit positive changes in the functioning of the cardiovascular system. Subjects were randomly selected for an intervention group or a control group from a group of people who had visited the yoga center who had no history of yoga practice but had showed a desire to learn. Detailed clinical histories were taken and individuals with a history of yoga training, or habits such as smoking or alcohol consumption were excluded from participating. A history of pulmonary, cardiovascular, renal or diabetic related disease resulted in exclusion from the research study. Baseline measurements and information were recorded, including age, height, weight, body mass index (BMI), heart rate, and BP (before and
after the practice of pranayama and meditation). Participants were instructed to lie in a supine position and relax for thirty minutes in a quiet room. HR was recorded via the right radial artery in beats per minute and BP was recorded in millimeter of mercury (Hg) via sphygmomanometer (Diamond) by auscultation. Between 5:00 and 6:00 p.m., readings of HR and BP were taken three separate times at 15 minute intervals, averaged and mean arterial BP was calculated.

Subjects received uniform diet and pranayama training for a period of 15 days. The training consisted of varied types of pranayamic breathing practices (Vibhagiya - sectional breathing; Nadishuddi Pranayama - alternate nostril breathing; Kabalabathi - cleansing breath; Bahya Pranayama - the external breathing; and Cooling Pranayama). Post testing was done after the 15-day period and revealed a significant reduction in resting HR, systolic, diastolic and mean arterial BP. The results were also significant for men and women separately. This study concluded that normal, healthy individuals who practice pranayamic breathing on a regular basis improve their cardiovascular functions.

The effect of pranayama on stress reduction and autonomic responses was investigated by Bhimani et al. (2011). Autonomic tone and reactivity were the main points of interest in this study and were measured through HRV. Subjects were newly admitted medical college students, with similar/comparable social and economic status, dietary habits, and psychosocial natures. Subjects were excluded if they were practicing stress relieving or relaxation techniques, taking drugs, consuming significant quantity of beverages that could affect the ANS or if they had major illness. Fifty-nine medical students (27 males and 37 females) meeting all criteria participated in an orientation session, filled out a stress questionnaire, and had initial autonomic functioning tests performed. Autonomic testing included the following: measuring autonomic tone via HRV testing, autonomic reactivity to handgrip dynamometry, cold pressor and a lying to standing test. The order of the tests was kept constant throughout the study. No control group was used. Following baseline measurements, the subjects participated in pranayama for one-hour sessions, 5 days per week, for a total of two months. Pranayama sessions included: Kapalabhati, External
Kumbhaka (Bahya), Easy Comfortable Pranayama (Sukha Purvaka), Surya Bhedan, Ujjayi, Sitkari and Sitali. Post testing was performed via the stress questionnaire and autonomic functioning tests. The results for reduced stress were significant when comparing pre and post data. Systolic BP reduced significantly in lying to standing tests after the pranayama intervention. Diastolic BP rose significantly in autonomic reactivity testing (isometric handgrip dynamometer test and cold pressure test) after pranayama. Data revealed significant positive changes in HRV under resting conditions. There were reductions in very low frequency (VLF) and LF domains after the pranayama training period. There were significant increases in HF and the HF/LF ratio post testing, all of which indicated an increase in parasympathetic activity influence on the heart. A trend (p = 0.09) was observed for an increase in the time domain measure of RMSSD. These particular changes indicate a healthier balance between sympathetic and parasympathetic influences, with a stronger emphasis developing towards parasympathetic dominance.

Wang et al., 2010, examined the effect of slow breathing on BP and HRV (specifically, SDNN) in postmenopausal women with prehypertension. Subjects performed 20-min breathing sessions at 6 breaths per min twice a day for 30 days. Half of the subjects did a total of 10 sessions (once every 3 days) with the assistance of biofeedback (combined group). For biofeedback, subjects viewed an EMG of their frontalis muscle and were asked to relax that muscle as they did slow breathing. There was not a control group that did neither slow breathing training nor biofeedback. Following training, both groups experienced a significant decrease in systolic BP (8 mm Hg in the combined group and 4 mm Hg in the breathing only group). The combined group also experienced a significant decrease in diastolic BP of 4 mm Hg. Wang et al. reported that both groups experienced an increase in SDNN following training. However, this finding was an artifact of their methodology. The baseline value was obtained during the first 5 min of the initial 20-min breathing session. The "trained" value was obtained during the last 5 min of the final 20-min session. The authors also measured SDNN during the first 5 min of the
final session, and it was not different from the baseline value. Therefore, breathing training, with or without biofeedback, had no effect of SDNN measured under similar conditions. However, a 20-min slow breathing session did cause an acute increase in SDNN.

Curiati et al., 2005, investigated whether meditation could reduce sympathetic activation in elderly patients that had optimally treated chronic heart failure (CHF). Nineteen patients 74.8 + 6.7 years old with CHF participated in the study. All were receiving diuretics, angiotensin-converting enzyme inhibitors, and a maximum carvedilol dose or spironolactone. Subjects were divided into a control group or directed into a two-month treatment intervention. Baseline levels of norepinephrine were established by drawing blood, Minnesota Living with Heart Failure Questionnaire (MLWHFQ) scores were taken, oxygen consumption (VO$_2$) and the ventilatory equivalent for carbon dioxide production (V$_E$/VCO$_2$) by cardiopulmonary testing (CPT), left ventricular ejection fraction (LVEF) and left ventricular diastolic diameter index (LVDDi) measured by echocardiography were taken at the beginning of the study. Control group subjects attended a weekly meeting while the intervention group listened to a 30-minute meditation audiotape twice a day. The first stage of the tape involved a 10-minute session of slow deep breathing with the aim of reducing the breathing rate and taking full diaphragmatic breaths known as “complete yoga breathing”. The second ten-minute section was dedicated to focusing on the word “peace” and gentle efforts to keeping other thoughts away while engaging in the “relaxation response” technique. The final ten minutes were dedicated to focusing on a mental image of a healthy heart. The results after 2 months revealed significant positive changes when compared to the pre assessed testing. Reduced sympathetic overall activity, improved quality of life and improved ventilatory efficiency were markers of success for this study. Meditation reduced norepinephrine levels significantly versus the control group and improved MLWHFQ total score. The intervention also reduced the V$_E$/VCO$_2$ slope. No changes were seen in LVEF, LVDDi and VO$_2$. The results support this meditation protocol, which also included twenty minutes total of slow breathing practice per day, as a possible treatment option for CHF patients in the future.
Dabhade et al., 2012, investigated the effect of pranayama breathing exercises on QT-interval dispersion (QTd) in stable subjects with arrhythmias who had ejection fractions of less than forty percent. Subjects were required to have a diagnosed presence of arrhythmia with ECG evidence of less than forty percent ejection fraction, a stable medical regimen for 2 weeks prior to the study, no active ischemia, no vascular procedures within 3 months, and no prior myocardial infarction within 8 weeks. Subjects were excluded if they were on class IA or III anti-arrhythmic medications, could not complete pranayama breathing sessions, did not have sinus rhythm at the beginning or end of practice sessions, or if they had a complete bundle branch block. Out of the initial 27 that enrolled, 12 were excluded. The remaining subjects were taking diuretics, angiotensin-converting enzyme inhibitors, and digoxin. Twelve of the patients were taking nitrates and aspirin. Before entering a pranayama session, subjects underwent symptom-limiting exercise testing with a treadmill protocol. The patients were given individual exercise prescriptions at 70 – 85% of their maximum heart rate based off of their treadmill testing results before each pranayama session. Standard ECG measurements were taken with QT, JT and RR interval measurements performed. All of the patients completed a 12-week intervention of 36 pranayama sessions that were 45 minutes in length. The breathing exercises included: Bhastrika pranayama (10 min); Kapalbhati pranayama (10 min); Anilom-vilom pranayama (15 min); Bharmari (5 times a day); and Udgit pranayama (5 times a day). The amount of exercise at the prescribe heart rate was not disclosed in the study. After the 12-week intervention, there was no increase in exercise capacity or peak oxygen consumption. The ECG analysis revealed significant decreases in all intervals when comparing pre to post values. Significant changes in repolarization dispersion occurred in all twelve of the patients with ischemic cardiomyopathy. The results indicate that pranayama significantly impacts ventricular repolarization in patients with arrhythmia. Additional research is needed to investigate the potential of how this ventricular repolarization may decrease sudden death in arrhythmia patients.
Dhungel et al. performed an interesting study in 2008 on the effect of an alternate nostril breathing exercise and how it affects cardio-respiratory functions. Thirty-two males and four females who were healthy nonsmokers participated in the study. Subjects were free of cardio-respiratory diseases and were not on medications. Age, height, weight and BMI were recorded. All participants were sedentary and of a similar background socioeconomically. No control group was used. Baseline ventilation rate, HR, BP, peak expiratory flow rate (PEFR) and pulse pressure (PP) were recorded. The subjects were taught the process of alternate nostril breathing (ANB), which involved a slow, steady breathing rate. Subjects practiced the given intervention for 4-weeks. All measurements were recorded again following an ANB session. The results indicate a significant increment in PEFR and pulse pressure. Diastolic BP and heart rate lowered significantly. These results indicate that regular practice of slow alternate nostril breathing increases parasympathetic activity.

Pal et al. performed a research study in 2004 that examined how autonomic functions were affected by breathing exercises that were performed regularly for a 3-month period. Sixty male undergraduate medical students were divided into equal groups of thirty with one being the "slow breathing exercise" (SBE) treatment group and the other acting as the "fast breathing exercise" (FBE) group. All participants were evaluated and found to be free from major health problems and were nonsmokers. Autonomic function testing was done before and after the designated 3-month treatment period. Prior to taking autonomic measurements, each individual rested in a supine position. ECG measurements were taken in a lying position and HR was recorded. Subjects were also asked to stand up quickly and the heart changes were noted via polygraph and HR changes were recorded. This protocol was done a total of three times with 5-min rest periods in between. Heart rate response was also recorded in relationship to deep breathing practices. Subjects were instructed on proper rest before the test and proper inhalation and exhalation techniques to fully properly execute a deep breath in a standardized manner. The protocol was repeated three times and results recorded. Finally, the groups were also asked to
perform Valsalva maneuvers and given careful instructions on how to do so in a standardized fashion. Bradycardia and tachycardia ratios were calculated on all subjects for pre and post results. All results were recorded. The SBE participants were instructed on exactly how to perform breathing exercises that were standardized but that closely mimic pranayama practices and include unilateral nostril breathing as the basis of the breathing practice. The participants performing the fast breathing exercises were asked to maintain a seated posture while quickly and deeply inhaling and exhaling for one minute. This was followed by a 3-min rest period. They performed this task eight to ten times over a period of thirty minutes. The SBE and FBE groups practiced their assigned breathing intervention for thirty minutes in the morning and thirty minutes in the evening during the 3-month period. The subjects returned and participated in post testing at the end of the training period. The compilation of data gathered resulted in significant changes in autonomic functions in all testing values including HR, BP, the deep breathing protocol, Valsalva ratio, bradycardia and tachycardia ratio in the SBE group while the FBE group did not see significant changes. The HR consistently was altered and responded in a calmer state in those that had practiced the SBE protocol. The authors state that that their findings support the improvement of vagal tone through the use of pranayama breathing practices. They suggest that left nostril breathing or the use of alternate nostril breathing is the best practice to help shift a decrease in the sympathetic influence and increase in the parasympathetic effect based on experiences within the study and other literature reviewed. Further research is needed to confirm these results.

Veerabhadrappa et al., 2011, studied the effect of yogic bellows on cardiovascular autonomic reactivity in 50 male subjects 18 - 25 years of age for a period of 12 weeks. Subjects had similar diet, activity levels, and did not smoke, consume alcohol or take drugs. A detailed history was taken and no evidence of cardiovascular disease was present. Participants were not considered trained athletes or experienced yoga practitioners. Baseline tests were recorded via portable BP monitor (Omron, Inc, Japan) and ECG. Tests performed were: heart rate response to
Valsalva maneuver (Valsalva ratio), HR response to deep breathing (deep breathing difference - DBD), and BP response to standing. Mukh Bhastrika training was carried out from 7 - 7:30 in the morning, 5 days a week for the 12-week training period. The training involved sitting in padmasana (keeping the body, neck and head erect while sitting), closing the mouth, then proceeding to inhale and exhale quickly ten times as deeply as possible "like bellows of the blacksmith, with a hissing sound." This was followed with a rapid release of air and an immediate deep long diaphragmatic inhalation and releasing of the air as slowly as possible. This completed one round of Bhastrika. Students were directed to take a few normal breaths and then restart and practice up to a total of 3 rounds. Subjects were re-tested at the end of the 12-week period and the results revealed significant changes in the basal HR from 73.3 ± 11.0 to 59.9 ± 10.1 bpm when compared to preceding values; Valsalva ratio significantly increased from 1.54 ± 0.04 to 1.61 ± 0.02; maximum and minimum heart rates achieved during deep breathing were increased after Mukh Bhastrika training compared to control values as well as DBD and HR; BP response to standing reduced significantly in the intervention group when compared to controls as did postural fall BP on standing. The results indicate that the practice of this breathing protocol positively affects autonomic functions in healthy volunteers.

**Effect of Acute Breathing Training**

Driscoll and DiCicco published a study in 2000 that involved "metronome breathing," which was paced at 12 breaths per minute. Eight subjects (3 male, 5 female) were recruited from Parker College of Chiropractic and asked to abstain from the usage of caffeine 12 hours before the initial testing visit, abstain from exercise the evening before and the day of the testing, and to not eat 4 hours prior. Subjects were seated comfortably for 15 minutes in a quiet room and BP was measured every 2.5 minutes through sphygmomanometry. Subjects breathed normally for 3 recording periods and then for 3 recording periods subjects were asked to pace their inhalation and exhalation to a metronome (12 breaths per minute). Results revealed that metronome
breathing increased HF power, decreased LF/HF power and reduced the CV% of LF power when compared with normal breathing. The metronome breathing also increased BP fluctuations at the right and left radial arteries. These findings indicate increased parasympathetic activity that is shown through increased HF power in the ECG and systolic BP results.

Ghiya and Lee performed research in 2012 that compared paced breathing (PB) and alternate nostril breathing (ANB). Twenty healthy subjects (12 females and 8 males) participated in the study. They had no prior experience with performing ANB, abstained from caffeine for testing, and filled out questionnaires. The height and weight of each participant was measured and their BMI was calculated. Each subject attended a practice session where they were provided demonstrations of the two breathing methods and practice time. The second visit for participants involved them performing the same breathing exercises in a randomized order with 5 minute rest; 30 minute intervention; 5 minute rest; 30 minute intervention; 5 minute rest. ANB was performed in a seated posture while inhaling through the left nostril, holding the breath for a moment, keeping both nostrils closed, exhaling from the right nostril, and keeping the left nostril closed. This was followed by the reverse pattern starting with the right nostril. ANB was performed at 5 breaths per minute for 30 minutes. PB was performed while breathing normally but at a slow rate of 5 breaths per minute for a period of 30 minutes. Investigators gave cues to prompt proper adherence to timing. An ECG was performed on the participants the entire time they were present for the second visit during their rest periods and while they performed both of the breathing exercise interventions. Each session of ANB or PB was preceded by 5 minutes of rest, seated quietly, but monitored, and also followed by 5 minutes of the same during which time they were asked to breathe normally. The results revealed that both groups had a significant increase in both the post-ANB and post-PB when compared to the pre-ANB and pre-PB values for lnTP (pre 8.5 ± 0.1, post-ANB 9.5 ± 0.2, post-PB 9.8 ± 0.2), lnLF (pre 7.6 ± 0.2, post-ANB 8.5 ± 0.1, post-PB 8.7 ± 0.2) and lnHF (pre 6.6 ± 0.2, post-ANB 8.0 ± 0.3, post-PB 8.4 ± 0.3) values. Because of the similar findings, logic indicates that there is elevated ANS modulation of
the heart after either intervention practice, with the common training point being the breathing rate, rather than the breathing type. This is a significant finding that may be helpful to research.

A study performed by Pramanik et al., 2009, examined the effect of pranayama on HR and BP. Thirty-nine subjects, ages 25-40 years had their BP taken after 5 minutes of rest. They were carefully instructed in how to perform slow pace bhastrika pranayama breathing exercises for a period of 5 minutes. These individuals were also questioned about their mood during the study. A second group was administered 20 mg of Buscopan orally. HR and BP were recorded after thirty minutes. The subjects taking the parasympathetic nervous system (PNS) blocker were then instructed in the same pranayama as the first group. BP and HR were taken again after the intervention for each group had been completed. Results revealed a significant decrease in both the systolic and diastolic BP measurements as well as a decrease in their second HR measurement in the participants that did not receive the PNS blockers, but practiced pranayama. Participants receiving the PNS blocker showed no significant changes after practicing pranayama. The investigators concluded that slow paced breathing practices significantly affect the ANS though the enhancement of the PNS.

An office worksite chair-based meditation and yoga intervention study was performed by Melville et al. in 2012 in order to examine physiological and psychological markers of stress. This study examined acute conditions in which 20 subjects, aged 39 ± 10 years, completed 3 different conditions 24 hours or more apart: 15 minutes of yoga postures, 15 minutes of guided meditation and a control condition. Subjects were sedentary, English speaking, full-time employees who were willing to participate in all of the study conditions. Testing was completed on location at the subjects’ office workspace during normal scheduled working hours and with all other normal habits in place, such as diet and beverage consumption patterns, with the exception of cell phone usage. Baseline testing included 5 minutes of testing and a gathering of physiological and psychological information within the confines of their workspace. This was immediately followed by one of the intervention conditions (yoga, meditation, or control). The
yoga condition consisted of basic yoga postures performed while seated in a chair. Each posture was held for six breaths, with the session lasting 15 minutes. The meditation condition consisted of guided breathing via headphones which involved deep breathing patterns for 15 minutes. The control condition mimicked the baseline condition, with the individual seated at their desk performing basic low key tasks. In both the yoga and meditation conditions, ventilation rate was significantly lower than during the control condition, being approximately 10, 12 and 15 breaths per minute, respectively. Meditation significantly reduced systolic BP (control 115 ± 12 mm Hg, meditation 110 ± 13 mm Hg) and diastolic BP (control 77 ± 11 mm Hg, meditation 76 ± 11 mm Hg), and also decreased HR. Regarding HRV, meditation significantly increased SDNN, LF, TP, and LF/HF ratio.

Raghuraj et al., 1998, examined the effects of two breathing techniques that were expected to have opposite effects to see their impact on HRV. The two selected yogic breathing methods were: kapalabhati (breathing at high frequency) and nadisuddhi (alternate nostril breathing). Twelve healthy male volunteers ranging in age from 21 to 33 years who were familiar (average experience of 19.7 months) with both techniques participated. Subjects were seated in a dimly lit room in a comfortable seated posture while 5-minute recordings were made, approximately 3 hours following their last meal. Testing occurred on two separate days and at the same time of day. Information was gathered via ECG at 500 Hz. Results indicate a significant increase in LF power and LF/HF ratio with lower HF following kapalabhati (high frequency breathing), while there were no significant changes after alternate nostril breathing. The results suggest that high frequency breathing caused increased sympathetic activity with parasympathetic withdrawal and that HRV is a useful tool to use in conjunction with HR.

Once having reviewed the literature available related to breathing protocols and how they interact with autonomic function and HRV, it was clear that further research needed to be performed to advance the area and gain greater understanding. Several issues arose upon careful
review of the research that had been performed prior to this study. The methodology was not clear in some of the studies, multiple interventions were mixed (breathing, yoga, meditation), and there was potential bias or prior knowledge as to the expected outcome in some cases where participants had already been practicing an intervention prior to the study. Many of the studies appeared to be laced with expectancy that yogic breathing was the causation of the autonomic changes that have presented within research rather than seeking sound science. Ghiya and Lee, 2012, created ground breaking work in the comparison of ANB and paced breathing performed at the same rate by inexperienced practitioners, with both interventions producing an effect on the ANS and therefore, HRV. This particular work certainly warranted additional research to back up its findings.

Despite the flaws in the available literature, there appeared to be evidence that there was a particular component of pranayamic /slow breathing / paced breathing that revealed significant findings within research. Heart rate was lowered in several of the studies in a significant manner (Ankad et al., 2011; Dhungel et al., 2008; Pal et al., 2004; Veerabhadrappa et al., 2011). BP was lowered significantly in several of the studies as well (Ankad et al., 2011; Dhungel et al., 2008; Veerabhadrappa et al., 2011). Opposing effects were found in some studies with specific pranayamic practices such as in Telles et al., 1994. Overall, it appears that breathing practice has the potential to positively impact autonomic function in both healthy and disease populations. The determination of the amount of training needed to sustain a therapeutic effect, as indicated by changes in HRV, appears to be a well-founded consideration for research.
CHAPTER III
METHODOLOGY

This chapter provides the procedures undertaken for this research study. Research design, sample population, instrumentation, and data collection procedures are discussed in detail.

Research Design

The type of research performed was an experimental design that involved an intervention of slow breathing (similar to that used by Curiati et al., 2005; Dane et al., 2001; Ghiya et al., 2012; Mohan et al., 1986; Pal et al., 2004; Pramanik et al., 2009) for 30 minutes (as used by Pal et al., 2004; Raghuraj et al., 2008; Veerabhadrappa et al., 2011) that were executed 2, 3 or 5 times weekly over a 4-week time period. Individuals participating in the research study were matched for sex, age and SDNN and then randomly assigned to one of the 3 intervention groups or a control group.

Subjects & Sample Population

The general population for this study was healthy volunteers aged 18 – 50 years from the Hampton Roads area. Subjects were free of pulmonary and cardiovascular diseases and did not have diabetes or use tobacco. Female subjects did not consider themselves to be pregnant. Subjects were not on any medications that interfered with autonomic function testing results. This included medications such as steroids, hormone-replacement therapy drugs, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, beta-blockers or any other medications that are known to interfere with the ANS. Subjects agreed to maintain their normal activity level and diet throughout the course of the 4-week period. A screening questionnaire (Appendix A) was used to determine eligibility prior to obtaining written, informed consent.
Approval for the study was obtained from the Old Dominion University Institutional Review Board.

Subjects were recruited via ODU email (Appendix B), word of mouth, and class announcements. Subjects acknowledged the ability to set aside time for the initial meeting, baseline measurements and follow up measurements at the end of the study and up to 30 minutes 2, 3, or 5 times a week for 4 weeks in order to qualify to participate in the study.

Procedures

Initial Screening

Once potential subjects were recruited, an initial interview was set up to screen participants individually. Subjects arrived at the Human Performance Laboratory (HPL) at a pre-arranged time and completed the health screening questionnaire to determine eligibility. The procedures and risks of the study were explained and the subject was given the opportunity to read the informed consent document (Appendix C) and ask any questions prior to providing informed consent. Diet information such as a list of food, drinks, drugs and stimulants to avoid prior to testing were reviewed at this time (antihistamines, allergy medications, caffeine supplements, guarana extract, energy supplements containing caffeine in synthetic and natural forms, coffee, tea, soda [items such as Red Bull, Monster Energy, Jolt, Full Throttle, RockStar Drinks, Arizona Green Tea, Java Monster, Go Girl Drinks, Propel Energy Water, Starbucks, Mountain Dew, Coca Cola, etc.], and weight control items with caffeine (e.g., Dexatrim). When time permitted and the guidelines were met, baseline testing was performed at this time. If not possible, an appointment was scheduled at the convenience of the subject to return to the HPL. In preparation for the second appointment, participants were asked to adhere to the preparation guidelines and otherwise maintain a normal diet.
Baseline Testing

On the day of testing, the test administrator confirmed exercise, food and caffeine abstinence for the prior 3 hours before testing procedures began. The subject was fitted with an Actiheart device that detected heart rate (HR) and transmitted this information to a computer for HRV analysis (Actiwave Cardio, Camntech). The subject was asked to lie supine and relax as still as possible for 10 minutes while heart rate was recorded. During this 10-minute period, the subject listened to a recording of breathing sounds at 6 breaths per minute, and was asked to match his or her breathing to the sounds. Each inhalation lasted approximately 4 seconds and each exhalation lasted approximately 6 seconds. The tester recorded the last 5 minutes of the heart rate data. Frequency domain measures (LF and HF in milliseconds, LF/HF ratio, LF and HF in normalized units), and time domain measures (SDNN and RNSSD) were later analyzed via Actiwave Software (Camntech). At the end of the 10-minute period, the subject’s BP and heart rate (HR) were measured via a digital BP cuff.

Group Assignment

Subjects were matched based on sex, age and SDNN. Matched groups of four subjects were randomly assigned to either the 2 times per week, 3 times per week or 5 times per week breathing practice groups, or to the control group. Those assigned to the control group were notified by phone or e-mail of their control assignment. Those assigned to a breathing intervention were scheduled for breathing training instruction.

Breathing Training

Subjects assigned to a training group were asked to report to the HPL to prepare for their at home protocols. They were seated in a quiet location. Written instructions were provided to each of the participants (Appendix D). If the participant wished to use a timing device that they owned during this breathing training, they were allowed to bring it into the training area with
them (e.g., stopwatch, phone). After remaining seated quietly for 2 – 3 minutes, the subject was asked to begin slow paced breathing at the rate of 6 breaths per minute for 30 minutes. Each inhalation lasted approximately 4 seconds and each exhalation lasted approximately 6 seconds. The subject was encouraged to relax and count silently to four during inhalation and then count silently to six during exhalation. Subjects had access to investigators throughout the training as needed to ask questions.

Subjects participating in an intervention group practiced the slow breathing protocols for 2, 3 or 5 times per week for thirty minutes per session for a total of four weeks. They were required to keep a training journal (Appendix E) and asked to maintain their regular habits and lifestyle in regards to exercise, sleep and diet. Reminders to maintain the journal were provided weekly via email. Control group participants were asked to maintain their normal activities and lifestyle.

Daily Breathing Protocol Training Log

A daily log of breathing protocol practice was kept by all participants in the 3 intervention groups. Notations were made of any breathing challenges that came up during the 4 weeks (e.g., upper respiratory infections, repeated conflicts, interruptions) in order to be able to assist the participant to track and structure their breathing practice and also evaluate if there was a health interference issue that could have marred the test results. One page breathing logs were provided by the researchers and instructions were reviewed with each client about requirements for adherence.

Post Testing

All subjects returned after 4 weeks to repeat the testing procedures. The time of day was approximately the same (within 1 hour) as pre-testing for each subject. As with the initial testing,
subjects were asked to refrain from eating, exercising or ingesting caffeine for 3 hours prior to testing.

**Statistical Analysis**

A two-way (4 groups x 2 times) ANOVA with repeated measures on one factor (time) was performed to compare the results of the groups for HR, systolic BP, diastolic BP and the HRV measures. A one-way ANOVA was performed on pre-post delta values to determine if changes in any variables that occurred with training were different between groups. When significant F-tests were found, post hoc testing was performed via T-tests. Significance was judged at the 0.05 level.
CHAPTER III
RESULTS

Subjects

In this research, 49 participants (18 males, 31 females) were recruited and participated in the pre-testing session. Six of the initial subjects who completed pre-testing withdrew from the study due to: reported disinterest (3), a lack of time for participation (2) or admission of engaging in behaviors which disqualified them from the study, such as smoking (1). Forty-three participants (14 males, 29 females) completed the entire study, and their demographics are presented in Table 1. All subjects in all training groups reported 100% compliance in performing the prescribed number of breathing sessions.

Table 1: Subject Characteristics by Group Placement

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Two Times Per Week</th>
<th>Three Times Per Week</th>
<th>Five Times Per Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age</td>
<td>24</td>
<td>24</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>SD</td>
<td>4</td>
<td>7</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Males</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Females</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Blood Pressure and Heart Rate Measures

BP and heart rate measures taken at rest before and after training are presented in Table 2.
Table 2: Results (Mean and SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Two Times Per Week</th>
<th>Three Times Per Week</th>
<th>Five Times Per Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Systolic BP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>103</td>
<td>107*</td>
<td>100b</td>
<td>94**</td>
</tr>
<tr>
<td>SD</td>
<td>10</td>
<td>10</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td><strong>Diastolic BP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>64c</td>
<td>68*</td>
<td>65c</td>
<td>61*</td>
</tr>
<tr>
<td>SD</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td><strong>Heart Rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>60</td>
<td>61</td>
<td>59</td>
<td>56**</td>
</tr>
<tr>
<td>SD</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td><strong>SDNN</strong></td>
<td>Mean</td>
<td>0.011</td>
<td>0.112</td>
<td>0.097</td>
</tr>
<tr>
<td>SD</td>
<td>0.044</td>
<td>0.044</td>
<td>0.042</td>
<td>0.026</td>
</tr>
<tr>
<td><strong>RMSSD</strong></td>
<td>Mean</td>
<td>0.080</td>
<td>0.082</td>
<td>0.088</td>
</tr>
<tr>
<td>SD</td>
<td>0.041</td>
<td>0.043</td>
<td>0.051</td>
<td>0.024</td>
</tr>
<tr>
<td><strong>HF</strong></td>
<td>Mean</td>
<td>549</td>
<td>482</td>
<td>777</td>
</tr>
<tr>
<td>SD</td>
<td>500</td>
<td>458</td>
<td>1039</td>
<td>225</td>
</tr>
<tr>
<td><strong>LF</strong></td>
<td>Mean</td>
<td>2813</td>
<td>2570</td>
<td>2044</td>
</tr>
<tr>
<td>SD</td>
<td>2211</td>
<td>1587</td>
<td>1829</td>
<td>1309</td>
</tr>
<tr>
<td><strong>HF/LF Ratio</strong></td>
<td>Mean</td>
<td>5.46</td>
<td>8.66</td>
<td>3.75</td>
</tr>
<tr>
<td>SD</td>
<td>2.93</td>
<td>7.47</td>
<td>1.36</td>
<td>2.70</td>
</tr>
</tbody>
</table>

*a* Pre value significantly (p < 0.05) different than that of the 3 times and 5 times per week groups;

*b* Pre value significantly (p < 0.05) different from that of the 5 times per week group;  
* Pre value significantly (p < 0.05) different from that of the 3 times and 5 times per week groups.  
*Post value is significantly different from its corresponding Pre value (p < 0.05);  
** Post value is significantly different from its corresponding Pre value (p < 0.01);  
*** Post value is significantly different from its corresponding Pre value (p < 0.001).

The two-way ANOVA on systolic BP indicated a significant (p < 0.05) main effect for group, significant main effect for time (p < 0.01) and significant (p < 0.001) group x time interaction. Post hoc testing revealed that the control group had a significantly (p < 0.05) higher pre value than either the 3 or 5 times per week group, and the 2 times per week group had a higher pre value than the 5 times per week group. Further, there was a significant increase
following training in the control group (p < 0.05) and significant decreases following training in all 3 training groups. The deltas observed in the 3 training groups were not significantly different from each other, but were significantly (p < 0.05) different from that of the control group. Results are illustrated in Figure 1.

Figure 1: Resting Systolic BP Before and After Training

![Systolic BP graph](image)

\[a\] Pre value significantly (p < 0.05) greater than that of groups 3 and 5; \[b\] Pre value significantly (p < 0.05) greater than that of group 5; \[*\] Post value significantly different from Pre value (p < 0.05); \[**\] Post value significantly different from Pre value (p < 0.01); \[***\] Post value significantly different from Pre value (p < 0.001); C: control group; 2: 2 times per week group; 3: 5 times per week group; 5: 5 times per week group.

The two-way ANOVA on diastolic BP revealed a significant (p < 0.05) main effect for group but not for time. Group x time interaction was significant (p < 0.05), with the control group
and 2 times per week group's pre values significantly (p < 0.05) greater than those of the 3 and 5 times per week groups. There was a significant (p < 0.05) increase following training in the control group and a significant (p < 0.05) decrease in the 2 times per week training group (Figure 2).

Figure 2: Resting Diastolic BP Before and After Training

![Graph showing resting diastolic blood pressure before and after training for different groups.]

* Pre value significantly (p < 0.05) greater than those of groups 3 and 5. *Post value significantly different from Pre value (p < 0.05); C: control group; 2: 2 times per week group; 3: 3 times per week group; 5: 5 times per week group.

The two-way ANOVA on heart rate revealed a significant (p < 0.01) main effect for time, but not for group, and a significant (p < 0.01) interaction term. There were differences in the pre values between any of the groups. There were significant decreases in the 2 times per week (p
< 0.05) and 5 times per week (p < 0.001) training groups (Figure 3). The deltas for these two groups were not significantly different from each other, but were from that of the control group.

Figure 3: Resting Heart Rate Before and After Training

*Post value significantly different from Pre value (p < 0.05); ***Post value significantly different from Pre value (p < 0.001); C: control group; 2: 2 times per week group; 3: 3 times per week group; 5: 5 times per week group.

Heart Rate Variability Measures

No significant differences from pre to post were found in any of the HRV measures for any of the groups, as presented in Table 2.
CHAPTER IV
DISCUSSION

The purpose of the study was to measure what effects different frequencies of a standardized slow breathing protocol would have on autonomic nervous system function over a 4-week period in healthy volunteers as determined by measurement of HRV and BP, specifically to determine if a lower frequency of practice than typically employed would be effective.

Blood Pressure and Heart Rate Measures

It was hypothesized that slow breathing practice performed 2, 3 or 5 times a week for thirty minutes would decrease resting BP and heart rate values when compared to controls. This hypothesis was supported, for the most part. Systolic BP decreased in all training groups. The twice a week group also experienced decreases in HR and diastolic BP, while the 5 times per week group decreased HR. There was an expectation that the 5 times per week group would be most likely to show changes in these variables and the 2 times per week group the least likely, but this was not the case. There was little difference between the groups, with the twice per week group showing more significant changes than the other frequencies of training, in that it was the only group that exhibited decreases in all 3 variables, while the 3 times per week group decreased only in systolic BP and the 5 times per week group in systolic BP and HR.

BP training studies have been a topic of interest within recent years, with many studies producing significant findings. Anderson et al. (2010) and Elliot et al. (2004) demonstrated significant BP reduction utilizing device-guided slow breathing. Their interventions involved applications of 15 minutes daily over a period of four and 8 weeks, respectively. Slow device-guided breathing as studied by Anderson and colleagues resulted in significant decreases in the
daytime measurements of systolic BP (pre 146 ± 1 mm Hg, post 141 ± 1 mm Hg) and diastolic BP (pre 90 ± 1 mm Hg, post 87 ± 1 mm Hg) when compared to controls following a 4-week intervention. This particular training study provided a paced breathing pattern similar to our study, but only utilized fifteen minutes of breathing training per session. In comparison, our longer thirty-minute intervention produced slightly greater reductions in BP and, in addition, saw a significant reduction in HR in two of the training groups. The protocol followed by Elliott et al. lasted 8 weeks. The largest reduction in systolic BP (pre 150 ± 7 mm Hg, post 135 ± 12 mm Hg) and diastolic BP (pre 83 ± 9 mm Hg, post 79 ± 9 mm Hg) occurred in those utilizing the device the most (180 minutes or more per week). Significant reductions were also found in subjects who were “low users” (less than 180 minutes of use per week).

Our HR results were similar to those of Dhungel et al. (2008). They found significant decreases in HR (pre 77 ± 4 bpm, post 74 ± 3 bpm) and diastolic BP (pre 79 ± 5 mm Hg, post 74 ± 5 mm Hg) with a slow breathing protocol among healthy volunteers with a mean age of 25 years over a 4-week period. Dhungel et al. did not utilize a control group to help confirm their findings. Ankad et al., 2011, had healthy but otherwise sedentary, 20-60 year olds perform a daily, 45-minute, slow paced ANB protocol over a 15-day period. This resulted in a significant reduction in systolic BP (pre 127 ± 12 mm Hg, post 123 ± 12 mm Hg) and diastolic BP (pre 80 ± 10 mm Hg; post 77 ± 10 mm Hg) when compared to controls.

Our BP and HR results compare well to those of Dhungel et al. and Ankad et al. We found that systolic BP decreased by 7, 4 and 6 mm Hg across the 3 groups (2, 3 and 5 times per week, respectively), while Ankad et al. found a 4 mm Hg decrease. We found a 4 mm Hg decrease in diastolic BP in the twice per week group, while Dhungel et al. and Ankad et al. found decreases of 5 and 3 mm Hg, respectively. We observed decreases in HR of 3 and 4 bpm in the 2 and 5 times per week groups, respectively, while Dhungel et al. found a 3 bpm decrease. The present research offers the validity of a control group to further solidify the significance of our
findings, whereas Dhungel et al. did not. The control group demonstrated increases in both systolic and diastolic BP, which we cannot explain.

Our subjects were young and healthy and thus began the study with low values of BP (average of 98/61 mm Hg across the 3 training groups) and HR (59 bpm average). These were somewhat lower than those in the healthy population studies that Dhungel et al. and Ankad et al. performed, yet, the breathing training was still able to produce reductions in these measures.

**Heart Rate Variability Measures**

It was hypothesized that the slow breathing practice performed 2, 3 or 5 times a week for thirty minutes would increase several HRV measures (SDNN, RMSSD, HF and HF/LF) when compared to baseline values. It was also hypothesized that slow breathing practice performed 2, 3 or 5 times a week for 30 minutes would decrease the HRV LF measure when compared to baseline values. These hypotheses were not supported, as none of the HRV measures was changed following training in any of the groups.

Previous studies have demonstrated that an acute bout of slow paced breathing alters HRV in comparison to normal, uncontrolled breathing, suggesting improved parasympathetic tone (Driscoll and DiCicco, 2000; Ghiya and Lee, 2012; Melville et al., 2012). Driscoll and DiCicco, 2000, found significantly increased HF, decreased LF and an improved LF/HF ratio. Ghiya and Lee, 2012, found significant increases in HF, LF and TP. Melville et al., 2012, reported a significant decrease in LF and increases in TP, SDNN and improved LF/HF ratio. Within these studies it was common to test for SDNN and RMSSD, but only Melville et al. saw changes in SDNN, and none saw a change in RMSSD. Slow breathing is expected to improve HRV because ventilation is a major factor responsible for variability in the heart rate. HR increases during inhalation and decreases during exhalation. Thus, slow, controlled breathing increases the difference between higher and lower heart rates during the breathing cycle.
However, the question remains whether this acute effect translates to a chronic increase in HRV if slow breathing is practiced on a regular basis.

Bhimani et al., 2011, performed research that involved training 5 days per week with daily practice of slow pranayama for one hour over a period of 8 weeks. They found a significant decrease in LF and significant increases in HF and HF/LF. They also observed a trend for increased RMSSD. No control group was used to validate these research findings, which leaves this research subject to criticism. Subjects studied by Bhimani et al. performed substantially more training than did ours: the duration per session was twice as long, the frequency per week was the longest of our groups, and the duration of the study was 4 weeks longer than our study. Perhaps the greater practice resulted in HRV effects that our study did not obtain. This does not explain, however, why our 2, 3 and 5 day training schedule with 30 minutes of breathing practice per day was long enough to make an impact on other autonomic variables such as heart rate and systolic and diastolic BP.

Wang et al., 2010, had subjects practice slow paced breathing twice a day, 20 min each session, for 30 straight days. Half of the subjects combined the breathing with supervised biofeedback on 10 of the days. They reported that both groups experienced decreased BP and increased SDNN at the end of training. However, HRV was measured at both the start and the end of a 20-min breathing session post training, while it was only measured at the start of a session for the pre training value. There was no change in SDNN from pre- to post-training when comparing data collected at the start of the sessions. Therefore, these results actually confirm that slow breathing acutely improves HRV (in accord with other acute studies cited above), but that training does not alter it. Of the 3 studies that have used a period of breathing training to affect HRV – the current study, Bhimani et al. and Wang et al. – only Bhimani et al. found an effect, and no control group was used. Moreover, Bhimani et al. stated that HRV was measured using “standard procedures,” but no details were provided, leaving open the question as to whether the
breathing pattern or the timing of the data collection may have differed in the pre- and post-tests conducted, as done by Wang et al.

A limitation in our study was that training sessions were not supervised and subjects were asked to record their training on a provided journal. It is not known if the subjects actually performed the amount of training they recorded. This particular limitation could affect the outcome of the HRV variables across all 3 training groups. Further, subjects were asked to refrain from caffeine and food for 3 hours prior to baseline testing and post testing. It wasn’t known if the subjects actually complied with these requests, therefore, testing outcomes for HRV could have been affected by eating patterns that were not thoroughly controlled for in this experiment. These limitations are typical of training studies, including prior studies that measured HRV following breathing training (Bhimani et al., 2011; Wang et al., 2010).

Conclusion

The main purpose of this study was to measure what effects different frequencies of a standardized breathing protocol would have on the ANS following a 4-week training period via measurement of HRV, BP and HR. Prior studies found adaptations using similar training with a frequency of at least 5 days per week. Thus, we expected our 5 times per week group would exhibit significant results and wished to determine whether lower frequencies of 3 or even 2 times per week would be effective. For the reduction of BP and HR, we found that 2 times per week was as or more effective than 3 or 5 times per week. Thus, no minimum frequency was revealed to be ineffective.

On the other hand, despite the claims made in two previous studies (Bhimani et al., 2011; Melville et al., 2012), HRV did not change as expected. No measure of HRV examined in this study (HF, LF, HF/LF, SDNN, RMSSD) was affected by the intervention, regardless of the frequency of practice. While we cannot eliminate the possibility that a training program with a duration longer than 4 weeks may be effective, we conclude that breathing training does not alter
resting measurements of HRV. While studies have shown an acute effect of slow breathing on HRV (Driscoll and DiCicco, 2000; Ghiya and Lee, 2012; Melville et al., 2012), one other training study did not find a true chronic effect (Wang et al., 2010), and the one study that found an effect (Bhimani et al., 2011) did not use a control group.

This study did confirm that autonomic functions are positively affected by the introduction of a 4-week breathing intervention, as evidenced by decreases in systolic and diastolic BP and HR. The practice of slow breathing clearly has beneficial effects and has potential as a complementary non-pharmacological method to lower BP and HR.
REFERENCES


Anderson DE, McNeely JD and Windham BG. Regular slow-breathing exercise effects on blood pressure and breathing patterns at rest. J Hum Hypertens. 2010;24:807-813.


Phongsuphap S, Ponsupap Y, Chandanamattha P and Lursinap C. Changes in heart rate variability during concentration meditation. *Int J Cardiol.* 2008;130:481-484.


APPENDIX A

SCREENING QUESTIONNAIRE

Name ___________________________  Sex _____  Age _____  Date __________

Phone ___________________________  email ___________________________

Do any of the following apply to you?
___ 1. Do you have any form of heart or vascular disease?
___ 2. Do you have any form of pulmonary disease?
___ 3. Do you have diabetes?
___ 4. Do you take any medication that may affect your heart rate, such as beta-blockers?
___ 5. Do you use any form of tobacco product?
___ 6. If you are female, do you think you may be pregnant?
APPENDIX B

RECRUITMENT E-MAIL

Dear ODU Students, Faculty and Personnel,

New research is being developed in the area of heart rate variability testing. Research within the past several decades has shown heart rate variability (HRV) to be a valid and reliable tool that can be used to determine the basic state of an individual’s autonomic health. A research study on campus is currently attempting to scientifically establish the frequency of slow breathing practice necessary to produce a therapeutic effect on HRV over the course of a four-week period.

The reason you are receiving this email is to ask if you would participate in a research study at Old Dominion University on heart rate variability and slow breathing training. If you participate, you could help determine the frequency of breathing practice necessary to maintain better health for individuals.

To participate, be 18 – 50 years old and pass a simple health screening questionnaire. The testing requires an initial visit and a personalized assignment for a four-week period that can be practiced at home. At the end of four weeks, another lab visit will be scheduled to repeat the testing measures for comparison. All visits may be scheduled at your convenience.

Please contact Jennifer Brown at jbrow126@odu.edu, or call 757-472-2316, if you have any question or you are interested in participating.

Thank you for your time,
Jennifer Brown
APPENDIX C
INFORMED CONSENT DOCUMENT
OLD DOMINION UNIVERSITY

PROJECT TITLE: Optimizing chronic slow breathing training to cause a therapeutic effect on heart rate variability

INTRODUCTION
The purposes of this form are to give you information that may affect your decision whether to say YES or NO to participation in this research, and to record the consent of those who say YES. The research project will take place in the Human Performance Laboratory (SRC 2003) at Old Dominion University.

RESEARCHERS
David P. Swain, PhD, Responsible Project Investigator
Jennifer Brown
Carmine Grieco, PhD
Samantha Vowell

DESCRIPTION OF RESEARCH STUDY
Heart rate and blood pressure are controlled by the nervous system. The practice of slow breathing on a regular basis may affect this.

If you agree to participate in this study, you will be scheduled at a convenient time at the Human Performance Laboratory for testing. On the day of testing you will be asked to refrain from exercising, eating or consuming any caffeine containing products for three hours prior to the testing session. Upon arrival you will be fitted with a chest strap that will measure heart rate. This device is very similar to a standard heart rate monitor, but gives more detailed information about your heart. You will also be fitted with a cuff on your arm for the measurement of blood pressure. You will lie on your back and asked to remain quiet for 10 minutes. During this time, you will be asked to breathe slowly, in time with an audio recording. After this, your blood pressure will be measured.

Within a short time you will be contacted and assigned to one of four groups. If you are in a breathing practice group, you will be asked to repeat the breathing exercise similarly to the testing session, but on your own, for 30 minutes each time. Written instructions will be provided. Depending on your group, you will do this 2, 3 or 5 times per week over the next 4 weeks. (If 2 times per week, perform the sessions approximately three days apart; if 3 times per week, perform the sessions approximately every other day; if 5 times per week, any 5 days of your choosing per week is appropriate.) You will be asked to keep a log to record each time you do the practice, and the time of day when you do it. Reminders will be sent out via email to help you remember to stay on track during the four-week time period. If you are assigned to the control
group, you will not perform any breathing practice over the next 4 weeks. If you have any questions during the study, an investigator will be available to answer your concerns. We ask you to continue your normal lifestyle (sleeping, eating and exercise habits) over the course of the study. You will be asked to return after four weeks at a convenient time to repeat the initial testing procedures.

Approximately 30 people between the ages of 18 and 50 years, who are without any serious health limitations will be participating in this study. Your total time commitment will depend on your group assignment, and will range from 2 to 12 hours over the entire study period.

EXCLUSIONARY CRITERIA
You should have completed a health screening questionnaire to determine if you are eligible for the study. You must be between the ages of 18 and 50 years. You should not participate if you have heart disease, pulmonary disease, diabetes, take medications that affect heart rate (such as beta-blockers), use tobacco products, or think you may be pregnant.

RISKS AND BENEFITS
RISKS: There are no known risks associated with this study. However, as with any research, there is some possibility that you may be subject to risks that have not yet been identified

BENEFITS: There is no guarantee that you will personally benefit from taking part in this study. It is possible you may benefit by learning your blood pressure.

COSTS AND PAYMENTS
You will not receive any payment for your time and participation in this study. If you are a student in a participating Exercise Science course, you will receive 2 or 5 extra credit points (2 for control subjects, 5 for intervention subjects) for completing the study. You do not have to participate in this study, or any study, to obtain this credit. An alternative means of obtaining this credit will be made available to students in participating courses.

NEW INFORMATION
If the researchers find new information during this study that would reasonably change your decision about participating, then they will give it to you.

CONFIDENTIALITY
Information collected about you will be kept confidential by the researchers. The results of this study may be used in reports, presentations, and publications; but the researcher will not identify you.

WITHDRAWAL PRIVILEGE
It is OK for you to say NO. Even if you say YES now, you are free to say NO later, and walk away or withdraw from the study -- at any time. Your decision will not affect your relationship with Old Dominion University or otherwise cause a loss of benefits to which you might otherwise be entitled. The researchers reserve the right to withdraw your participation in this study, at any time, if they observe potential problems with your continued participation.
COMPENSATION FOR ILLNESS AND INJURY
If you say YES, then your consent in this document does not waive any of your legal rights. However, in the event of injury or illness arising from this study, neither Old Dominion University nor the researchers are able to give you any money, insurance coverage, free medical care, or any other compensation for such injury or illness. In the event that you suffer injury or illness as a result of participation in this research project, you may contact Dr. George Maihafer, the chair of the Institutional Review Board, at 757-683-4520, or the Office of Research, at 757-683-3460, who will be glad to review the matter with you.

VOLUNTARY CONSENT
By signing this form, you are saying several things. You are saying that you have read this form or have had it read to you, that you are satisfied that you understand this form, the research study, and its risks and benefits. The researchers should have answered any questions you may have had about the research. If you have any questions later on, then the researchers should be able to answer them:
Dr. David Swain, 757-683-6028
Ms. Jennifer Brown, 757-472-2316

If at any time you feel pressured to participate, or if you have any questions about your rights or this form, then you should call Dr. George Maihafer at 757-683-4520 or the Old Dominion University Office of Research, at 757-683-3460.

And importantly, by signing below, you are telling the researcher YES, that you agree to participate in this study. The researcher should give you a copy of this form for your records.

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<th>Subject's Printed Name &amp; Signature</th>
<th>Date</th>
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INVESTIGATOR'S STATEMENT
I certify that I have explained to this subject the nature and purpose of this research, including benefits, risks, costs, and any experimental procedures. I have described the rights and protections afforded to human subjects and have done nothing to pressure, coerce, or falsely entice this subject into participating. I am aware of my obligations under state and federal laws, and promise compliance. I have answered the subject's questions and have encouraged him/her to ask additional questions at any time during the course of this study. I have witnessed the above signature(s) on this consent form.

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<th>Investigator's Printed Name &amp; Signature</th>
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APPENDIX D

HOME INSTRUCTIONS FOR SLOW PACED BREATHING

1. Log the start time of your breathing session and the date.
2. Turn off all distractions such as televisions, phone ringers and find a quiet place that you can relax and concentrate.
3. You should be seated in a comfortable chair with a timer, stopwatch, clock or other device nearby to help assist in keeping time.
4. Relaxing the upper body and keeping an open airway, breathe normally (spontaneously) for 1 - 2 minutes, and then begin breathing at a rate of 6 breaths per minutes (bpm) for 30 minutes, with each inhale lasting 4 seconds (approximately) and each exhale lasting 6 seconds (approximately).
5. It is strongly suggested that you utilize a stopwatch such as one from a touch screen phone to help you keep your pace. Another option is to watch a second hand on a clock until you are able to more adequately know you are “on time”.
6. You may close your eyes and concentrate on the breathing pattern if you wish. Check in every few minutes on your breathing to see if you are breathing at the correct rate and adjust as necessary.
7. Be sure to log the ending time of your breathing practice when you are done and provide your initials for that session. If you have anything unusual that interferes, be sure to note it on the log (e.g., sinus problems, too cold in the room, interruptions) so that you can try to correct it if possible before the next slow paced breathing training session.

**If you have any concerns or questions, please do not hesitate to contact Jennifer Brown at 472-2316 or at jbrow126@odu.edu. Thank you so much for your time and effort in this research!**
APPENDIX E

Breathing Training Journal For: __________________________
If you have questions, Contact Jennifer Brown at jbrew12@odu.edu or 757-472-2316

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VITA

JENNIFER JOY BROWN
HUMAN MOVEMENT SCIENCES
2016 RECREATION CENTER, NORFOLK, VA 23529
CELL PHONE: (757) 472-2316, EMAIL: JBROW126@ODU.EDU

EDUCATION
2010 – Present – Old Dominion University, Norfolk, VA
  • Masters Studies in Higher Education toward MSEd, expected completion December 2012
2010 – Old Dominion University
  • Bachelor of Science in Exercise Science, May 2010, Old Dominion University

PROFESSIONAL DEVELOPMENT
2008 – Present – TOP Certification and Blackboard Trainings to Teach Online for VCCS
2006 – Present – Basic and Advanced Yoga Certifications, YogaFit
2004 – Present – Mat Pilates Certification, AAAI/ISMA
2003 – Present – Group Fitness Certification, IFTA
2002 – Present – Kickboxing Certification, IFTA
2001 – Present – Personal Trainer Certification, ACE

WORK EXPERIENCE
2005 – Present, Thomas Nelson Community College, Hampton, VA
Adjunct Faculty
  Responsible for teaching and the development of Physical Education courses including
  those listed below and: PED 107: Nutrition and Exercise, PED 111: Weight Training,
  and PED 154: Volleyball.

2005 – Present, Tidewater Community College, Portsmouth & Chesapeake, VA
Adjunct Faculty
  Responsible for teaching and the development of Physical Education courses including:
  PED 100: Pilates, PED 101: Aerobic Dance, PED 109: Yoga, PED 103: Aerobics,
  PED 206: Sports Appreciation, and PED 116: Lifetime Fitness and Wellness.
  Additional responsibilities have included the development and delivery of Distance
  Education for PED 116, and PED 206.

2002 – Present, Hampton Roads Fitness,
Owner and Operator
  Developed on location personal training and post-rehabilitation company

  Manager of Elite Health and Fitness, a fitness center specifically developed for
  physicians, medical staff and post rehabilitative patients. Developed land and water
  based interactive training programs for post-rehab patients; Marketing director for
  physical therapy practice and fitness center.