Effects of inspiratory muscle training on heart rate variability

Anuja Choudhari
Western Washington University

Follow this and additional works at: https://cedar.wwu.edu/wwuet

Part of the Kinesiology Commons

Recommended Citation
Choudhari, Anuja, "Effects of inspiratory muscle training on heart rate variability" (2010). WWU Graduate School Collection. 89.
https://cedar.wwu.edu/wwuet/89

This Masters Thesis is brought to you for free and open access by the WWU Graduate and Undergraduate Scholarship at Western CEDAR. It has been accepted for inclusion in WWU Graduate School Collection by an authorized administrator of Western CEDAR. For more information, please contact westerncedar@wwu.edu.
EFFECTS OF INSPIRATORY MUSCLE TRAINING ON HEART RATE VARIABILITY

By

Anuja Choudhari

Accepted in Partial Completion

Of the Requirements for the Degree

Master of Science

Moheb A. Ghali, Dean of the Graduate School

ADVISORY COMMITTEE

Chair, Dr. Lorraine Brilla

Dr. Kathleen Knutzen

Dr. Brandi Row
MASTER’S THESIS

In presenting this thesis in partial fulfillment of the requirements of a master’s degree at Western Washington University, I grant to Western Washington University the non-executive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms including electronic format via any digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe any rights of others. I warrant that I have obtained written permission from the owner of any third party copyrighted materials included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, research and non-commercial reproduction of this work for educational purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without any written permission.

Anuja Choudhari

November 2, 2010
EFFECTS OF INSPIRATORY MUSCLE TRAINING ON HEART RATE VARIABILITY

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Anuja Choudhari
November 2010
Abstract

The purpose of this investigation was to evaluate the effect of six weeks of inspiratory muscle training (IMT) on heart rate variability (HRV) in young and older adults. Twelve young (age 22.25 ± 2.13 years) and 14 older adults (age 72.5 ± 6.44 years) participated in the study. The training protocol included using IMT device set at a resistance equivalent to 80% of maximal inspiratory pressure (MIP), five days per week. HRV data were obtained by electrocardiogram prior to and following six weeks of IMT, along with pulmonary function measures (vital capacity, forced expiratory volume in one second, maximal ventilatory volume). The HRV variables included total power (TP), very low frequency (VLF), low frequency (LF), and high frequency (HF) powers. In addition, normalized LF (LFnu), normalized HF (HFnu) and LF to HF ratio (LF/HF) were analyzed. The results from the young group demonstrated a significant increase in HFnu (pre 0.22 ± 0.039, post 0.243 ± 0.059 nu, p < 0.05), and a significant decrease in LFnu (pre 0.779 ± 0.039, post 0.756 ± 0.059 nu, p < 0.05) and LF/HF (pre 3.651 ± 0.756, post 3.278 ± 0.787, p < 0.05). In the older group, HFnu decreased significantly (pre 0.236 ± 0.076, post 0.205 ± 0.03 nu, p < 0.05) along with significant increases in LFnu (0.763 ± 0.076, post 0.794 ± 0.03 nu, p < 0.05) and LF/HF (pre 3.551 ± 1.08, post 3.94 ± 0.574, p < 0.05). No significant change was observed in any of TP, LF or HF. Both the groups demonstrated a significant increase in inspiratory muscle strength as determined by MIP (Young: pre 1.06 ± 0.33, post 1.68 ± 0.38; Older: pre 0.72 ± 0.24 cm, post 0.98 ± 0.14 cmH2O) (p < 0.05) and the pulmonary function measures. The results of this study demonstrated that IMT had a training effect on not only inspiratory muscle strength but also HRV. However, the training response varied within groups. The young group demonstrated a shift in autonomic balance towards parasympathetic modulation, while the older group demonstrated decrease in parasympathetic modulation.
Acknowledgements

I want to thank Dr. Brilla for her constant support and guidance throughout all the phases of this study. She always encouraged me to explore further and motivated me to strive for more. She always answered all my questions and doubts with time and patience. I also want to thank Dr. Kathy Knutzen for her unwavering support and encouragement throughout this study and the graduate program. She has put forth a perfect example of positivity and hard work I always aspired to. I want to express my gratitude to Dr. Brandi Row for being very supportive and motivating. Her feedbacks have always helped me to become a critical reader, a better analyst and a better writer. My special thanks to Teresa Hahn who has been a great help in the recruitment and training of young subjects of this study, and Cody Bludorn for his help in the training sessions. I would like to acknowledge all volunteers who enthusiastically participated in this study. I thank the unyielding support of friends and family, and Kushal for his endless support, encouragement and understanding.
# Table of Contents

Abstract……………………………………………………………………………………………… iv
Acknowledgements…………………………………………………………………………….. v
List of List of Tables and Figures……………………………………………………………... viii
List of Appendices…………………………………………………………………………….. ix

**Chapter I: The problem and its scope**

- Introduction………………………………………………………………………………….. 1
- Purpose of the Study……………………………………………………………………….. 2
- Hypotheses………………………………………………………………………………..... 3
- Significance of the Study……………………………………………………………….... 3
- Limitations of the Study…………………………………………………………………… 3
- Definition of Terms………………………………………………………………………. 4

**Chapter II: Review of Literature**

- Introduction………………………………………………………………………………….. 8
- Physiology of Heart Rate Variability………………………………………………….. 8
- Measurement of Heart Rate Variability…………………………………………… 11
- Heart Rate Variability in Clinical Conditions……………………………………….. 14
- Physiological influences on HRV……………………………………………………. 15
  - Age……………………………………………………………………………………… 15
  - Short term stability of HRV………………………………………………………….. 18
  - Aerobic exercise training……………………………………………………………. 19
  - Resistance training………………………………………………………………….. 23
- Modulatory Effect of Respiration on HRV…………………………………………... 24
  - Genesis of respiratory sinus arrhythmia………………………………………… 25
  - Effect of respiration rate and pattern on HRV………………………………….. 26
- Inspiratory Muscle Training …………………………………………………………… 29
  - Inspiratory muscle training and autonomous nervous system………………… 33
- Summary………………………………………………………………………………….. 37
Chapter III: Methods and Procedures

Introduction .................................................................................................................. 39
Description of the Study Population ....................................................................... 39
Design of the Study ..................................................................................................... 40
Data collection Procedures ....................................................................................... 40
  Instrumentation and measurement technique for pulmonary function ........ 41
  Instrumentation for HRV measurement ................................................................. 42
  Measurement techniques and procedures for HRV .............................................. 42
  Data processing ....................................................................................................... 43
  Training procedures ............................................................................................... 44
Data Analysis ............................................................................................................. 45

Chapter IV: Results and Discussion

Introduction .................................................................................................................. 46
Subject Characteristics ............................................................................................. 46
Results ......................................................................................................................... 47
  Heart rate variability ............................................................................................... 47
  Pulmonary function measures and MIP ................................................................. 51
Discussion of results .................................................................................................. 55
  Limitations ................................................................................................................ 59
Summary of results ..................................................................................................... 60

Chapter V: Summary, Conclusions, and Recommendations

Summary ....................................................................................................................... 62
Conclusions .................................................................................................................. 62
Recommendations ....................................................................................................... 63
References .................................................................................................................... 65
List of Tables and Figures

Table 1. Physical characteristics of subjects…………………………………………. 47
Table 2. Descriptive data of HRV……………………………………………………. 48
Table 3. Descriptive data of pulmonary function measures and MIP……………… 51
Figure 1. Mean LFnu in younger and older groups…………………………………… 49
Figure 2. Mean HFnu in younger and older groups…………………………………… 50
Figure 3. Mean LF/HF in younger and older groups………………………………… 50
Figure 4. Mean vital capacity in younger and older groups………………………… 52
Figure 5. Mean FEV1 in younger and older groups…………………………………… 53
Figure 6. Mean MVV in younger and older groups…………………………………… 53
Figure 7. Mean MIP in younger and older adults……………………………………… 54
List of Appendices

Appendix A

Informed consent ................................................................. 84
Human Subjects Activity Review Form ....................................... 86

Appendix B

Medical background ............................................................... 93
Physical Activity log ............................................................... 95
Data collection sheet .............................................................. 96
Training record ................................................................. 97

Appendix C

PowerBreathe Force Chart ....................................................... 99

Appendix D

Descriptive subject characteristics ......................................... 101
Heart rate variability data included in statistical analysis ............. 102
Pulmonary function measures and MIP data .......................... 104
Chapter I

The Problem and Its Scope

Introduction

It has been well established that heart rate (HR), measured as the time between successive heart contractions (R-R intervals), normally fluctuates at rest (Pagani et al., 1986). These variations over time between consecutive heart beats are quantified by heart rate variability (Rajendra, Paul, Kannathal, Lim, & Suri, 2006). The Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Malik et al., 1996) defined heart rate variability (HRV) as the oscillation in the interval between consecutive heart beats as well as oscillation between consecutive instantaneous heart rates.

HRV is a simple and powerful non-invasive tool to investigate autonomic nervous system (ANS) activity and cardiac autonomic control in particular (Akselrod et al., 1981; Montano, Cogliati, Dias da Silva, Gnicchi-Ruscone, & Malliani, 2001; Pomeranz et al., 1985; Rajendra et al., 2006). It reflects the interaction of the sympathetic and parasympathetic nervous systems to control heart rate (Akselrod et al., 1981; Pagani et al., 1986). Thus, HRV is widely used to investigate sympathetic-parasympathetic autonomic balance. HRV is associated with pathogenesis in various clinical conditions, and analysis of heart rate variability is vital in surveillance of these conditions (van Ravenswaaij-Arts, Kollee, Hopman, Stoelinga, & van Geijn, 1993).

A reduced HRV has been identified as a strong predictor of risk related to adverse events in healthy individuals and patients with a large number of diseases. This reflects the vital role that ANS plays in maintaining health. High heart rate variability is a sign of good adaptability which implies a healthy individual with well functioning autonomic control.
mechanisms. An attempt to improve and possibly correct autonomic imbalance will substantially decrease mortality (Kleiger, Miller, Bigger, & Moss, 1987). There are several studies that have investigated the effects of external mitigating factors such as exercise, relaxation, meditation and breathing patterns, in an attempt to improve HRV. Respiration is a powerful modulator of heart rate variability, baroreflex and chemoreflex sensitivity (Bernardi, Porta, Gabutti, Spicuzza, & Sleight, 2001). Respiratory activity is also known to influence heart rate through both anatomical and physiological mechanisms. There are studies that have observed favorable effects of different breathing patterns and yoga exercises on HRV. It is reasonable to suggest that manipulation of respiration may prove to be helpful in the HRV profile derangement. Inspiratory muscle training (IMT), as its name suggests, is a form of resistance training for inspiratory muscles. IMT may provide an option to manipulate the respiratory function and the related physiological mechanisms affecting heart rate. It is proposed that IMT may also have an effect on cardiovascular regulation and therefore HRV.

**Purpose of the Study**

The purpose of this study was to determine the effect of six weeks of IMT on HRV variables in young (age 18 to 30 years) and older adults (age > 60 years). The HRV analysis included measurements by frequency domain method before and after the training program. The variables measured were total power (TP), high frequency power (HF), and low frequency power (LF). Additionally, LF and HF were expressed in normalized units as LFnu and HFnu which represent their values relative to total power. LF to HF ratio (LF/HF) was determined to evaluate sympathovagal balance. The study also investigated the differences between the two groups in their response to IMT in terms of the changes in HRV variables.
**Hypotheses**

The null hypothesis is that there will be no significant difference between pre- and post measures of HRV after six weeks of IMT training. Additionally, it is hypothesized that both the young and old groups will exhibit a similar response to training.

**Significance of the Study**

Most of literature on IMT is restricted to its application in improving respiratory dynamics in health and disease. Further, it has been shown to be effective in sports and performance. However, the studies that examined the effects of IMT on the autonomic nervous system are very limited. This study is relevant as it allows for further investigation into whether IMT can enhance HRV variables and thereby cardiac autonomic control. This study also explores differences, if any, in young and old adults in their training response to IMT in terms of autonomic regulation. IMT may provide an effective intervention to increase HRV.

**Limitations of the Study**

1. External validity may be affected by the population studied, as the results can only be applied to populations with similar characteristics.

2. The resistance applied to the inspiratory muscles when using the IMT apparatus cannot be adjusted to any exact value. Resistance on the IMT device can be adjusted; however there is no way of determining the precise load on the muscles.

3. Subject adherence is important to study. It was emphasized that subjects should attend all training sessions and subjects were dropped if they missed more than three sessions.

4. Individual subject’s respiration pattern and rate were not controlled during training.
5. The study subjects may have been involved in regular exercise or physical activity. To control for exercise effect, subjects were asked to keep same exercise routine without any major changes during the study duration.

6. Environmental factors are known to affect HRV. To minimize potential influence of such factors, the laboratory was kept at consistent room temperature and external noise was limited.

7. Any stress or emotional disturbance due to life events occurred during the time of study was not controllable. To minimize the effect of emotions and stress on HRV measures, a simple visual analog scale was used to ensure no change in stress level and emotional disturbance during ECG recording.

8. Instrumentation may have provided a source of error. A standardized protocol of measurement was followed for each subject. All measurements were completed by the same investigator.

**Definition of Terms**

Autonomic nervous system (ANS): centers, nuclei, tracts, ganglia, and nerves involved in the regulation of smooth muscle, cardiac muscle, and glandular secretions at the subconscious level (Martini & Nath, 2009).

Baroreflex: a reflexive change in cardiovascular activity that responds to changes in blood pressure by adjusting cardiac output and peripheral resistance (Martini & Nath, 2009).

Electrocardiogram (ECG): an ECG is the recording of the electrical activity generated by the cells of the heart that reaches the body surface (Wagner & Marriott, 2007).

Forced expiratory volume in one second (FEV₁): the volume of air exhaled during the first second of forced vital capacity expressed in liters (American Thoracic Society, 1994).
Heart rate (HR): a measure of cardiac activity usually expressed as the number of heart contractions per minute (Pagani et al., 1986).

Heart rate variability (HRV): oscillation in the interval between consecutive heart beats as well as oscillation between consecutive instantaneous heart rates (Malik et al., 1996).

High frequency power (HF): frequency domain measure of HRV which refers to power in the high frequency range of 0.15-0.4 Hz (Malik et al., 1996).

HFnu: HF power in normalized units which represent the relative values of HF component in proportion to the total power minus very low frequency (VLF) component (Malik et al., 1996).

Inspiratory muscle training (IMT): pressure threshold loading that requires user to overcome negative pressure, resulting in improved strength of inspiratory muscles (Caine, Waller, & Wilcox, 2003).

Low frequency power (LF): frequency domain measure of HRV which refers to power in the low frequency range of 0.04-0.15 Hz (Malik et al., 1996)

LFnu: LF power in normalized units which represent the relative values of LF component in proportion to the total power minus VLF component (Malik et al., 1996)

Maximal inspiratory pressure (MIP): Maximal inspiratory pressure is commonly used to measure inspiratory muscle strength. It reflects the force-generating ability of the combined inspiratory muscles during a brief quasi-static or isometric-like contraction (Volianitis, McConnell, & Jones, 2000). Also abbreviated as PImax, it is defined as the largest negative pressure averaged over 1 second during maximal inspiratory effort from residual volume (Larson et al., 1993). The residual volume is the amount of air left in lungs after maximal exhalation. Thus, the maximal inspiratory effort should be preceded by maximal exhalation.
Maximal voluntary ventilation (MVV): the largest volume that can be breathed into and out of the lungs with voluntary effort during a 10- to 15-s interval (Heliopoulos et al., 2003). To measure the MVV, the subject is required to breathe as deeply and rapidly as possible. The test is conducted for a specific interval, usually 10, 12, or 15 seconds and extrapolated to one minute (Heliopoulos et al., 2003).

Normal to Normal interval (N-N interval): intervals between adjacent normal QRS complexes resulting from sinus node depolarization (Malik et al., 1996). It is commonly used in the analysis of heart rate variability. Abnormal QRS complexes are indicated by morphological abnormalities in QRS complex on an ECG and are not often included in HRV analysis.

Parasympathetic nervous system (PNS): division of autonomic nervous system generally responsible for activities that conserve energy and lower metabolic rate. Parasympathetic stimulation slows down heart rate and slightly decreases heart muscle contractility (Martini & Nath, 2009).

R-R interval: the period between successive QRS complexes on an electrocardiogram.

Respiratory sinus arrhythmia: heart rate variability in synchrony with respiration, which is characterized by decrease in R-R interval during inspiration and increase during expiration (Yasuma & Hayano, 2004).

Sympathetic nervous system (SNS): division of autonomic nervous system primarily concerned with the elevation of metabolic rate. Sympathetic stimulation increases heart rate and contractility (Martini & Nath, 2009).

Sympathovagal balance: the balancing interaction between sympathetic and parasympathetic branches of the ANS.
Total power (TP): a marker of global HRV defined as variance of N-N intervals over the temporal segment in the frequency range of approximately $\leq 0.4$ Hz (Malik et al., 1996).

Vagus nerve: nerve of the parasympathetic nervous system whose motor components affect the heart and control smooth muscles and glands within areas monitored by its sensory nerves (Martini & Nath, 2009).

Very low frequency power (VLF): frequency domain measure of HRV which refers to power in the very low frequency range $\leq 0.04$ Hz (Malik et al., 1996).

Chapter II
Review of literature

Introduction

Heart rate variability (HRV) is a popular noninvasive tool to estimate cardiac autonomic modulation because it is easily measured without interfering with neural functions of the control mechanisms. Analysis of these fluctuations has been useful in providing information regarding the physiology of the active autonomic control branches (Akselrod et al., 1981; Pomeranz et al., 1985). The purpose of this chapter is to review literature pertinent to physiology behind HRV, various methods of measuring HRV and physiological influences on HRV. The first section describes the physiological mechanisms involved in heart rate variability. In second section, various methods to measure HRV are explained with more emphasis on frequency domain method. Various frequency domain variables are described with their physiological correlates. Many clinical conditions are associated with derangement of HRV profile indicating its importance in pathogenesis. The third section provides a brief about HRV derangement in various clinical conditions. HRV is influenced by several physiological and maturational factors. The fourth section restricts the discussion to the effects of aging and exercise on HRV. The modulatory effect of respiration is described separately in the fifth section. Inspiratory muscle training (IMT) is a promising way to improve breathing energetics at preventive as well as intervention level. The sixth section reviews the literature pertinent to IMT and describes a few studies that have evaluated the possible effect of IMT on autonomic regulation.

Physiology of Heart Rate Variability

The cardiovascular system is constantly challenged by a wide variety of stimuli. In order to respond to these challenges and achieve dynamic stability, the cardiovascular system
is tightly regulated by the autonomic nervous system (ANS). The ANS is comprised of control centers in the brain stem, spinal cord, hypothalamus, portions of the cerebral cortex, and by various reflex mechanisms that direct afferent signals to these control centers.

Efferent signals are transmitted throughout the body via either sympathetic or parasympathetic pathways (Lee, Wood & Welsch, 2003). The ANS reacts rapidly to a wide range of external and internal stimuli and maintain dynamic stability through control of heart rate (HR), blood pressure and several other factors. Heart rate, in particular, reacts in a complex manner to these stimuli. It exhibits autonomically mediated self organization through frequent small adjustments, expressed as heart rate variability. These fluctuations are observed even at rest. The exact mechanism behind HRV is not established. It is believed to be resulting from the interplay among several factors including respiratory movements, intrathoracic pressure changes, peripheral vascular resistance and renin-angiotensin system (Akselrod et al., 1981; Elghozi, Laude, & Girard, 1991). However, the autonomic nervous system and its component branches mediate the effect of these factors over heart rate.

The sinus node is densely innervated by both sympathetic and parasympathetic divisions. They modulate the intrinsic rate of pacemaker cells of the sinus node which is reflected in the heart rate (Pumprla, Howorka, Groves, Chester, & Nolan, 2002). The ANS has both cardiovascular stimulatory and inhibitory control centers in the higher brain centers. The cardioinhibitory center is found in nucleus ambiguous and dorsal nucleus of the vagus nerve (Lee et al., 2003). The vagal efferents from these sites that constitute parasympathetic supply to heart, act to decrease both heart rate and contractility. Conversely, a cardiostimulatory center believed to be located in the lateral medulla exerts its effect through
the sympathetic pathways (Lee et al., 2003). Thus, sympathetic activation increases heart rate and conduction velocity through cardiac conduction system together with an increase in contractility (Pumprla et al., 2002). The parasympathetic influence on HR is mediated by the vagus nerve via synaptic release of acetylcholine (ACh) which is rapidly hydrolyzed by acetylcholinesterase in the sinus node. Thus, the vagal effect is brief owing to the short latent period of ACh (Malik et al., 1996; Pumprla et al., 2002). The sympathetic activity is mediated by synaptic release of epinephrine and norepinephrine, which are reabsorbed and metabolized relatively slowly (Pumprla et al., 2002). These differences result in both of these autonomic branches operating at different frequencies and thresholds, as reflected in HR variations.

Modulation of the ANS is largely attributed to the presence of various autonomic reflexes. There are three major cardiovascular reflexes: the baroreflex, chemoreflex and cardiac sympathetic afferent reflex (CSAR) (Du & Chen, 2007). The stretch-sensitive baroreceptors are located in the aortic arch and carotid sinus. The baroreflex is sympathoinhibitory and thus a depressed arterial baroreflex will result in increased sympathetic activity. It is a primary peripheral regulator of sympathetic outflow (Du & Chen, 2007). The chemoreflex, composed of chemoreceptors and located in the internal carotid and aortic bodies as well as brain stem, is sympathoexcitatory in nature (Du & Chen, 2007). Thus, chemoreflex activation results in increased sympathetic activity. The third reflex, CSAR is also sympathoexcitatory, and consists of sympathetic sensory nerve endings in the cardiac chambers and thoracic aorta. It is sensitive to both chemical and mechanical stimulation. Thus, cardioregulation is likely to result from a complex interaction of central integration and peripheral inhibitory and excitatory ANS reflexes (Du & Chen, 2007). HRV
mirrors the dynamic response of these reflexes and cardiovascular control centers in order to maintain homeostasis.

**Measurement of Heart Rate Variability**

Heart rate variability (HRV) is also described by several other terms such as cycle length variability, heart period variability, R-R variability and R-R interval tachogram. HRV remains the most commonly used term even though HRV variables represent variations in intervals between the consecutive heart beats (R-R interval) rather than variation in HR (Malik et al., 1996). HRV is measured from electrocardiogram (ECG) recordings in two common settings. First, HRV is assessed from short-term (2 to 5 min) ECG recordings under controlled laboratory conditions. Secondly, HRV is determined from long term (24 hr) ECG recordings while subjects perform their usual tasks (Kleiger, Stein, & Bigger, 2005). In either condition, the data obtained are used to quantify HRV variables by analyzing it using add-on software or dedicated HRV analyzers. The Task Force of European Society of Cardiology and the North American Society of Pacing and Electrophysiology describes standards of methods to measure HRV. These methods are categorized into non-linear and linear methods.

The non-linear method attempts to quantify the structure or complexity of the R-R interval time series, while the linear methods quantify R-R interval on various time and frequency scales (Kleiger et al., 2005). The methods commonly used to measure non-linear properties of HRV include detrended fluctuation analysis, correlation function, Hurst exponent, fractal dimension and Lyapunov exponent (Vanderlei, Pastre, Hoshi, Carvalho, & Godoy, 2009). The linear methods include analysis in time and frequency domains, and the time domain methods are the simplest to perform (Malik et al., 1996; Stein & Kleiger, 1999).
In these methods, the intervals between adjacent normal complexes, called normal to normal interval (N-N interval), are measured over a period of time and various indices are derived by statistical or geometrical methods (Malik et al., 1996). HRV analysis in the frequency domain is mathematically more complex, but it is perhaps the most widely used technique.

In the frequency domain method, power spectral density (PSD) analysis yields information about the distribution of amount of variance (power) of periodic oscillations of heart rate as a function of frequency (Malik et al., 1996). PSD can be calculated by using non-parametric or parametric methods. The non-parametric method typically uses a simple algorithm, mostly Fast Fourier Transform-FFT, and is preferred over autoregression techniques used in the parametric method. PSD analysis of short term recording decomposes HRV into fundamental spectral components that are defined as follows: 1) Total power (TP), the variance of N-N intervals over the temporal segment in the frequency range of approximately ≤ 0.4 Hz; 2) High frequency component (HF), power in high frequency range of 0.15-0.4 Hz; 3) Low frequency component (LF), power in low frequency range of 0.04-0.15 Hz; 4) Very low frequency component (VLF), power in very low frequency range ≤ 0.04 Hz. Spectral analysis of long term analysis often includes an additional ultra low frequency component (ULF).

These spectral components are correlated to branches of ANS and their regulatory mechanism in terms of physiological origin and frequency range. The high frequency component (HF) is parasympathetically mediated (Akselrod et al., 1981; Pomeranz et al., 1985; Raeder et al., 1987). The studies that investigated the effect of muscarinic parasympathetic blockade with glycopyrrolate or atropine have demonstrated a decrease in HF power with parasympathetic withdrawal (Akselrod et al., 1981; Pomeranz et al., 1985;
Saul, 1990). Similarly, in instances of vagotomy, HF power was completely abolished (Raeder et al., 1987). High frequency power reflects respiratory modulation of R-R intervals which occurs around 0.25 Hz (respiratory sinus arrhythmia) (Yasuma & Hayano, 2004). Respiration acts as a primary modulator of vagal activity and thus plays a vital role in modulating HF power. The LF component is influenced by both sympathetic and parasympathetic nervous activity (Akselrod et al., 1981; Pomeranz et al., 1985; Raeder et al., 1987; Saul, 1990). When sympathetic activity was blocked and arterial pressure was increased, the parasympathetic activity was reflexively increased, and increase in LF power was observed (Akselrod et al., 1981). On the other hand, under the condition of parasympathetic blockage with an accompanying decrease in arterial pressure, sympathetic activity was reflexively increased, and LF power was increased (Akselrod et al., 1981). Thus, increasing the activity of either sympathetic or parasympathetic nervous system augmented the LF power. HRV in the low frequency band is primarily modulated by baroreflex response to blood pressure fluctuations mediated by a combination of sympathetic and parasympathetic drives (Bernardi et al., 1994; Pomeranz et al., 1985; Stein & Kleiger, 1999). The VLF component is far less defined and its physiological correlates are not well established. Changes in autonomic activity associated with the renin-angiotensin-aldosterone, thermoregulation and peripheral vasomotor systems are thought to be related to VLF (Taylor, Carr, Myers, & Eckberg, 1998). It is proposed that they depend primarily on the presence of parasympathetic outflow and diminish with parasympathetic block (Taylor et al., 1998).

Measurement of VLF, LF and HF power components is usually made in absolute values of power (ms$^2$). Because VLF cannot be measured accurately from short term
recordings, LF and HF are also reported in normalized values which represent the relative values of each power component in proportion to the total power minus VLF component. Normalization tends to control the effect of changes in total power on the values of HF and LF (Malik et al., 1996; Sandercock & Brodie, 2006). Another expression in the form of a ratio (LF/HF) provides efficient use of power spectral analysis of HRV to assess a shift in sympathovagal balance (Montano et al., 1994; Pagani et al., 1986). Increase in LF/HF indicates the progression of sympathetic excitation (increasing LF) over concomitant vagal withdrawal (increasing both HF & LF), and consequently a shift to sympathetic excitation (Montano et al., 1994; Pagani et al., 1986). Malliani, Pagani and Lombardi (1994) supported evaluation of LF and HF component in normalized units and evaluation of sympathovagal balance by LF/HF ratio in numerous physiologic and physiopathologic conditions.

**Heart Rate Variability in Clinical Conditions**

High heart rate variability is a sign of good adaptability which implies a healthy individual with well functioning autonomic control mechanisms. A reduced HRV (low TP, low HF) has been identified as a strong predictor of risk related to adverse events in healthy individuals and patients with a large number of diseases.

Deranged HRV variables have been associated with mortality related to several cardiovascular pathologies. In myocardial infarction and diabetic autonomic neuropathy, low or reduced values of HRV variables are strongly and independently associated with mortality and adverse prognosis (Kleiger, Miller, Bigger, & Moss, 1987; La Rovere, Bigger, Marcus, Mortara, & Schwartz, 1998; Steeds et al., 2004). Similarly, in patients of chronic heart failure (CHF), reduction in HRV variables is an independent predictor of high risk of death (Nolan et al., 1998; Ponikowski et al., 1997). It has been observed that HRV analysis has
better prognostic value than other conventional clinical measurements and risk factors evaluation (Nolan et al., 1998; Tsuji, et al., 1996; Tsuji et al., 1994). Low heart rate variability has been inferred as a risk factor for life threatening ventricular and sudden death in clinical conditions of chronic heart failure, cardiac arrest and diabetes (Dougherty & Burr, 1992; Galinier et al., 2000; Kataoka, Ito, Sasaki, Yamane, & Kohno, 2004; La Rovere et al., 2001). Several other significant clinical conditions that are associated with low HRV include hypertension, metabolic syndrome and chronic obstructive pulmonary disorder (COPD) (Liao et al., 1998; Pagani et al., 1996; Singh et al., 1998). This reflects the vital role that ANS plays in maintaining health. Any intervention to improve HRV (increase TP, HF, lower LF/HF ratio) thus has applications in several clinical conditions as well as health promotion.

**Physiological Influences on HRV**

As age advances, the cardiovascular and nervous systems go through changes. These physiological changes associated with maturation of both the systems affect heart rate variability. The cardiovascular system is readily influenced by transient external stimuli that can change the sympathetic-parasympathetic interaction. Mechanical, gravitational, emotional, acoustic or thermal stimulation are only a few amongst many possible stimuli. Exercise is one such potent stimulus that promotes long standing changes in HRV. This section reviews the influence of aging and the effect of both aerobic and resistance exercise training on heart rate variability.

**Age influences.** HRV changes with advancing age. HRV is high in gestational and postnatal life. Infants have a high sympathetic activity as indicated by high LF power. It decreases quickly between ages of five and 10 years as the parasympathetic system matures (Finley, Nugent, & Hellenbrand, 1987). In adolescence, the advanced cardiovascular
parasympathetic system predominates against a strong sympathetic system (Korkushko, Shatilo, Plachinda, & Shatilo, 1991). With advancing age, heart rate variability decreases gradually over years.

Schwartz, Gibb, & Tran (1991) studied HRV in 56 healthy subjects from age 20 to 81 years and observed total power, high frequency and low frequency components of HRV to decrease with aging. Shannon, Carley, & Benson (1987) investigated HRV in 33 healthy male subjects (aged 9 to 62 years) and observed a decline in total power and its LF and HF components with aging. Several other studies have observed similar decline in time and frequency domain HRV variables (Antelmi et al., 2004; Beckers, Verheyden, & Aubert, 2006; Melo et al., 2005; O'Brien, O'hare, & Corrall, 1986; Yeragani, Sobolewski, Kay, Jampala, & Igel, 1997; Zhang, 2007).

Yeragani et al. (1997) studied HRV in 33 healthy subjects aged from six to 61 years. HRV was obtained from a 24-hour Holter ECG and analysed by power spectral method. The adult group (35.4 ± 10.4 years) demonstrated significantly lower total power, HF, LF and higher LF/HF ratio (TP: 4.5 ± 0.4 bpm$^2$, LF: 1.8 ± 0.5 bpm$^2$, HF: 0.5 ± 0.8 bpm$^2$, LF/HF: 3.8 ± 1.7) in comparison with children group (11.1 ± 2.1 years) (TP: 4.8 ± 0.3 bpm$^2$, LF: 2.0 ± 0.4 bpm$^2$, HF: 1.3 ± 0.5 bpm$^2$, LF/HF: 2.3 ± 0.8). It was suggested that the increase in LF/HF probably reflects a steeper decline in HF power with age.

Antelmi and associates (2004) studied time and frequency domain HRV variables in 653 apparently healthy subjects. The HRV was analyzed in both time and frequency domains and compared within subjects categorized in six age strata (< 19, 20-29, 30-39, 40-49, 50-59, > 60 years). Comparison between age groups showed that all time domain variables (SDNN, SDANN, rMSSD, pNN50) and frequency domain variables (LF, HF, VLF)
decreased with age. HF, rMSSD, and pNN50 which are indexes of parasympathetic modulation decreased significantly until the fourth decade and decreased nonsignificantly in the older age groups. The LF/HF increased with age, with significant increase from the second to the fifth decade.

These observations are attributable to age related marked morphological and functional changes in cholinergic and adrenergic innervations of the heart. It is well known that baroreflex activity decreases with aging (Ebert, Morgan, Barney, Denahan, & Smith, 1992; Monahan, 2007). There is an impairment of the parasympathetic component while the sympathetic component can be well maintained (Ebert et al., 1992; Ferrari, Radaelli, & Centola, 2003; Monahan, 2007). With advancing age neurotransmitters are not as effective at the level of target organs (Lakatta, 1993). The endogenous effect of acetylcholine (Ach) is diminished with age. In addition, the number of cholinergic receptors (M2 receptors) and their response to acetylcholine decreases with age leading to decreases in cardiac parasympathetic activity (Brodde et al., 1998; Brodde & Leineweber, 2004; Poller, Nedelka, Radke, Pönicke, & Brodde, 1997). In adults, along with impairment of cholinergic system with aging, low HF power is attributable to attenuation of respiratory sinus arrhythmia or respiratory modulation of R-R interval (Pagani et al., 1996; Shannon et al., 1987). Similar age related reductions are also observed in adrenergic modulation with decrease in β-adrenergic receptor density and responsiveness (Brodde & Leineweber, 2004; Lakatta, 1993). Despite age related impairment in sympathetic tone, there appears a relative prevalence of sympathetic influences upon heart rate control against a background of more markedly decreased parasympathetic influences (Korkushko et al., 1991).
Short term stability of heart rate variability. It is clear that heart rate variability declines with aging. These changes occur slowly over the years. Heart rate variability is demonstrated to be stable over relatively short periods.

Kleiger et al. (1991) assessed the stability of HR variability over time in fourteen normal subjects aged 20 to 55 years. HRV was analyzed from 24-hour ambulatory electrocardiograms performed three and 65 days apart. The HRV variables analyzed included both the time domain (SDNN, rMSSD, rNN50) and the frequency domain (LF, HF, TP) measures. The mean and standard deviations of these measures were virtually identical within the time range studied.

Another study by Tarkiainen et al. (2005) assessed the stability of short term HRV over a three to four month period in 89 subjects with stable coronary artery disease (age 40-83 years). The HRV assessments were performed from a 40-minute ECG recordings consisting of five minute periods of rest, paced breathing and standing, a six minute submaximal exercise test and a 10-minute period of recovery. The coefficient of variation (CV) varied between 5.1–16.7% for the 40-minute and 6.0–37.1% for the 5-minute time domain variables and 4.4–11.0 % for the 40-minute and 7.2–16.5 % for the 5-minute frequency domain measurements. The mean of the RR intervals and the total power showed the highest stability over time.

Heart rate variability does not vary much and remains stable unless there are present any physiological influences like exercise training, breathing exercises or similar interventions. The following sections elaborate on the effects of such physiological influences.
**Aerobic exercise training.** There are many studies supporting that HRV is affected by exercise training and physical activity status. Cross-sectional differences between trained/athletes and recreationally active/sedentary individuals support the notion of increased parasympathetic and/or decreased sympathetic drive with physical activity or training status. The cross sectional study by Mølgaard, Sørensen, & Bjerringard (1991) evaluated 24-hour holter monitoring in 140 apparently healthy subjects aged 40 to 77 years. The subjects were classified as active, intermediate and passive, according to their participation in leisure time physical activity. It was observed that high physical activity (in active subjects) was independently associated with significantly higher values of mean R-R interval, standard deviation of R-R intervals (SD) and percentage of successive R-R interval differences.

When assessed during the resting condition, active young and older men had lower heart rates and higher values of HRV variables in both time and frequency domains when compared to sedentary men (Melo et al., 2005). The young active group was significantly different from young sedentary group in values of R-R interval (active 1034 ± 100.3 ms, sedentary 874 ± 123.8 ms), RMSSD index (active 63 ± 19.9, sedentary 34 ± 14.6), LFnu (active 27 ± 12.2 NU, sedentary 35 ± 16.9 NU), HFnu (active 72 ± 12.2 NU, sedentary 65 ± 16.9 NU), LF/HF (active 0.41 ± 0.3, sedentary 0.65 ± 0.6). Similarly, smaller sympathetic driven variables and greater parasympathetic driven variables were observed in the old active group when compared to the old sedentary group, reported as RR interval (active 1109 ± 186.3 ms, sedentary 893 ± 93.8 ms), RMSSD index (active 48 ± 22.4, sedentary 27 ± 9.2), LFnu (active 43 ± 9.9 NU, sedentary 46 ± 7.4 NU), HFnu (active 56 ± 9.9 NU, sedentary 54 ± 7.4 NU), LF/HF (active 0.81 ± 0.3, sedentary 0.87 ± 0.2). In both active
young and older men, LF/HF was lower than that in sedentary adults indicating higher parasympathetic activity associated with physical activity status. It can also be observed that the active and sedentary older men demonstrated lower HF power and higher LF power, and thus greater LF/HF compared to younger men.

Rennie et al. (2003) in their cross-sectional study investigated HRV in 3328 apparently healthy civil servants (age 45-68 years). Leisure time physical activity was categorized as moderate (≥3 to <5 metabolic equivalent hours per week) and vigorous (≥5 metabolic equivalent hours per week). It was observed that lower heart rates and higher HF power were associated with moderate activity ($p < 0.05$) and vigorous activity ($p < 0.01$).

When parasympathetic activity was assessed by 24 hour recordings in endurance trained and untrained young men, HF power was significantly greater in endurance trained men (during the day: 852 versus 177 ms$^2$, $p < 0.005$; during the night: 1,874 versus 427 ms$^2$, $p < 0.005$ and over the entire 24 hour 1,165 versus 276 ms$^2$, $p < 0.001$) (Goldsmith, Bigger, Steinman, & Fleiss, 1992). This indicates that parasympathetic activity is substantially greater in trained men.

Similarly, the power spectrum of HRV measured in physiological conditions of 45 minute resting, 10 min standing, and 15 min steady state exercise at 50% maximum workload was assessed in endurance athletes and sedentary controls (Dixon, Kamath, McCartney, & Fallen, 1992). Though there was no significant difference in response to standing and exercise induced changes in HRV, in the resting condition the LF peak power was significantly reduced ($p < 0.05$) in athletes (54 ±9.9 beats.min$^{-1}$Hz$^{-1}$) when compared to controls (69.6 ±19.5 beats min$^{-1}$ Hz$^{-1}$). The endurance athletes had higher HF peak (62.2 ± 10.7 beats min$^{-1}$ Hz$^{-1}$) than controls (43.7 ± 22.4 beats min$^{-1}$ Hz$^{-1}$), while the area contained
within HF band was greater \( p < 0.01 \) in athletes \( (54 \pm 4.4 \%) \) versus controls \( (43.3 \pm 11.4\%) \). This association of higher HRV (HF) with training status suggests that HRV may be improved with training.

The observations from the studies that have investigated the effects of exercise training on HRV variables support this notion. Results from these studies imply the modification of HRV with training by means of increase in global HRV and variables related to parasympathetic component. However, contradictions exist about the adequate volume of training necessary to achieve significant beneficial results. Exercise training of variable duration and intensity influences heart rate variability.

Sandercock, Grocott-Mason, and Brodie (2007) examined changes in HRV measures after eight weeks of cardiac rehabilitation in 38 cardiac patients. The training included aerobic training at an intensity of 70 % of maximum heart rate. HRV was analyzed from five minute ECG before and after training. The results demonstrated significant increases in SDNN (pre 29 ± 15 ms, post 35 ± 17 ms, \( p < 0.015 \)) and RMSSD (pre 26 ± 14, post 33 ± 16, \( p < 0.095 \)). The HF and LF measured in log units, increased significantly (HF: pre 3.8 ± 1.0, post 4.3 ± 0.3, \( p < 0.024 \); LF: pre 2.4 ± 1.2, post 3.0 ± 2.4, \( p < 0.014 \)). These results suggest increased parasympathetic modulation of heart rate with aerobic training.

Tulppo et al. (2003) evaluated the effect of eight weeks of moderate (30 min exercise sessions) and high (60-min exercise sessions) volume at an intensity of 70-80% of maximum HR. The subject population consisted of twenty healthy sedentary males in each of the training groups and fifteen subjects in the control (no training) group. The normalized HF increased \( p < 0.01 \) and LF/HF decreased \( p < 0.05 \) in both training groups, while the HRV
in control group remained unchanged. The high volume training had no significantly greater result compared to moderate volume aerobic training.

Similarly, Carter, Banister, & Blaber (2003) investigated the effect of a 12-week training program in young (age 19-21 years) and middle aged runners (age 40-45 years). The training included 45-60 min of running at an intensity of 70-90% of subjects’s maximum heart rate. The young group demonstrated an increase in TP (pre 1161.82 + 59.85 ms², post 2010.82 + 59.85 ms², p < 0.001) and HF (pre 362.26 ± 82.61 ms², post 926.68 ± 82.61 ms², p < 0.01) The middle aged group also increased TP (pre 528.94 + 59.85 ms², post 727.24 + 59.85 ms², p < 0.05) and HF (pre 49.18 +82.61 ms², post 168.19 + 82.61 ms², p < 0.01). The aerobic exercise influenced HRV in both age groups. Younger individuals experienced higher total power and high-frequency power prior to and following training program; however, older individuals experienced a greater overall training response (Carter et al., 2003).

Melanson and Freedson (2001) investigated the effect of a 16-week aerobic training in previously sedentary adult males and found significant increases in pNN50, rSMMD, and HF. The most important finding from this study was that significant changes in HRV were apparent after only 12 weeks of training at 70-80 % of heart rate reserve. Another study (Killavuori, Toivonen, Naveri, & Leinonen, 1995) reported a significant increase in HF power by 22-25% after three months of training at a workload corresponding to 50-60% of peak oxygen consumption.

A cross-sectional study (Buchheit, Simon, Piquard, Ehrhart, & Brandenberger, 2004) analyzed HRV in young subjects divided in three groups according to their weekly training load (sedentary subjects (< 2 hr/wk), moderately trained (4-6 hr/wk) and highly trained (> 18
h/wk)). Except for the lower heart rate and higher SDNN, RMSSD and HF during slow wave sleep, highly trained subjects did not show any significantly greater differences during waking periods in the aforementioned variables as compared to the moderately trained subjects.

Thus, it is common observation that exercise training using moderate to high intensity lasting about eight to twelve weeks elicited significant changes in HRV. Increase in HRV following aerobic training has been documented over a range of age groups. In the study by Levy et al. (1998), standard deviation of R-R interval at rest was increased by 68% in older (age 60 to 82 years) men and 17% in young (age 24 to 32 year) in response to six months of training. It is important to note that the older individuals experience a greater overall training response (Carter et al., 2003). The observation is applicable to both genders.

**Resistance exercise training.** The effect of resistance training on HRV has been explored in the literature to a much lesser extent than aerobic training. The results are inconsistent among studies and many studies were unable to show any effect of resistance training on HRV. Multi week resistance training studies have examined the effects of programs ranging from four weeks to six months duration with the resistance levels varying from 60 - 85% of one repetition maximum. The training mode most commonly included isotonic weight training, weight machines, isometric dynamometer and leg ergometry (Collier et al., 2009; Figueroa, Kingsley, McMillan, & Panton, 2008; Selig et al., 2004).

Collier et al. (2009) evaluated the effect of a four week resistance training program in individuals with mild hypertension. The training program included three sessions per week with three sets of ten repetitions of various resistance exercises. The log-transformed heart rate variability measure did not demonstrate an significant improvement with resistance
training (TP: pre $7.45 \pm 0.32 \text{ ms}^2$, post $7.46 \pm 0.34 \text{ ms}^2$; HF: pre $5.98 \pm 0.28 \text{ ms}^2$, post $5.76 \pm 0.42 \text{ ms}^2$; LF: pre $6.1 \pm 0.35 \text{ ms}^2$, post $6.0 \pm 0.39 \text{ ms}^2$). Another study by Sloan et al. (2009) examined the effect of 12-week resistance training using similar protocol in healthy sedentary young adults and reported no improvement in any of HRV variables assessed.

Conversely, prolonged resistance training has resulted in favorable changes in HRV (Figueroa et al., 2008; Selig et al., 2004). Figueroa and associates (2008) investigated the effect of 16 weeks of resistance training in women with fibromyalgia. After resistance training, there were significant increases in total power, square root of standard deviation of R-R interval and high frequency power. Similar increases in HF power were observed in patients with chronic heart failure after 3 months of resistance training (Selig et al., 2004).

Though the results have been inconsistent, it is important to note that HRV is affected by training, aerobic or resistance training. The studies reviewed further indicated that HRV is influenced by not only intrinsic factors such as age or disease status, but also external factors like training influence the parasympathetic and sympathetic modulation of heart rate. The other observation from these studies is that HRV does not change significantly over short duration, unless there is any exercise intervention (Buchheit et al., 2004; Melanson & Freedson, 2001; Sandercock, Grocott-Mason, & Brodie, 2007; Tulppo et al., 2003).

**Modulatory Effect of Respiration on HRV**

Respiration is the most consistent and important external modulator of heart rate variability. The circulatory and respiratory systems are interconnected anatomically and physiologically. Respiratory sinus arrhythmia (RSA) is one of the physiological interactions between respiration and cardiovascular system. This section explores on how respiration contributes to genesis of heart rate variability, majorly RSA. The heart rate changes with
changes in respiration rate and pattern. This section also elaborates on how respiration rate and pattern affect heart rate variability.

**Genesis of respiratory sinus arrhythmia.** Respiratory sinus arrhythmia (RSA) is heart rate variability in synchrony with respiration, characterized by the R-R interval on an ECG shortening during inspiration and increasing during expiration (Yasuma & Hayano, 2004). RSA primarily contributes to the high frequency component of HRV that occurs at respiratory frequency > 0.25 Hz (Bernardi et al., 1989; Kleiger et al., 2005; Pumprla et al., 2002; Saul, 1990; Stein & Kleiger, 1999). The origin of RSA is debatable. Among several factors suggested to be involved in genesis of RSA are the baroreflex mediated responses to arterial pressure fluctuations, modulation of vagal-cardiac motonurone firing by phasic afferent pulmonary and thoracic stretch receptor inputs, and phasic mechanical stretch of sinus node (Bernardi et al., 2001; Koh, Brown, Beightol, & Eckberg, 1998).

The most prominent mechanism behind RSA is the arterial baroreceptor response (Bernardi et al., 2001). The baroreflex is stimulated by inspiratory increase in venous return with subsequent changes in arterial pressure. Thus, the influence of breathing on autonomic activity may be secondary to respiratory related fluctuations on arterial baroreceptor activity (Davidson, Goldner, & McCloskey, 1976; Davis, McCloskey, & Potter, 1977; Elghozi et al., 1991). It is also demonstrated in the study by Piepoli et al. (1997) where RSA was restored during apnea by stimulating carotid baroreceptors by cycled neck suction at the frequency of the previous respiration. Conversely, RSA was considerably suppressed by neck suction appropriately phased so as to smooth out respiration related blood pressure changes. However, the strong correlation between systolic pressure and R–R intervals at respiratory
frequencies reflects the influence of respiration on these two measures, rather than arterial baroreflex physiology (Badra et al., 2001; Eckberg, 2003).

Koh et al. (1998) demonstrated the effect of mechanical lung inflation on R-R interval and arterial pressure fluctuations in nine healthy young adults undergoing elective orthopedic surgery. Following sedation and induced muscle paralysis, the subjects were ventilated with intermittent positive pressure ventilation or high frequency jet ventilation. The R-R interval spectral power (index of RSA) declined dramatically with sedation and muscle paralysis, but was greater during mechanical ventilation. It was suggested that phasic inputs from pulmonary and thoracic stretch receptors make small but statistically significant contribution to respiratory sinus arrhythmia. Bernardi et al. (1989) observed a reduced RSA that persisted after transplantation despite the fact that recently transplanted heart is denervated; a direct influence of myocardial wall stretch on sinus node independent of autonomic influence was suggested for this observation. On the similar background, Raeder et al. (1987) observed a minimal degree of fluctuations in heart rate variability in an anesthetized dog after denervation (bilateral cervical vagotomy). These fluctuations were abolished by equalization of intrathoracic pressure indicating that non-neural mechanisms contributed to these fluctuations (later identified as RSA). Thus, it may imply from above that respiration can exert its modulatory effect on heart rate variability independent of autonomic nervous system.

**Effect of respiration rate and pattern on HRV.** There is a clear interaction between the frequency of breathing and heart rate variability. This can be seen in healthy subjects or in subjects with autonomic dysfunction of various origins. Radelli et al. (2004) observed that slow controlled breathing at the rate of six breaths/min in healthy young males
was associated with a significant reduction in blood pressure and a significant increase in R-R interval. When evaluated in patients with chronic heart failure, slow breathing resulted in significant increases in baroreflex sensitivity (Bernardi et al., 2002).

Hirsch and Bishop (1981) investigated the influence of breathing frequency and tidal volume on RSA in healthy subjects (age 22-78 years). They found that RSA increased with a respiratory rate of six breaths/min and decreased as breathing rate was increased. It may imply that breathing at a lower frequency appears to be more effective on autonomic modulation of heart rate control than breathing at higher frequency. This study also study observed increases in amplitude of RSA with increases in tidal volume thereby concluding that RSA amplitude depends on both the depth and frequency of breathing.

Brown et al. (1993) investigated the influence of breathing frequency (6, 7.5, 10, 15, 17-1, 20 and 24 breaths/min) and tidal volume on R-R interval power spectra in healthy subjects (age 23-32 years). It was observed that R-R interval power in LF and HF spectra declined significantly with increasing breathing frequency. It appeared that breathing frequency influences R-R interval power spectra in both low frequency and high frequency and more likely redistributes autonomic outflow within the respiratory cycle without altering the levels of autonomic traffic. Similar to the study by Hirsch and Bishop (1981), they observed that breathing rate more than 10 breaths/min yielded reduced levels of R-R interval power in an inverse relationship to breathing rate.

The respiratory modulation of cardiac autonomic control is also supported by studies investigating the training effects of various breathing patterns in yoga. Pranayam or pranayamic breathing involves conscious inhalation, retention and exhalation. Pal, Velkumary & Madanmohan (2004) investigated the effect of three months of pranayama on
heart rate. The study involved 30 male volunteers (age 17-19 years) each in fast (fast inspiration and expiration for a minute) and slow breathing (5-breaths/min) groups. After three months, heart rate in response to standing and deep breathing along with valsalva ratio were evaluated. The heart rate response was significantly lower in the slow breathing group when compared to the control and fast breathing groups. Another study (Raghuraj, Ramakrishnan, Nagendra, & Telles, 1998) evaluated two breathing techniques in yoga-Kapalabhati (rapid and forceful breathing for a minute at a rate of 120 breaths per minute) and Nadisuddhi (very slow alternate nostril breathing for 15 minutes). Twelve male volunteers (age 21-33 years) with an average experience of 19.7 ± 12.8 months in these two breathing techniques participated in this cross-sectional study. HRV was assessed before and after each practice on separate days. The results showed a significantly increased low frequency (LF) power and LF/HF, and decreased high frequency (HF) power following Kapalabhati. The authors attributed the observations to sympathetic excitation with rapid breathing causing LF to increase. The decrease in HF was attributed to initial higher HF power. There were no significant changes following Nadisuddhi. Varying breathing rate and depth has influence on sympathetic and parasympathetic balance thereby affecting HRV.

Respiration is a powerful modulator of heart rate variability and it plays an important role in its genesis of RSA and its distribution. Respiratory variations in frequency and depth influences autonomic control mechanisms. It may imply that the changes in respiratory pattern can be used to manipulate ANS control over cardiovascular system. The respiratory patterns are easily manipulated voluntarily; training of some aspects of respiratory function may temporarily or permanently influence cardiovascular modulation.
**Inspiratory Muscle Training**

One possible method to train respiratory function is inspiratory muscle training (IMT). IMT, as its name suggests, is training of inspiratory muscles against resistance. Devices have been developed to improve inspiratory muscle strength. Most of the devices use some variation of threshold pressure loading. Training at threshold pressure permits loading at a quantifiable intensity by providing near flow-independent resistance to inspiration (Caine & McConnell, 2000). This is typically achieved by IMT devices requiring subjects to create a negative pressure to overcome the resistance when inhaling and working against resistance, thereby strengthening the inspiratory muscles of the body (Gething, Williams, & Davies, 2004). The resistance is usually set by spring-loaded mechanism.

IMT typically uses the basic principles of resistance training. Ventilatory muscles have shown similar adaptations to training as that of other skeletal muscles by using training principles (frequency, intensity, duration) that apply for any other striated muscle (Koessler et al., 2001). The specific functional improvement and adaptive changes in the structure of ventilatory muscles in response to inspiratory training are also similar to those changes seen in limb muscles (Ramirez-Sarmiento et al., 2002). This study demonstrated that inspiratory training for five weeks, five times a week and at an intensity of 40 to 50% of MIP in patients with COPD, induced increases in proportion of type I fibers (approximately 38%, $p < 0.05$) and in the size of type II fibers (approximately 21%, $p < 0.05$) of external intercostals.

It is observed that IMT results in a significant increase in contracted diaphragm thickness and velocity of movement of diaphragm during quiet breathing (Darnley et al., 1999; Enright, Unnithan, Heward, Withnall, & Davies, 2006). The study by Enright et al. (2006) examined the effects of eight weeks of IMT at 80% of MIP, three times a week in
healthy young subjects (age, 21yrs) and observed a significant increase in the contracted diaphragm thickness (Tdi.cont) and diaphragm thickening ratio (abbreviated as TR, is ratio of diaphragm thickness during maximal inspiratory pressure maneuver at functional residual capacity, to mean thickness while relaxing at functional residual capacity) (Tdi.cont: pre 4.1 mm, post 4.6 mm, \( p < 0.05 \); TR: pre, 3.8, post 4.1, \( p < 0.05 \)).

Despite these observations, there is yet no clear agreement that the training principles always apply to the inspiratory muscles to exhibit similar training response as skeletal muscles. However, inspiratory muscles improve in strength and endurance in response to inspiratory training. The inspiratory muscle strength is often assessed directly by measuring MIP, while endurance of inspiratory muscles is assessed by measuring sustained maximal inspiratory pressure (SIP) or performance in various functional tests. IMT improves inspiratory strength and endurance in healthy as well as clinical population (Inbar, Weiner, Azgad, Rotstein, & Weinstein, 2000; Volianitis et al., 2001; Sturdy et al., 2003).

The study by Inbar et al. (2000) was a randomized controlled trial with twenty well-trained endurance athletes. The training group completed 10-week IMT training using inspiratory muscle trainer for 30 min, six times a week, while the control group received similar training with the same device but with no resistance. The training group started training with at a resistance of 30% of MIP for one week. The resistance was then increased by 5% each session to reach 80% of their MIP, at the end of first four weeks and then adjusted every week to 80% of the new MIP achieved. The training group demonstrated a significant increase in inspiratory muscle strength (MIP) (pre 142.2 ± 24.8 cmH₂O, post 177.2 ± 32.9 cmH₂O, \( p < 0.005 \)). The inspiratory strength remained unchanged in the control group. The inspiratory endurance (maximal pressure that can be tolerated for 60 sec)
increased significantly (pre 121.6 ± 13.7 cmH$_2$O, post 154.4 ± 22.1 cmH$_2$O, $p < 0.005$), but not in the control group.

Gething, Passfield, & Davies (2004) compared the effects of six weeks of IMT at maximal (100% MIP) & submaximal (80% MIP) intensities in healthy young subjects. The training was conducted three times per week and each session consisted of ten attempts at maximal or submaximal intensity. The increase in MIP was significantly greater for the maximal intensity group (32 ± 19 cmH$_2$O, $p = 0.011$) than the submaximal intensity group (37 ± 25 cmH$_2$O, $p = 0.001$) relative to the control group without training.

IMT has been investigated in various clinical conditions and it has shown to improve the inspiratory function. The study by Sturdy et al. (2003) examined the effect of eight weeks of IMT in subjects with moderate to severe COPD. The training consisted of 25 minute sessions three times per week at an intensity of 70% of MIP. On completion of training, muscle strength (PImax) increased by 32 ± 27 % ($p < 0.05$) and endurance (maximum pressure generated against a progressively increasing load [Pthmax]) increased by 56 ± 33 % ($p < 0.05$).

Similarly, the aforementioned controlled study by Enright et al. observed an increase in inspiratory strength by 41%, as assessed from MIP (pre 90 cmH$_2$O, post 127 cmH$_2$O, $p < 0.01$). The inspiratory muscle endurance (SIPmax) also improved by 36% in the training group (pre 504 pressure-time units, post 688 pressure-time units, $p < 0.01$). The control group who did not participate in any form of training demonstrated no change in MIP and SIPmax. Another studies using high intensity IMT (60% and 70% of PImax) have also demonstrated increase in inspiratory muscle strength and endurance in chronic obstructive pulmonary disorder (Covey et al., 2001; Koessler et al., 2001; Riera et al., 2001)
In the study by Laoutaris et al. (2004), patients with chronic heart failure received IMT training using 15% of SIPmax (control) and 60% of SIPmax (training), three times in a week for 10 weeks. The training group significantly increased both maximum inspiratory pressure (83 ± 5.7 versus 111 ± 6.8 cmH₂O, \( p < 0.001 \)) and SIPmax (367360 ± 41111 versus 527822 ± 51358 cmH₂O/sec × 10⁻¹, \( p < 0.001 \)) when compared to the control group. An improvement in peak VO₂ and six minute walking distance was also observed. This study also observed a significant reduction in resting heart rate in the training group (80 ± 3 bpm versus 77 ± 3.3 bpm, \( p < 0.05 \)).

Using lower intensity for IMT is not uncommon. The randomized placebo-controlled study by Dall’Ago et al. (2006) evaluated the effect of IMT in sixteen patients with chronic heart failure. The 12-week IMT program included 30-min sessions using 30% of SIPmax, seven times a week. This study found increases in PImax as well as walking distance. IMT significantly increased inspiratory muscle strength and resulted into generalized improvements in expiratory pulmonary function in persons with multiple sclerosis (Fry, Pfalzer, Chokshi, Wagner, & Jackson, 2007). This study used a 10-week IMT exercise protocol where three sets of 15 repetitions were performed daily. The initial resistance used was 30% of maximal inspiratory pressure (MIP) and it was progressed every week 1-or 2-cmH₂O according to RPE and patients’ symptoms. The results also showed significant improvement in expiratory outcomes including FEV₁, forced vital capacity (FVC) and mid-expiratory flow rate.

Jong et al. (2001) conducted a controlled trial to evaluate the effect of IMT in patients with cystic fibrosis. The training group performed IMT using 40% of PImax, 20 min a day, five days a week for six weeks. The training intensity for control group was 10% of PImax.
After six weeks of training, mean inspiratory muscle endurance (% PImax) increased from 49% to 64% and the change in inspiratory muscle endurance in the training group was significantly higher than in the control group.

The observations in studies evaluating IMT are primarily dependent on training modes, intensity, duration and the initial fitness level of the study population. IMT has been widely explored for its influence in sports and performance, as well as clinical conditions. The majority of the studies that tested the effects of IMT have used a three to five days per week training protocol. The duration of IMT programs vary most commonly from six weeks to ten weeks. The intensity used for IMT varies widely between 40% -80% of the subject’s maximal inspiratory pressure (MIP) or maximal sustained inspiratory pressure (SIPmax). Most studies evaluating IMT in clinical conditions have used lower intensities like 30-40%, while the protocols using high intensity in clinical population are not uncommon. The higher intensity using 60% to 80% of MIP was widely used in sports training and performance (Inbar, Weiner, Azgad, Rotstein, & Weinstein, 2000; Volianitis et al., 2001).

It is clear that IMT improves inspiratory muscle strength and endurance. It also improves vital capacity and forced expiratory volume and exerts some influence on expiratory measures (Fry et al., 2007). IMT also improves dyspnea and quality of life (Laoutaris et al., 2004; Riera et al, 2001) and performance (Romer, McConnell, & Jones, 2002; Voliantis et al, 2001). The effect of IMT is not restricted to these parameters. A few studies have investigated the acute and prolonged influence of IMT on parameters other than respiration.

**Inspiratory muscle training and autonomous nervous system.** Chiappa et al. (2008) investigated in a controlled trial if inspiratory muscle training could result in
exaggerated peripheral vasoconstriction in resting and exercising limbs of subjects with chronic heart failure. They found that after the four weeks of training with 60% of maximal inspiratory pressure, the inspiratory muscle loading resulted into a more marked reduction in resting calf blood flow and an attenuated rise in the exercising forearm blood flow. These observations were attributed to inspiratory muscle baroreflex mediated by sympathetic activation. Other significant changes observed with high intensity IMT include reduction in resting heart rate (Gething, Passfield, & Davies, 2004; Laoutaris et al., 2004). The mechanism behind the observed decrease in heart rate remains undetermined.

Another study by Laoutaris et al. (2008) assessed the effect of IMT on autonomic activity, endothelial function, and N-terminal pro-brain natriuretic peptide (NT-proBNP) levels in patients with chronic heart failure. IMT protocol included exercising at 60% (high intensity) & 15% (low intensity) of SIPmax, three times a week for 10 weeks. All measurements were taken before and after training, and included HRV analysis from 24-hour ECG recording, forearm blood flow by venous plethysmography and NT-proBNP from serum sample. High intensity IMT improved PI max and SIPmax without any changes in HRV, endothelial function, and NT-proBNP levels. The low intensity group demonstrated minimal increase in only the SIPmax. With a similar background, another study by the same authors observed again that IMT did not have an effect on autonomic function (Laoutaris et al., 2005).

The above mentioned studies were conducted on COPD or CHF subjects. IMT also did not show any effect beyond respiratory parameters in healthy subjects (Stillings, Gonzales, & Scheuermann, 2006). This study involved training healthy young subjects three days per week, 30 min a day for five weeks. The intensity used was 70% PImax and it was
gradually increased throughout training period as subjects adapted to the resistance. At the end of training, subjects improved FVC, FEV\textsubscript{1} and maximal voluntary contraction, but IMT did not affect resting HR (Pre, 64 ± 2 bpm; Post, 65 ± 1 bpm), LF (Pre, 43.3 ± 7 nu; Post, 50.1 ± 4.3 nu) or HF (Pre, 46.5 ± 6.1 nu; Post, 43.7 ± 3.8 nu) components.

Contradictory to these observations, Hepburn et al. (2005) observed a significant increase in HF peak (13.2 ± 5.7 NU) and decrease in LF peak (10.2 ± 5.5 NU) after six weeks of respiratory training. Eighteen healthy subjects (age, 36 to 88 years) used a Hepburn heart and lung exerciser (HHALE) for training. HHALE has a mouthpiece through which the subject inspires and expires into a rebreathing bag via a filter which provides resistance to airflow. The authors claimed that breathing the same used air increases the depth of ventilation and eventually depth of inspiration sufficient to take in fresh air across the filter. The resistance was gradually increased by adjusting the volume of rebreathing bag. The protocol used for training consisted of 30-min per day, six days per week. The exact mechanism related to these changes in HRV could not be indentified but it was proposed that respiratory training contributed to central or peripheral mediators of HRV.

Mello et al. (2008) investigated the effect of 12-week IMT using inspiratory resistance of 30% in six patients with CHF. They evaluated sympathetic activity by muscle sympathetic nerve activity (MSNA), forearm blood flow by venous occlusion plethesmography, and heart rate variability from power spectrum analysis. The results indicated an increase in inspiratory force, a 15% reduction in MSNA (40±1 vs. 33 ± 1 burst/min); an increase in forearm blood flow (0.8 ± 0.1 mL/min/100g); a decrease in LF % (45 ± 5 vs. 39 ± 3 Units); an increase in HF % (40 ± 5 vs. 61 ± 3 Units) and a decrease in LF/HF.
The addition of resistance during inspiration resulted in increased HRV and change in RSA characteristics (Calabrese, Perrault, Dinh, Eberhard, & Benchetrit, 2000). This study evaluated four conditions of resistive load breathing in seven healthy volunteers and acute responsive changes in HRV to these conditions. A flowmeter mounted on a face mask and connected to a differential pressure transducer was used. Four levels of resistance were applied throughout breathing cycle, and end tidal CO$_2$ (FCO$_2$) and ECG were obtained continuously for eight minutes. The resistance was created by using increasing tube thickness connected to flowmeter and the scouring pads at the end of face mask. The resistance was calculated by using a mouth pressure-flow plot on a breath-by-breath basis ($R_0$ = 0.76 ± 0.02 cmH$_2$O/sec (control), $R_1$ = 3.25 ± 0.16 cmH$_2$O/sec, $R_2$ = 5.24 ± 0.30 cmH$_2$O/sec, $R_3$ = 8.25 ± 0.37 cmH$_2$O/sec, and $R_4$ = 12.51 ± 0.63 cmH$_2$O/sec). The results indicated that the applying resistive loads resulted in lengthening of the respiratory period and increase in tidal volume without any change in FCO$_2$. Mean R-R interval remained unchanged whereas TP, HF, LF and RCF (respiratory centered frequency spectral power components), and RSA increased significantly with increasing load. The HRV in condition $R_4$ was increased to two fold that for $R_0$. The observed increase in RSA could probably be accounted for by either increase in intrathoracic pressure and the ensuing stimulation of baroreceptor or changes in respiratory pattern following resistive loading.

Another study (Witt, Guenette, Rupert, McKenzie, & Sheel, 2007) observed the effect of five week IMT (six days per week) on HR and mean arterial pressure (MAP) response to a resistive breathing task. The resistive breathing task was designed to increase metabolic requirements and compromise diaphragm perfusion by combing resistance and a prolonged breath cycle. Sixteen healthy young men (age 25.8 ± 0.8 years) were randomized into an
experimental group (intensity of 50% of MIP) and a control group (intensity of 10% of MIP). After training, the experimental group showed lower HR and MAP response than the pre-training response to resistive breathing task, while the control group demonstrated similar response to pre-training. This observation was attributed to attenuation of sympathetic activity and reduced activity of chemosensitive afferents within inspiratory muscles by resistive inspiratory work.

**Summary**

Studies evaluating the effect of aerobic exercise and resistance exercise training reported improved HRV. This suggests that HRV can be modified with the external mitigating stimulus in healthy as well as clinical conditions. Currently, it is understood that the changes in the breathing pattern influences autonomic control over cardiovascular system. The breathing pattern can be highly manipulated voluntarily; training of some aspects of breathing function may temporarily or permanently influence autonomic cardiovascular modulation. IMT may provide such an option. However, studies investigating influences of IMT on the cardiac autonomic control are limited and the results are variable. This makes it difficult to draw inferences on the possible effects of IMT. Different subject population within studies adds to this difficulty. The studies that yielded a positive effect on HRV have used some forms of respiratory resistance training. It may imply that IMT using a pressure threshold device will produce similar results. Laoutaris and colleagues (2008) speculated that their results would have been in favor of IMT, if HRV was measured for a shorter time at rest rather than 24 hours. There are also speculations that more demonstrable effects may be obtained with higher intensity of IMT. Stillings et al. (2006) speculated that the effect of IMT can be observed prominently in older adults or
conditions associated with reduced HRV. This investigation further explores if higher intensity, 80% of MIP, results in beneficial changes in HRV measured from short term recording. This study also assesses the IMT induced changes in HRV, if any, in older adults as contrasted with young adults.
Chapter III
Methods and Procedures

Introduction

This study was designed to evaluate the effects of six weeks of inspiratory muscle training (IMT) on the heart rate variability (HRV) in young and older adult populations. This chapter describes the subject population as well as the design of the study. Methods and procedures relating to the data collection, measurement techniques, training protocol and analysis are discussed.

Description of the Study Population

The study population consisted of 12 healthy young (age 18 to 30 years) and 14 older (age > 60 years) adults. The subjects were recruited from the community by placing descriptors of the study at university boards, local senior centers and retirement communities. Each subject was required to complete medical background form (Appendix B). Subjects with a history of chronic respiratory or neuromuscular disease, previous thoracic surgery or trauma were excluded. The subjects with body mass index (BMI) more than 40 were excluded for the chest wall compliance limited by obesity. Potential subjects were also excluded if they reported currently smoking, an acute infection, dementia, respiratory disorders, dysrhythmias, unstable angina pectoris or congestive heart failure on medical background form. Further criteria for exclusion included the current use of any heart rate-altering medications or the use of such medications within the previous year. Each subject was determined to have an apparently healthy pulmonary function if they demonstrated normal pulmonary function, defined as vital capacity (VC) and forced expiratory volume (FEV<sub>1</sub>). The study was reviewed by Human Subjects Committee at Western Washington
University (Appendix A) and informed consent (Appendix A) was obtained from every subject.

**Design of the Study**

The design of the study was a within subjects quasi-experimental design. The eligible subjects were divided into two groups according to age, young group with adults aged 18-30 years and older group with adults aged more than 60 years. The study did not include controls for either group. It is well established that without any intervention, HRV is stable over months without any significant changes.

After initial evaluation, all subjects started with the inspiratory muscle training for six weeks. At the conclusion of training, all tests to measure respiratory function and HRV were repeated and results were compared to pre-training values.

**Data Collection Procedures**

Upon arrival for the initial evaluation, the procedures, any potential risks involved and possible benefits of participation in the study were reviewed with each participant, and the informed consent (Appendix A) was obtained.

At the initial evaluation, each subject needed to complete medical background form (Appendix B). Each subject’s height (in centimeters) and body mass (in kilograms) were determined using a stadiometer and balance beam scale. A physical activity log (Appendix B) was provided to each subject to complete over three days including two weekdays and one weekend day. This activity log assigned a kilocalorie expenditure value per kilogram body weight in fifteen minute intervals to corresponding exertion levels of categorized physical activities, ranging in intensities from one to nine, with one being activities such as sleep and nine being activities such as heavy resistance exercise (Bouchard, 1997). These values were
summed in tabular form and then converted to reveal an average daily caloric expenditure in kilocalories using Microsoft Excel.

**Instrumentation and measurement technique for pulmonary function.** Each subject was assessed for pulmonary function by measuring VC and FEV\textsubscript{1} using a Pneumoscan spirometer (KL engineering, Northridge, CA) and following American Thoracic Society (1994) guidelines. All measurements were made with the subject in a sitting position. The subject put the mouth piece mounted on the flow-meter, in their mouth and the nose clip was secured. To measure VC and FEV\textsubscript{1}, subjects were instructed to perform a maximal inspiration at a self selected pace and exhale with the maximal force. Three measurements were obtained and were expressed in liters. The best trial was used in the analysis. Maximal voluntary ventilation (MVV) was also measured using the spirometer. To measure the MVV, the subject was required to breathe as deeply and rapidly as possible for 12 seconds. The respiratory volume value so obtained was extrapolated to one minute and the result of the test was recorded as flow rate in liters per minute.

Maximal inspiratory pressure (MIP) was tested at the initial evaluation and then every week during training. MIP is a useful measure of inspiratory muscle strength and it was also used to determine the training intensity. It is measured during maximal inspiratory effort against an occluded airway at residual volume. MIP was measured through a device developed by the engineering staff at Western Washington University’s instrument center. Three MIP trials were conducted in upright standing position. The highest value was recorded in centimeters of water (cm H\textsubscript{2}O) and used in analysis and determination of training intensity.
Before measuring MIP, a clean mouthpiece was attached to breathing hose connected to the inspiratory pressure measuring device. The FLUKE multimeter device connected to the inspiratory pressure measuring device was set to measure the maximum value of inspiratory pressure. The subject stood close to the inspiratory pressure measuring box and applied the nose clip. The mouthpiece was placed in the mouth, and then subject inhaled and exhaled with the breathing hose valve open. Immediately following exhalation through the breathing hose, the valve was closed when the subject indicated readiness to inhale maximally. The subject inhaled as forcefully as possible. The multimeter displayed the subject’s MIP value.

**Instrumentation for HRV measurement.** Measures of HRV were taken with a standard 3-electrode, 1-lead electrocardiogram (ECG) setup which examined lead II. Disposable adhesive electrodes were used for the subject-ECG interface. The ECG device was an MP100 system (BIOPAC Systems Inc., Goleta CA) interfaced with a Pentium III computer. AcqKnowledge software (BIOPAC Systems Inc., Goleta, CA) was used for data recording, collection, and HRV data reduction. All HRV measurements were taken by the same experimenter to reduce experimental error.

**Measurement techniques and procedures for HRV.** Subjects were instructed to eat a light breakfast at least two hours before testing. They were also instructed to wear clothing which would allow easy access to the wrists and ankles. Subjects were asked to lie still and comfortably on a padded examination table in the laboratory. Every effort was made to keep the laboratory quiet and at a comfortable room temperature. Before placing the electrodes on the skin, the subject was presented with a simple visual analog scale (VAS) (Data Collection Sheet, Appendix B) to rate the level of stress or anxiety at the moment. The
procedure was not progressed until subject was comfortable and relaxed. At the post-training ECG recording, a difference of one cm more or less than VAS score at pre-training ECG recording was accepted. Otherwise, the post-training ECG recording was postponed and performed another day.

The HRV test required the placement of three electrodes configured to examine lead II. The electrode wires were color coded black (left arm [LA]), white (right arm [RA], and red (left foot [F]) and were equipped with clips for adhesive electrodes placed at their terminal ends. The proximal ends of the wires were plugged into the ECG port of the MP100 system. The subject’s skin at the anterior aspects of the right and left wrists, and medial aspect of the left ankle were inspected visually for cleanliness. An alcohol swab was then used to prep these areas for optimal ECG signal acquisition. The electrodes were clipped to the terminal ends of LA, RA, and F wires and were placed on the medial aspects of the left and right wrist and left ankle, respectively.

During data collection, the experimenter observed the ECG for line noise or artifacts and abnormalities in rhythm. ECG data were recorded for five minutes. The subject was instructed and encouraged to remain still and relaxed for the entire measurement period. If the subject spoke or moved in any way, the test was terminated and started over.

**Data processing.** From the raw ECG acquisition, HRV variables were obtained with the aid of the AcqKnowledge software which utilized algorithms conforming to frequency domain guidelines established by the Task Force of European Society of Cardiology and The North American Society of Pacing and Electrophysiology (BIOPAC 2007, Malik et al. 1996). The power frequency for HRV variables were defined by the task force guidelines as
Total power (TP), \( \leq 0.4 \) Hz; High frequency (HF), 0.15- 0.4 Hz; Low frequency (LF), 0.04-0.15 Hz; Very low frequency (VLF), \( \leq 0.04 \) Hz.

**Training procedures.** IMT apparatus used for the study was a commercially available product (PowerBreathe, Southam, Warwickshire, UK). PowerBreathe is a pressure-threshold device which requires continuous application of inspiratory pressure throughout inspiration in order for the inspiratory regulating valve to remain open. IMT resistance setting was set at 80% of the MIP throughout training period. This number was rounded down to nearest lower number on the PowerBreathe Force Chart (Appendix C) to determine the resistance setting.

Prior to training, the subjects were familiarized with the IMT device. Training included five sets of 12 repetitions on the IMT device five days per week for six weeks. Subjects were instructed to breathe at a relaxed, self selected comfortable pace to discourage hyperventilation. They were instructed to stop if they felt light headedness or nauseous. Training sessions lasted approximately 15 minutes. Each subject was allowed a recovery between sets. After each training session, rating of perceived exertion (RPE) values were obtained and recorded. Any discomfort was noted. Every week the resistance setting was adjusted to 80% of new MIP owing to the adaptation and improvement in inspiratory muscle strength.

Training was performed in a quiet room with no distractions, and the same instructions were given to all subjects, thereby ensuring that they were being motivated in a consistent manner during the training period. As much as possible, IMT was performed at the same time of the day for each session. It was emphasized that subjects do not miss more than three sessions in six weeks.
Data Analysis

To evaluate possible differences between pre- and post training HRV parameters and pulmonary function measures, a two-way repeated measures analysis of variance (ANOVA) was conducted using the factors Group (young and older) and Time (pre and post training). HRV variables (TP, LF, HF, LFnu, HFnu, LF/HF ratio) were compared before and after IMT within and between the two groups. The data analysis was performed with Excel 2004 (Microsoft Inc., Redmond, WA) and PAWS version 17 (Chicago, IL). The significance was accepted at $p < 0.05$. 
Chapter IV

Results and Discussion

Introduction

The current study was instigated to evaluate the effects of inspiratory muscle training (IMT) on heart rate variability (HRV) in young and older adults. HRV and pulmonary function variables were assessed prior to and following six weeks of IMT. The current chapter describes subject characteristics and results pertaining to data analysis from pre-and post-training evaluations. This is followed by a discussion of these results.

Subject Characteristics

In the beginning of study, a total number of 17 young and 15 older subjects volunteered for the study. Five of the young participants were unable to complete six weeks of training and were excluded from the study. Results of one older subject were not included in the study owing to higher visual analog score for stress during post-training electrocardiogram (ECG) recording and failure to report for a second recording. As a result, the final subject population consisted of 12 young (6 males, 6 females) and 14 older (5 males, 9 females) adults. The subject characteristics are given in Table 1.
Table 1. Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young (N= 12)</th>
<th></th>
<th>Older (N= 14)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22.25</td>
<td>2.13</td>
<td>72.5</td>
<td>6.44</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.66</td>
<td>8.4</td>
<td>166.57</td>
<td>7.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.89</td>
<td>8.05</td>
<td>73.37</td>
<td>8.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.95</td>
<td>2.07</td>
<td>26.43</td>
<td>3.2</td>
</tr>
<tr>
<td>Total energy expenditure (Kcal/day)</td>
<td>3326</td>
<td>722</td>
<td>3114</td>
<td>454</td>
</tr>
</tbody>
</table>

SD= standard deviation, BMI= body mass index

Results

**Heart rate variability.** The dependent HRV variables included total power (TP), low frequency power (LF), high frequency power (HF), normalized LF (LFnu), normalized HF (HFnu) and LF/HF ratio. These HRV variables were derived from five minute ECG recordings obtained before and after six weeks of IMT. Every subject completed VAS to report the level of stress at both of the recordings and the scores at both occasions were within a permitted difference of 1-cm. Table 2 provides a descriptive summary of HRV data among young and older subjects.
Table 2. Descriptive data of HRV

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young Pre-IMT</th>
<th>Young Post-IMT</th>
<th>Older Pre-IMT</th>
<th>Older Post-IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
<td>SD</td>
</tr>
<tr>
<td>TP (s^2)</td>
<td>15.773</td>
<td>6.484</td>
<td>17.399</td>
<td>6.507</td>
</tr>
<tr>
<td>VLF (s^2)</td>
<td>10.301</td>
<td>4.111</td>
<td>11.376</td>
<td>4.106</td>
</tr>
<tr>
<td>LF (s^2)</td>
<td>4.203</td>
<td>1.726</td>
<td>4.485</td>
<td>1.690</td>
</tr>
<tr>
<td>HF (s^2)</td>
<td>1.269</td>
<td>0.714</td>
<td>1.537</td>
<td>0.880</td>
</tr>
<tr>
<td>LFnu (nu)</td>
<td>0.779</td>
<td>0.039</td>
<td>0.756</td>
<td>0.059</td>
</tr>
<tr>
<td>HFnu (nu)</td>
<td>0.220</td>
<td>0.039</td>
<td>0.243</td>
<td>0.059</td>
</tr>
<tr>
<td>LF/HF</td>
<td>3.651</td>
<td>0.756</td>
<td>3.278</td>
<td>0.787</td>
</tr>
</tbody>
</table>

SD = standard deviation, TP = total power, VLF = very low frequency, LF = low frequency, HF = high frequency, LFnu = normalized LF, HFnu = normalized HF, nu = normalized units

To assess the possible changes in the HRV variables, a two-way repeated measures ANOVA test was used. The test revealed that there was no significant interaction between time and group for TP, VLF, LF, and HF (p > 0.05). On further examination into main effects for time and group, none of these variables indicated significant differences. A significant interaction effect between time and group was observed for LFnu (F = 6.11, p = 0.02, ηp^2 = 0.203) as shown in Figure 1. The main effects of time and group were examined, but no significant effect was observed for either time (F = 0.13, p = 0.71, ηp^2 = 0.006) or group (F = 0.36, p = 0.55, ηp^2 = 0.015). There was an increase of 4% and a decrease of 2% in LFnu in the young and older group respectively.
Figure 1. Mean LFnu in young and older groups

Comparison of pre-and posttest HFnu values also indicated significant interaction effect between group and time ($F = 6.11, p = 0.02, \eta^2_p = 0.203$) (Figure 2). There was no significant main interaction effect for time ($F = 0.13, p = 0.71, \eta^2_p = 0.06$) or group ($F = 6.11, p = 0.02, \eta^2_p = 0.203$). The young subjects demonstrated an increase of 10% in HFnu, while the older subjects demonstrated a decrease of 13% in HFnu.
Similar responses were observed for LF/HF ratio where a significant interaction effect was observed ($F = 5.50, p = 0.02, \eta_p^2 = 0.187$) (Figure 3), but no significant main effect of either time ($F = 0.00, p = 0.962, \eta_p^2 = 0.00$) or group ($F = 1, p = 0.32, \eta_p^2 = 0.04$). The ratio decreased by 10% in the young adults and increased by 10% in the older adults.

**Figure 2. Mean HFnu in young and older groups**

**Figure 3. Mean LF/HF in young and older groups**
To summarize, the significant interaction effect for LFnu, HFnu, LF/HF suggests that the independent variables of the study (IMT and age) were not independent. Thus, it may be proposed that the effect of IMT depended on age group or age-groups varied in their training response to IMT. This is also indicated by the changes in the opposite direction for groups. The changes in LFnu, HFnu and LF/HF were significant in both the groups despite opposite training response.

**Pulmonary function measures and MIP.** Pulmonary function and maximal inspiratory pressure (MIP) were evaluated before and after training to assess the effectiveness of training regime. Pulmonary function measures included vital capacity (VC), forced expiratory volume in 1-second (FEV₁), and maximal voluntary ventilation (MVV). MIP values measured during only pre-and post training evaluations were included in the statistical analysis. Table 3 provides descriptive data of pulmonary function and MIP.

**Table 3. Descriptive data of pulmonary function and MIP**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young</th>
<th>Older</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-IMT</td>
<td>Post-IMT</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
</tr>
<tr>
<td>VC (liter)</td>
<td>4.09</td>
<td>1.03</td>
</tr>
<tr>
<td>FEV₁ (liter)</td>
<td>3.35</td>
<td>0.95</td>
</tr>
<tr>
<td>MVV (liter/min)</td>
<td>139.75</td>
<td>32.91</td>
</tr>
<tr>
<td>MIP (cm H₂O)</td>
<td>1.06</td>
<td>0.33</td>
</tr>
</tbody>
</table>

VC=vital capacity, FEV₁= forced expiratory volume in 1-second, MVV= maximal voluntary ventilation, MIP= maximal inspiratory pressure.
A two-way repeated measures ANOVA test was used to compare the differences between pre-and post-training pulmonary function measures and MIP. The test revealed that there was not a significant interaction effect between time and group for VC \((F = 0.51, p = 0.48, \eta_p^2 = 0.02)\), but there was significant main effect of time from pre-test to post-test \((F = 4.09, p = 0.05, \eta_p^2 = 0.146)\) as shown in Figure 4. The young group and older group had an increase of 7% and 5% in vital capacity respectively. There was also significant main effect of group \((F = 33.98, p = 0.00, \eta_p^2 = 0.58)\).

**Figure 4. Mean vital capacity in young and older groups**

![Graph showing mean vital capacity comparison between young and older groups](image)

IMT= inspiratory muscle training

When comparing \(FEV_1\) values, there was a significant interaction effect between time and group \((F = 4.40, p = 0.04, \eta_p^2 = 0.15)\) (Figure 5). On further examination, the main effects for both the group \((F = 29.96, p = 0.00, \eta_p^2 = 0.55)\) and time \((F = 11.09, p = 0.03, \eta_p^2 = 0.31)\) were significant. The young subjects demonstrated an increase in \(FEV_1\) by 8%, while the older subjects improved by 3%.
Figure 5. Mean FEV₁ in young and older groups

![Graph showing FEV₁ in young and older groups]

FEV₁ = forced expiratory volume in one second

Similar results were observed for MVV (Figure 6) where there was a significant interaction effect \( (F = 11.13, \ p = 0.00, \ \eta_p^2 = 0.31) \) followed by significant main effect of time \( (F = 22.54, \ p = 0.00, \ \eta_p^2 = 0.48) \) and group \( (F = 24.28, \ p = 0.00, \ \eta_p^2 = 0.50) \). The overall increase in MVV was 16% in the young group and 4% in the older group.

Figure 6. Mean MVV in young and older groups

![Graph showing MVV in young and older groups]

MVV = maximal voluntary ventilation
ANOVA test results for maximal inspiratory pressure revealed significant interaction effect between time and group ($F = 14.65, p = 0.00, \eta^2 = 0.379$). The main effects were examined and were found significant for time ($F = 93.10, p = 0.00, \eta^2 = 0.795$) and group ($F = 24.72, p = 0.00, \eta^2 = 0.50$) (Figure 7). The young group demonstrated an increase of 50% in MIP, while the older group demonstrated an increase of 37%.

**Figure 7. Mean MIP in young and older adults**

![Mean MIP graph](image)

MIP = maximal inspiratory pressure

To summarize, the significant main effect of time for VC is consistent across groups implying no interaction effect. The significant main effect of group, backed by the greater increase in means for the young group, reveals significantly greater improvement in VC for the young group as opposed to the older group. For FEV₁, MVV, and MIP, a significant interaction was observed indicating that the effect of IMT depended on the group. However, the significant main effects for both the time and group further revealed that the improvement in FEV₁, MVV, and MIP was significantly greater in the young group as opposed to the older group.
**Discussion of Results**

The current investigation compared changes in HRV measures in the young and older adults after six weeks of IMT. The significant changes in LFnu, HFnu and LF/HF between and within the young and older group \((p < 0.05)\) showed that there was a training effect of IMT. However, there were no significant changes in TP, LF and HF \((p > 0.05)\). Therefore, the null hypothesis that there will be no difference in HRV measures before and after IMT cannot be completely rejected. Similarly, the other null hypothesis that there will be no difference in responses from both the groups cannot be rejected.

Because VLF cannot be measured accurately from short term recordings, LF and HF are also reported in normalized values which represent the relative values of each power component in proportion to the total power minus VLF component. Normalization tends to control the effect of changes in total power on the values of HF and LF (Malik et al., 1996; Sandercock & Brodie, 2006). Thus, normalized LF and HF reflect the changes in HRV profile more effectively. A significant training effect of IMT is apparent in both the young and older adults for these variables.

The VLF component is not heavily evaluated due to its physiological correlates being largely unknown. The high frequency component (HF) is parasympathetically mediated (Akselrod et al., 1981; Pomeranz et al., 1985; Raeder et al., 1987), and the LF component is influenced by both sympathetic and parasympathetic nervous activity (Akselrod et al., 1981; Pomeranz et al., 1985; Raeder et al., 1987; Saul, 1990). There is no known method of precisely determining the contribution of each branch with the LF power spectra. LF/HF is an indicator of the sympathetic-parasympathetic balance in autonomic nervous system (ANS). The decrease in LF/HF in the young adults is attributed to relatively greater increase
in HF when compared to the increase in LF. The culmination of such results may imply a shift in balance towards parasympathetic modulation. In older adults, LF/HF increased despite simultaneous non-significant decrease in both LF and HF. These observations may suggest that IMT resulted in a shift in balance away from parasympathetic modulation.

Currently, there are no other known IMT studies evaluating the simultaneous effect of IMT on HRV in similar age groups. In addition, the available studies used different populations and different methods to evaluate HRV, making the comparison of results from the current study difficult. Stillings, Gonzales, & Scheuermann (2006) evaluated HRV in healthy young adults (age 25 ±5 years) prior to and following a five weeks IMT (70% MIP, 3-days/week, 30 min/day) and found no significant changes in resting heart rate, normalized LF or normalized HF. The investigations by Laoutaris et al. (2008) and Mello et al. (2008) included subjects with chronic heart failure. Laoutaris and colleagues demonstrated no significant changes in time and frequency domain HRV variables obtained from 24-hour ambulatory ECG monitoring after 10 weeks of IMT (3-days/week). On the other hand, Mello et al. (2008) observed a significant decrease in LF, increase in HF and decrease in LF/HF ratio. The study used 12-week IMT at 30% MIP, however the training protocol and method of HRV measurement are not available for further comparison.

The exact mechanism attributable to the observed changes in HRV profile is not well established and is beyond the scope of the current study. The speculations may include attenuation of sympathetic activity through reduced activity of chemosensitive afferents within fatigued inspiratory muscles by resistive inspiratory work (Witt et al., 2007). The IMT protocol in the current study used an intensity of 80% of MIP. The rating of perceived
exertion for both the groups ranged from 13 to 15. It may be suggested that the IMT protocol was able to induce fatigue in inspiratory muscles.

Calabrese et al. (2000) demonstrated that breathing under resistive loading resulted in breathing pattern changes. While breathing in against resistance, the most of subjects in both the groups decreased the rate and increased the depth of breathing. Respiration is strong modulator of autonomous nervous system and HRV is influenced by the rate and depth of breathing by affecting the phasic inputs from pulmonary and thoracic stretch receptors. It is suggested that these phasic inputs from pulmonary and thoracic stretch receptors make small but statistically significant contribution to respiratory sinus arrhythmia (RSA) (Davidson, Goldner, & McCloskey, 1976; Davis, McCloskey, & Potter, 1977; Elghozi et al., 1991). Hirsch and Bishop (1981) have demonstrated increase in RSA with decrease in breathing rate and associated decrease in receptors phasic inputs. RSA is primary contributor to the high frequency component of HRV that occurs at respiratory frequency > 0.25 Hz (Bernardi et al., 1989; Kleiger et al., 2005; Pumphra et al., 2002). Brown et al. (1993) observed that R-R interval power in LF and HF spectra declined significantly with higher breathing frequency and vice versa. Thus, it may be suggested that change in breathing rate during IMT had a role in affecting HF spectra of HRV.

As the subject population in the current study showed a small heterogeneity of responses, it would be valuable to identify factors that are likely to be responsible for these differences. The older adults demonstrated higher mean values of TP, VLF, LF and HF, and lower mean LF/HF ratio at the beginning of the study. However, the between-group differences were not significant. This contrasts the common observation in previous studies that demonstrated decline in HRV with age (Schwartz, et al., 1991; Shannon et al., 1987;
Yeragani et al., 1997). These studies observed lower frequency domain HRV variables in older adults when compared to young adults. The possible explanation for the higher values in older subjects is that the most of older subjects were recruited from adult fitness program at Western Washington University and were moderately trained in aerobic and resistance exercises. The observations from the studies reviewed previously have indicated greater HRV in trained individuals (Melo et al., 2005; Rennie et al., 2003). It is also well established that HRV increases with regular aerobic exercises of moderate intensity (Buchheit et al., 2004; Sandercock et al, 2007; Tulppo et al., 2003). There are also investigations that have established increase in HRV with resistance training (Figueroa et al., 2008; Selig et al., 2004). Thus, the training status of older adults may have favored towards higher HRV.

When the mean total energy expenditure for both the groups were compared, no significant differences were revealed ($p > 0.05$). Carter et al. (2003) have observed that older adults have greater training potential to improve HRV than the young adults in response to same aerobic exercise training. Thus, it may be proposed that the older adults in the current study might have exhibited greater training response from the physical activity.

The other confounding factor is the time of ECG recording from the previous exercise. The time of ECG recording at pre-and post training evaluations and previous exercise activity was not actively controlled. Some of the older adults reported for their initial evaluation and ECG recording after they finished their regular scheduled 30-45 minutes long aerobic exercise session. The average time that elapsed between the exercise and ECG recording ranged from 45 minutes to an hour. Thus, sympathetic excitation and parasympathetic withdrawal associated with exercise might have influenced HRV data. However, it has been show that autonomous nervous system activity recovers to its pre-
exercise state within 30 minutes after exercise. These studies investigated the HRV during recovery from 30 to 40 minutes of moderate aerobic exercises and observed that the least time to recover HRV ranged from 15 minutes to 60 minutes (Gladwell, Sandercock, & Birch, 2010; Heffernan, Kelly, Collier, & Fernhall, 2006; Seiler, Haugen, & Kuffen, 2007). These observations are often contradicted by studies that observed altered autonomous nervous system as long as 24 to 48 hr after moderate to vigorous exercise (Hautala, 2001).

A recent study by Mendonca et al. (2010) demonstrated that women, compared to men, have greater parasympathetic input for cardiac autonomic function during rest and recovery from exercise. The study observed lower LF, higher HF and lower LF/HF ratio in women. The older group had proportionally higher number of females. Also, the larger standard deviations in the older group indicate the wide variations within the group. These facts may have contributed to the heterogeneity in responses.

The pulmonary function measures and MIP test results indicate that IMT was effective in improving not only inspiratory muscle strength, but also pulmonary function measures. This observation is consistent with other research using similar training protocol (Stillings et al, 2008; Gething et al., 2004). The young adults demonstrated higher increases in VC, FEV₁, MVV and MIP when compared to older adults. The between-group differences for these variables were statistically significant. The statistically significant increases of 58% and 37% in MIP in young and older adults respectively, suggest that six weeks of IMT was a successful training program.

**Limitations.** The current study has several limitations. First, the subjects voluntarily participated for the study with the interest of improving lung function. The non-randomized selection of subjects and their underlying interest of participation might have confounded the
results. Secondly, the subject’s efforts pose another limitation as the subject had to put forth maximal effort during MIP and pulmonary function evaluations. The third limitation is posed by the subject’s familiarity and comfort with testing procedures at the post-training evaluations.

The subjects were instructed to keep same exercise routine. The possible change in physical activity despite the advice was not actively assessed during the period of training. This poses another limitation to analysis of the results.

The time of the day when subjects completed the ECG recordings for pre-and post-training evaluations, was not essentially the same at the two occasions. Another important limitation is that the respiration rate was not controlled during ECG recording. There is no common agreement over controlled respiration rate during ECG recording. HRV can be adversely affected when the subjects attempt to control breathing at a rate set by metronome.

Lastly, the number of IMT devices available for training was limited and the training period of older group followed the training period of the young group. The training of the young subjects was completed mostly in Spring season, while that of older subjects extended into Summer. The seasonal influence on HRV is not unknown and may have confounded the results. The systematic difference in the calibration of the equipment over different time spans of training poses limitation of instrumentation error.

**Summary of Results**

Results of the current study demonstrate significant changes in LFnu, HFnu and LF/HF following six weeks of inspiratory muscle training. IMT did not appear to influence LF, HF, and TP in both the young and older groups. The training response varied between both the groups. A significant increase in strength of inspiratory muscles and pulmonary
function measures occurred in both the groups with the young group demonstrating greater training response.
Chapter V

Summary, Conclusions, and Recommendations

Summary

The purpose of this study was to investigate the effect of inspiratory muscle training (IMT) on heart rate variability. The study population consisted of 26 apparently healthy subjects (12 young, 14 older). Every subject had to complete a medical background form and physical activity log. The before and following training assessments included pulmonary function tests (VC, FEV₁, MVV) and five minutes recording of electrocardiogram (ECG). The dependent HRV variables (TP, LF, HF, LFnu, HFnu and LF/HF) were obtained from ECG recordings using AckKnowledge software. IMT protocol included five sets of 12 repetitions, five days per weeks for six weeks. The intensity for training was kept constant at 80% of maximal inspiratory pressure (MIP) by adjusting the resistance of the IMT device every week owing to the adaptation and improvement in inspiratory muscle strength as determined by MIP.

At the conclusion of six weeks of IMT, it was demonstrated that there was significant difference in pre-and post-training values of LFnu, HFnu and LF/HF ratio in both the groups. No significant effect was observed for TP, LF and HF. The training response varied between groups. The pulmonary function measures (VC, FEV₁, MVV) and MIP demonstrated significant increases in both the groups.

Conclusion

Results from this investigation indicate that six weeks of inspiratory muscle training had a significant effect on LFnu, HFnu and LF/HF ratio in both the young and older groups. The other variables TP, LF, and HF did not demonstrate any significant changes. However, the training response depended on group and varied within groups. The young group
demonstrated a shift in autonomic balance towards parasympathetic modulation, while the older group demonstrated decrease in parasympathetic modulation. The determination of exact mechanism is beyond the scope of this study. It may be proposed that IMT was associated with respiratory modulation of ANS, which was then reflected in changes in HRV profile. The results also demonstrated improved inspiratory muscle strength and pulmonary function measures in both the groups, where the young group experienced greater improvement than older group.

**Recommendations**

A high HRV is a sign of good adaptability. Any attempt to increase HRV is beneficial in many clinical conditions as well as health. The current study adds to the literature to better understand the effects of IMT on HRV. More investigations are needed to elaborate and examine the typical effects IMT can produce on HRV profile.

Recommendations for future research include:

1. Perform same or a similar study with larger subject pool.
2. Control precisely physical activity changes over the period of training.
3. Control precisely the time between ECG recordings and the previous exercise session.
4. Minimize influence of season by controlling the time of training and prevent systematic effects by having the two groups evaluated at the same time.
5. Perform same or a similar study using different subject population with diagnosed clinical conditions associated with low heart rate variability.
6. A longer duration of IMT may produce different results.

The recommendations for clinical application of IMT include use of a similar IMT protocol to increase inspiratory muscle strength and pulmonary function. The observations
from pulmonary function tests and MIP results are applicable to a wide range, from healthy to clinical subject population. The associated HRV changes need to be explored further, to facilitate the use of IMT to improve HRV.
References


Collier, S., Kanaley, J., Carhart, R., Jr., Frechette, V., Tobin, M., Bennett, N., . . . Fernhall, B. (2009). Cardiac autonomic function and baroreflex changes following 4 weeks of


Journal of Respiratory and Critical Care Medicine, 166(11), 1491-1497.
doi:10.1164/rccm.200202-075OC

Effects of moderate and vigorous physical activity on heart rate variability in a British
study of civil servants. American Journal of Epidemiology, 158(2), 135-143.
doi:10.1093/aje/kwg120

Inspiratory muscle training in patients with COPD: effect on dyspnea, exercise
performance, and quality of life. Chest, 120(3), 748-756. doi:10.1378/chest.120.3.748

effects of inspiratory muscle training. Medicine and science in sports and exercise,
34(5), 785-792.

autonomic control during exercise. Scandinavian Journal of Medicine and Science in

heart rate variability after eight weeks of cardiac rehabilitation. Clinical Autonomic


of Gerontology, 46(3), M99-106.


Appendix A

Informed consent

Human Subjects Activity Review form
The purpose of this study is to determine the effect of inspiratory muscle training (IMT) on measures of heart rate variability (HRV) in young and older adults. This study will contribute to the limited existing literature on the possible positive effects of IMT on heart rate variability. This study will also investigate the difference in response to IMT in young and older adults. The benefit of this research is that the subjects may develop increased inspiratory muscle strength and pulmonary function allowing improvement in exercise capacity and tolerance.

Before beginning of the program you will be required to complete a medical background check. If you have any relevant medical or physical limitations, you may not be eligible to participate in this study. You will be required to perform a pulmonary function test and maximal inspiratory pressure test (MIP) that evaluates lung parameters. These simple tests require subjects to inhale and/or exhale deeply and forcefully. You may not be eligible for study if your pulmonary test values do not fall within normal range.

The baseline data will be collected the first week of study and again during the last week of the study. The testing procedures will take place in the Physiology Lab (CV 210). In addition to previously mentioned data, your height and weight will be measured on stadiometer and balance beam scale. A five minute electrocardiogram (ECG) recording will be obtained with you lying comfortably on the cushioned table. You will be required to answer to a visual analog scale before the investigator starts the ECG recording. To allow analysis of caloric expenditure, you will be required to fill out a three-day physical activity record. A familiarization session will take place prior to training to explain the use of IMT device.

All participants will be required to complete six weeks of inspiratory muscle training using an IMT apparatus. It is a pressure-threshold device which requires continuous application of inspiratory pressure throughout inspiration in order for the inspiratory regulating valve to remain open. You will be required to use IMT device five days per week for six weeks. The approximate time for each session is 10 to 15 minutes. You should not miss more than three sessions to continue participation in the study. Every week, the MIP test is repeated to allow readjusting the resistance at a persistent load of 80% of MIP.

As with any exercise, muscle fatigue may be experienced. There are very minimal discomforts associated with pulmonary function test and IMT training. You may feel dizziness, light headedness, exhaustion during breathing maneuvers. All proper procedures will be taken to reduce these discomforts including testing and training in sitting positions, adequate rest between sets and close monitoring of participants. The training device requires subjects to use mouthpiece to blow into apparatus. The investigator will ensure that all mouthpieces used are cleaned between subjects to prevent cross-contamination. The ECG recording requires putting electrodes on subject’s skin. A new set of electrodes will be used for every subject.
All subjects will be assigned a reference number to assure that all records will be kept confidential. All records and signed documents will be kept in separate files that will be accessed only by reference number. Data for each individual from each testing session will not be shared with other subjects. Subject questions and concerns will be initially addressed during the orientation session and subjects should feel free to ask questions and voice concerns as the study progresses. Your participation in this study is greatly appreciated. This participation is voluntary and you may withdraw yourself from participation at any time. If you have any questions or comments during the course of the study please contact me at 425-614-9694 or email choudha@students.wwu.edu or Dr. Lorrie Brilla at 360-650-3056 or Lorrie.Brilla@wwu.edu. I will answer any questions you may have concerning the procedures. If you have any questions about your participation or your rights as a research participant, you can contact WWU Human Protections Administrator, (360) 650-3220.

Investigator: Anuja Choudhari

I have read and understand the procedures for the study described above. I am aware of the potential risks and I agree to participate as a subject in the study described above. I understand that I may withdraw from participation at any time during the course of the investigation.

Subject Signature: ________________________________   Date: _________________

A copy of the consent form will be provided to each subject.
Human Subjects Activity Review Form

Investigator: Anuja Choudhari

1. What is your research question, or specific hypothesis?

The purpose of this study is to investigate the effect of inspiratory muscle training (IMT) on the heart rate variability (HRV) in young and older adults. The null hypothesis is that there will be no significant difference between pre and post measures of heart rate variability after six weeks of IMT training in young and older adults. Additionally, it is hypothesized that both young and older group will exhibit similar response to training relative to HRV changes, if any.

A reduced HRV has been identified as a strong predictor of risk related to the adverse events in healthy individuals and patients with a large number of diseases. An attempt to improve and possibly correct the autonomic imbalance will substantially decrease the mortality (Kleiger, Miller, Bigger, & Moss, 1987). Given the modulatory effect of breathing on the heart rate variability, IMT may provide an effective intervention to increase the heart rate variability. But, the effect of inspiratory muscle training on HRV has not explored much in the literature and thus warrants further experimentation.

2. What are the potential benefits of the proposed research to the field?

Most of literature on IMT is restricted to its application in improving the respiratory dynamics in health and disease. The studies are very limited that examined the possible effects of IMT on the autonomic nervous system. Given the scarcity of literature, the potential benefits of this study include adding to scant literature regarding the effects of IMT on cardiac autonomic control. This study also allows exploring differences, if any, in young and older adults in their training response to IMT in terms of autonomic regulation.

3. What are the potential benefits, if any, of the proposed research to the subjects?

As a benefit of participation, the subjects will individually receive pulmonary function information and six weeks of monitored inspiratory muscle training. IMT has been shown to improve the pulmonary function and inspiratory muscle strength by training inspiratory muscles against resistance (Inbar, Weiner, Azgad, Rotstein, & Weinstein, 2000; Volianitis et al., 2001). This in turn may benefit the subjects to improve their exercise capacity and tolerance. A positive change in HRV, if observed, will be beneficial to subjects as it represents greater ability to adapt to stressors.

4. a) Describe how you will identify the subject population, and how you will contact key individuals who will allow you access to that subject population or database
The young subject population will be pooled from those who volunteer either based on responses to flyers around Western Washington University campus or from announcements made in Kinesiology and Physical Education classes. The older subject population will be pooled from healthy and active community dwelling older adults who responded to the descriptors of the study at local senior centers and retirement communities. The investigator will then contact the interested individuals to explain the study, its duration or expected time involvement, information regarding IMT and the benefits to be expected from performing this study.

b) Describe how you will recruit a sample from your subject population, including possible use of compensation, and the number of subjects to be recruited.

The study population will consist of approximately twelve healthy young (age 18 to 30 years) and twelve older (age > 60 years) adults. All interested subjects will complete a medical background (See Attached) and a pulmonary function test. The medical background will be used to identify and exclude any subjects with history of chronic respiratory or neuromuscular disease, previous thoracic surgery or trauma. Potential subjects will be excluded if they are current smokers, had acute infection, dementia, respiratory disorders, dysrhythmias, unstable angina pectoris or congestive heart failure. Further criteria for exclusion include the current use of any heart rate-altering medications or the use of such medications within the previous year. Each subject will complete the pulmonary function test to determine vital capacity (VC) and forced expiratory volume for 1 second (FEV$_1$). The subjects will be excluded if they do not demonstrate normal VC and FEV$_1$.

The first twelve subjects to respond and determined apparently healthy from the medical background and pulmonary function values will be chosen from each of the older and young subject pool. The remainder will be held in a reserve pool to replace any drop outs. Each subject will be provided with six weeks of inspiratory muscle training. No other compensation will be used.

5. Briefly describe the research methodology. Attach copies of all test instruments/questionnaires that will be used. Note: All attachments must be in final form; drafts are unacceptable.

The first day of the study will consist of orientation and preliminary testing conducted at the Carver Gym Physiology Laboratory of Western Washington University. The orientation will include a thorough explanation of testing and training procedures. The participants will be provided with the informed consent document (See Attached).

The baseline data will be collected prior to beginning of training. The baseline data includes: height, measured by stadiometer converted from quarter inch to the nearest centimeter units (2.54 cm = 1 inch), weight, using a balance beam scale with measurements converted to kilograms (2.2 lbs=1 kg), and the pulmonary function test using a Pneumoscan spirometer (KL engineering, Northridge, CA). VC and FEV$_1$
will be measured three times, following guidelines from American Thoracic Society guidelines (1994). The subjects are instructed to inhale maximally and exhale with maximal force through the mouthpiece mounted on Pneumoscan flowmeter. The highest value will be noted. All measurements will be made with subjects in sitting position. Additionally, maximal voluntary ventilation (MVV) will be measured using Pneumoscan spirometer. MVV is measurement of maximal amount of air subject breathes when breathing as deeply and as quickly as possible over the period of 12 seconds.

Maximal inspiratory pressure (MIP) reflects the force-generating ability of the combined inspiratory muscles and it is measured during maximal inspiratory effort against an occluded airway at residual volume. MIP will be measured through a device developed by the engineering staff at Western Washington University’s instrument center. Three MIP trials will be conducted in upright standing position and the highest value will be recorded in centimeters of water (cmH₂O). It will be assessed to provide a baseline for IMT training and it will be repeated every week to ensure a persistent training load.

All measurements will be taken by the same experimenter to reduce experimental error. Precise instructions for each subject will be enforced to ensure consistency for pulmonary function and HRV measurements. Prior to the HRV measurement, all subjects will be asked to refrain from vigorous exercise for at least 24 hours. They will also be instructed to wear clothing which would allow easy access to the wrists and ankles.

Measurements of HRV will be collected via five minute electrocardiogram (ECG). Subject will be asked to lie still and comfortably on the padded table. Every effort will be made to keep the area quiet and at a comfortable room temperature. The subject will then respond to visual analog scale (VAS) that will be used to monitor the level of stress or depression at the time of ECG recording. VAS is a 10-cm scale where left end represents no stress/depression and the right end represents maximum stress/depression. The subject will put a mark on the scale relating to level of stress or depression. The distance from left end will be measured and recorded. This data will be used to compare the stress/depression level at the pre- and post-training ECG recordings. At the post-training ECG recording, a difference of no more or less than one in VAS scores is acceptable. In the case of difference, the post-training ECG recording will be postponed and performed later another day. A standard 3-electrode, 1-lead ECG setup which examined lead II will be used. Disposable adhesive electrodes will be used for the subject-ECG interface. The electrode placement sites on the skin are the medial aspects of the right and left ankles, and the anterior of right wrist. All skin sites will be inspected visually and cleaned with alcohol swab prior to electrode placements. The subject is instructed to stay still and quiet until examiner indicates the end of test. The subject is reminded that they can freely interrupt test in case of any discomfort. The ECG device is an MP100 system (BIOPAC Systems Inc., Goleta CA) interfaced with a Pentium III computer. AcqKnowledge software (BIOPAC Systems Inc., Goleta, CA) will be used for data processing and data
reduction into HRV variables according to the guidelines established by the Task Force of European Society of Cardiology and The North American Society of Pacing and Electrophysiology (Malik et al. 1996).

Each subject will be required to complete a three day physical activity log (see attached). This activity log will be used to assign a kilocalorie expenditure value per kilogram body weight in fifteen minute intervals to corresponding exertion levels of categorized physical activities, ranging in intensities from one to nine, with one being activities such as sleep and nine being activities such as heavy resistance exercise (Bouchard, 1997). These values are summed in tabular form and then converted to reveal an average daily caloric expenditure in kilocalories using Microsoft Excel.

The IMT protocol includes five sets of 12 repetitions on IMT device five days per week for six weeks. An adequate recovery will be allowed between sets. IMT resistance setting for each subject will be determined by using 80% of his/her highest MIP value. Every week MIP will be reassessed and the training load will be readjusted to 80% of MIP. After each adjustment in training load, rate of perceived exertion (RPE) values will be obtained and any discomfort will be noted. Same instructions will be given to all subjects, thereby ensuring that they are being motivated in a consistent manner during the training period. As much as possible, IMT will be performed at the same time of the day for each session. After completion of training, pulmonary function test and HRV measurement will be repeated.

6. Give specific examples (with literature citations) for the use of your test instruments/questionnaires, or similar ones, in previous similar studies in your field.

Measurement and analysis using ECG to computer interface is widely used in studies which examine heart rate variability. Studies by Carter et al. (2003), Sandercock, Grocott-Mason, and Brodie (2007) examined the effects of exercise on HRV using such instrumentation. A few studies including Calabrese et al. (2000) & Stillings, Gonzales, Scheuermann (2006) have used similar instrumentation to assess changes in HRV in response to respiratory muscle training. Short term HRV measurement has also been used in previous study at Western Washington University (Barlond, 2009) and had been approved by the university human subjects committee.

IMT apparatus used for the training the inspiratory muscles is a commercially available product (PowerBreathe, Southam, Warwickshire, UK). PowerBreathe is a pressure-threshold device which requires continuous application of inspiratory pressure throughout inspiration in order for the inspiratory regulating valve to remain open. It has been used in previous studies at Western Washington University (Feutz, 2006; Jackson, 2008; Hahn, 2010).

7. Describe how your study design is appropriate to examine your question or specific hypothesis. Include a description of controls used, if any.
Heart rate variability reflects the interaction of the sympathetic (SNS) and parasympathetic nervous systems (PNS) to control heart rate (Akselrod, et al., 1981; Pagani, et al., 1986) and provides a powerful tool to investigate cardiac autonomic control. It is considered inexpensive, simple to use, and is also considered as accurate and reproducible. It is extensively used within research, however only a few studies have used in association with IMT. Similar IMT protocol is widely used in studies (Chiappa et al. 2008; Gething, Passfield, & Davies, 2004). There is no control group against any of the two study groups. HRV remains stable without any significant changes over months unless there is an intervention (Buchheit, et al., 2004; Melanson & Freedson, 2001; Tulppo, et al., 2003).

The design of study is within subjects quasi-experimental design. The two groups consisted of young and older eligible subjects. Comparisons will be made from baseline, between and within groups for HRV variables that included total power (TP), high frequency power (HF), normalized HF (HFnu), low frequency power (LF), normalized LF (LFnu), and LF/HF ratio. The data analysis will be performed with Excel 2004 (Microsoft Inc., Redmond, WA) and PAWS version 17 (Chicago, IL). The significance will be accepted at $p < 0.05$.

8. Give specific examples (with literature citations) for the use of your study design, or similar ones, in previous similar studies in your field.

Stillings, Gonzales, & Scheuermann (2006) examined the effect of inspiratory muscle training on HRV in healthy young subjects using a pre-post treatment design. The training included 30-min sessions, three days per week, for five weeks at an intensity of 70% maximal inspiratory pressure (PImax). The intensity was gradually increased throughout training period as subjects adapted to the resistance. The results however did not observe any effect on HRV.

Another study by Laoutarlis et al. (2008) assessed the effect of IMT on autonomic activity, endothelial function, and N-terminal pro-brain natriuretic peptide (NT-proBNP) levels in patients with chronic heart failure. IMT protocol included exercising at 60% of SIPmax, three times a week for 10 weeks. All measurements were taken before and after training and included HRV analysis from 24-hour ECG recording, forearm blood flow by venous plethysmography and NT-proBNP from serum sample. High intensity IMT improved PI max and sustained maximal inspiratory pressure (SIPmax), but HRV, endothelial function, and NT-proBNP levels were not altered.

Mello et al. (2008) investigated the effect of 12 week IMT using inspiratory resistance of 30% in patients with CHF. They evaluated sympathetic activity by MSNA, forearm blood flow by venous occlusion plethesmography, and heart rate variability from power spectrum analysis. The results indicated a decrease in LF, an increase in HF, and a decrease in LF/HF ratio.

In exercise science, repeated measures designs are popular and used regularly to assess human performance across multiple conditions. It is felt that evaluating using a within subjects design or even a single subject design, allows for a stronger
interpretation of the interventions at the individual level. Schutz et al. (1987) has provided guidance for many exercise science researchers utilizing a within subject design, and Bates (1996) highlights many of the problems assessing human performance using randomized, fixed designs.

9. Describe the potential risks to the human subjects involved.

The risks associated with the evaluation using pulmonary function test and ECG recording are very minimal. Subjects may feel light headedness or dizziness during the pulmonary function tests. When using PowerBreathe, subjects may feel little discomfort in chest wall and may feel exhausted. These discomforts often lessen as subject progress through training. Subjects are required to use mouthpieces and nose clips to inspire through the apparatus potentiating risk of cross-contamination.

10. If the research involves potential risks, describe the safeguards that will be used to minimize such risks.

During the ECG measurements, participants in this study will be lying down supine, still and quiet. The examination table will be at a height which allows each subject to easily translate to and from standing and lying positions. A new set of disposable adhesive-interfaced electrodes will be used for each subject. The pulmonary function tests will be performed in sitting position and every subject will be closely monitored during tests. The mouthpieces and nose clips used during tests and training, will be cleaned after each use to reduce risk of cross-contamination. Cleaning will consist of an initial rinsing of instruments with warm water, followed by submersion into 10% bleach solution and Alconox, a biodegradable laboratory detergent. The mouthpieces will be then rinsed and allowed to air dry. Every subject will be familiarized with PowerBreathe before beginning of training to ensure proper posture and technique.

11. Describe how you will address privacy and/or confidentiality.

Subjects identifiable information will be undisclosed during the study. During the presentation of this study, the participant data will be devoid of names or otherwise identifying features, and instead will include only a subject number in order to ensure confidentiality and anonymity. At no point will an individual’s identifiable data be released to public sources. All data will be maintained electronically and hard copy in a cabinet in a locked room.

12. N/A

13. N/A
Appendix B

Medical background

Physical Activity log

Data collections sheet

Training record
**Medical background**

Name: __________________________________________________ Subject Number: ____
Age: _____________ Sex: _______
Phone number: __________________

Emergency Contact Name: __________________________________
Emergency Contact Phone Number: ___________________________

**Have you diagnosed with any of the conditions below? If so, place check by the condition, and provide a date (month/year) of diagnosis by medical professional.**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Month/Year</th>
<th>Condition</th>
<th>Month/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td></td>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Bronchitis</td>
<td></td>
<td>Ischemic heart disease</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td></td>
<td>Myocardial infarction</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td></td>
<td>Congestive heart failure</td>
<td></td>
</tr>
<tr>
<td>Pleural effusion</td>
<td></td>
<td>Arrhythmia</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td></td>
<td>Arterial disease</td>
<td></td>
</tr>
<tr>
<td>Rheumatic fever or arthritis</td>
<td></td>
<td>Unstable angina</td>
<td></td>
</tr>
<tr>
<td>Dementia</td>
<td></td>
<td>Valvular disease</td>
<td></td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td></td>
<td>Stroke</td>
<td></td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td></td>
<td>Other (please list)</td>
<td></td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please describe any medications you are taking. List the medications and describe the condition.

___________________________________________________________________________
___________________________________________________________________________
___________________________________________________________________________
Please describe any drug allergies that you are aware of:
___________________________________________________________________________
___________________________________________________________________________

Please describe any spring allergies:
___________________________________________________________________________
___________________________________________________________________________

List any surgical interventions, hospitalizations for severe injuries, and disabilities. Please include month and year.
___________________________________________________________________________
___________________________________________________________________________
___________________________________________________________________________

Have you ever smoked? Yes or No (circle one)
If yes, are you still smoking? Yes or No (circle one)
If you have quit, when did you quit? ______________

Do you consume caffeine and/or alcohol? How often and how much do you drink?
___________________________________________________________________________
___________________________________________________________________________

How much sleep do you average in one night? Do you wake up feeling rested in the morning?
___________________________________________________________________________
___________________________________________________________________________

Do you exercise? How often and how long do you exercise for? What kind of exercises do you do?
___________________________________________________________________________
___________________________________________________________________________
___________________________________________________________________________
Physical activity log

Use this form to record daily physical activities from hour to hour over three days. Please include two weekdays and one weekend day.

Name: ________________________________________
Start date/day: __________________
End date/day: __________________

DAY:

<table>
<thead>
<tr>
<th>TIME</th>
<th>ACTIVITY</th>
<th>TIME</th>
<th>ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15 min</td>
<td>16-30 min</td>
<td>31-45 min</td>
<td>46-60 min</td>
</tr>
<tr>
<td>Mid night</td>
<td>Noon</td>
<td>1:00 am</td>
<td>1:00 pm</td>
</tr>
</tbody>
</table>

(Actual log included two more sheets as given here.)
Data Collection Sheet

**Pretest / Post-test**

Name: ________________________________________________ Subject number:  
Age: ____________
Height: ____________________________________  
Weight: ____________________________________  
BMI: ____________  

Maximal Inspiratory Pressure (MIP): ____________  

<table>
<thead>
<tr>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
</table>

Maximal voluntary ventilation (MVV): ____________  

Vital capacity (VC): ____________  

Forced expiration volume (FEV\(_1\)): ____________  

<table>
<thead>
<tr>
<th>VC</th>
<th>FEV(_1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td></td>
</tr>
<tr>
<td>Trial 2</td>
<td></td>
</tr>
<tr>
<td>Trial 3</td>
<td></td>
</tr>
</tbody>
</table>

Visual Analog Scale:  

<table>
<thead>
<tr>
<th>No</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress /Depression</td>
<td>Stress/ depression</td>
</tr>
</tbody>
</table>

Respiratory rate:
## TRAINING RECORD

Subject Number:

<table>
<thead>
<tr>
<th>WEEK</th>
<th>TRAINING LOAD ( 80% MIP)</th>
<th>POWERBREATHE POSITION</th>
<th>DAY</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>week 1</td>
<td></td>
<td></td>
<td>day 1</td>
<td></td>
</tr>
<tr>
<td>week 2</td>
<td></td>
<td></td>
<td>day 1</td>
<td></td>
</tr>
<tr>
<td>week 3</td>
<td></td>
<td></td>
<td>day 1</td>
<td></td>
</tr>
<tr>
<td>week 4</td>
<td></td>
<td></td>
<td>day 1</td>
<td></td>
</tr>
<tr>
<td>week 5</td>
<td></td>
<td></td>
<td>day 1</td>
<td></td>
</tr>
<tr>
<td>week 6</td>
<td></td>
<td></td>
<td>day 1</td>
<td></td>
</tr>
</tbody>
</table>

MIP Trial 1: Trial 2: Trial 3:
Appendix C

PowerBreathe Force Chart
### Powerbreathe Force Chart

<table>
<thead>
<tr>
<th>Position</th>
<th>Voltage</th>
<th>cm H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.196</td>
<td>19.6</td>
</tr>
<tr>
<td>1.5</td>
<td>0.316</td>
<td>31.6*</td>
</tr>
<tr>
<td>2</td>
<td>0.436</td>
<td>43.6</td>
</tr>
<tr>
<td>2.5</td>
<td>0.582</td>
<td>58.2*</td>
</tr>
<tr>
<td>3</td>
<td>0.728</td>
<td>72.8</td>
</tr>
<tr>
<td>3.5</td>
<td>0.893</td>
<td>89.3*</td>
</tr>
<tr>
<td>4</td>
<td>1.058</td>
<td>105.8</td>
</tr>
<tr>
<td>4.5</td>
<td>1.150</td>
<td>115.0*</td>
</tr>
<tr>
<td>5</td>
<td>1.241</td>
<td>124.1</td>
</tr>
<tr>
<td>5.5</td>
<td>1.412</td>
<td>141.2*</td>
</tr>
<tr>
<td>6</td>
<td>1.583</td>
<td>158.3</td>
</tr>
<tr>
<td>6.5</td>
<td>1.869</td>
<td>172.6*</td>
</tr>
<tr>
<td>7</td>
<td>1.989</td>
<td>186.9</td>
</tr>
<tr>
<td>7.5</td>
<td>2.109</td>
<td>198.9*</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>210.9</td>
</tr>
<tr>
<td>8.5</td>
<td>2.229</td>
<td>222.9*</td>
</tr>
<tr>
<td>9</td>
<td>2.350</td>
<td>235.0*</td>
</tr>
<tr>
<td>9.5</td>
<td>2.470</td>
<td>247.0*</td>
</tr>
<tr>
<td>10</td>
<td>2.609</td>
<td>260.9*</td>
</tr>
</tbody>
</table>

* = Estimated values
Appendix D

Raw subject data
### Descriptive subject characteristics

#### Young group

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Height (cm)</th>
<th>Pre-weight (kg)</th>
<th>Post-weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>TEE (Kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20</td>
<td>F</td>
<td>166</td>
<td>73.93</td>
<td>76.65</td>
<td>26.83</td>
<td>3324.81</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>M</td>
<td>192</td>
<td>92.53</td>
<td>87.99</td>
<td>25.10</td>
<td>3706.68</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>M</td>
<td>184</td>
<td>87.99</td>
<td>86.63</td>
<td>25.99</td>
<td>4175.75</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>F</td>
<td>175</td>
<td>71.66</td>
<td>71.66</td>
<td>23.40</td>
<td>3527.58</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>M</td>
<td>175</td>
<td>76.65</td>
<td>77.56</td>
<td>25.03</td>
<td>3026.63</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>F</td>
<td>175</td>
<td>81.19</td>
<td>80.28</td>
<td>26.51</td>
<td>3215.41</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>M</td>
<td>181</td>
<td>75.29</td>
<td>75.29</td>
<td>22.98</td>
<td>2250.42</td>
</tr>
<tr>
<td>13</td>
<td>20</td>
<td>F</td>
<td>172</td>
<td>64.18</td>
<td>61.68</td>
<td>21.69</td>
<td>2567.54</td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>M</td>
<td>187</td>
<td>77.79</td>
<td>75.74</td>
<td>22.25</td>
<td>3582.37</td>
</tr>
<tr>
<td>15</td>
<td>22</td>
<td>F</td>
<td>174</td>
<td>63.95</td>
<td>63.95</td>
<td>21.12</td>
<td>3485.28</td>
</tr>
<tr>
<td>16</td>
<td>22</td>
<td>M</td>
<td>186</td>
<td>87.54</td>
<td>86.18</td>
<td>25.30</td>
<td>4717.08</td>
</tr>
<tr>
<td>17</td>
<td>27</td>
<td>F</td>
<td>165</td>
<td>58</td>
<td>58.8</td>
<td>21.30</td>
<td>2336.00</td>
</tr>
</tbody>
</table>

#### Older group

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Height (cm)</th>
<th>Pre-weight (kg)</th>
<th>Post-weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>TEE (Kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>73</td>
<td>M</td>
<td>172</td>
<td>90.71</td>
<td>90.71</td>
<td>30.66</td>
<td>3642.91</td>
</tr>
<tr>
<td>3</td>
<td>83</td>
<td>M</td>
<td>174</td>
<td>69.39</td>
<td>69.85</td>
<td>22.92</td>
<td>2748.60</td>
</tr>
<tr>
<td>4</td>
<td>76</td>
<td>F</td>
<td>165</td>
<td>66.67</td>
<td>67.13</td>
<td>24.49</td>
<td>2896.32</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>F</td>
<td>165</td>
<td>73.02</td>
<td>71.21</td>
<td>26.82</td>
<td>3155.75</td>
</tr>
<tr>
<td>6</td>
<td>77</td>
<td>M</td>
<td>169</td>
<td>82.1</td>
<td>83.91</td>
<td>28.75</td>
<td>3735.23</td>
</tr>
<tr>
<td>7</td>
<td>78</td>
<td>F</td>
<td>162</td>
<td>71.21</td>
<td>70.76</td>
<td>27.13</td>
<td>3002.19</td>
</tr>
<tr>
<td>8</td>
<td>64</td>
<td>F</td>
<td>152</td>
<td>63.5</td>
<td>61.23</td>
<td>27.48</td>
<td>2818.07</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>F</td>
<td>166</td>
<td>52.61</td>
<td>52.65</td>
<td>19.09</td>
<td>2165.55</td>
</tr>
<tr>
<td>10</td>
<td>63</td>
<td>F</td>
<td>159</td>
<td>68.94</td>
<td>69.85</td>
<td>27.27</td>
<td>2890.53</td>
</tr>
<tr>
<td>11</td>
<td>67</td>
<td>F</td>
<td>168</td>
<td>86.63</td>
<td>86.63</td>
<td>30.69</td>
<td>3751.66</td>
</tr>
<tr>
<td>12</td>
<td>77</td>
<td>M</td>
<td>181</td>
<td>76.2</td>
<td>76.2</td>
<td>23.26</td>
<td>3209.54</td>
</tr>
<tr>
<td>13</td>
<td>63</td>
<td>F</td>
<td>162</td>
<td>65.31</td>
<td>64.86</td>
<td>24.89</td>
<td>2730.10</td>
</tr>
<tr>
<td>14</td>
<td>72</td>
<td>F</td>
<td>166</td>
<td>77.56</td>
<td>76.2</td>
<td>28.15</td>
<td>3330.44</td>
</tr>
<tr>
<td>15</td>
<td>72</td>
<td>M</td>
<td>171</td>
<td>83.46</td>
<td>82.1</td>
<td>28.54</td>
<td>3528.64</td>
</tr>
</tbody>
</table>
Heart rate variability data included in statistical analysis

<table>
<thead>
<tr>
<th>Young group</th>
<th>subject #</th>
<th>TP (s²)</th>
<th>VLF (s²)</th>
<th>LF (s²)</th>
<th>HF (s²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pre</td>
<td>post</td>
<td>pre</td>
<td>post</td>
</tr>
<tr>
<td>2</td>
<td>8.191</td>
<td>5.600</td>
<td>2.056</td>
<td>0.535</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.217</td>
<td>4.790</td>
<td>1.985</td>
<td>0.441</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>24.798</td>
<td>15.831</td>
<td>7.043</td>
<td>1.923</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20.705</td>
<td>13.823</td>
<td>5.321</td>
<td>1.562</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>14.765</td>
<td>9.746</td>
<td>3.811</td>
<td>1.208</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10.027</td>
<td>6.801</td>
<td>2.609</td>
<td>0.617</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>13.703</td>
<td>8.684</td>
<td>4.095</td>
<td>0.924</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>24.215</td>
<td>16.093</td>
<td>5.814</td>
<td>2.308</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>21.382</td>
<td>13.737</td>
<td>5.362</td>
<td>2.283</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>21.940</td>
<td>13.741</td>
<td>6.185</td>
<td>2.014</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>9.639</td>
<td>6.623</td>
<td>2.430</td>
<td>0.586</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Older group</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pre</td>
<td>post</td>
<td>pre</td>
<td>post</td>
</tr>
<tr>
<td>2</td>
<td>18.272</td>
<td>12.537</td>
<td>4.651</td>
<td>1.083</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>21.850</td>
<td>13.692</td>
<td>5.194</td>
<td>2.965</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>17.745</td>
<td>11.543</td>
<td>4.427</td>
<td>1.774</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15.809</td>
<td>10.719</td>
<td>4.131</td>
<td>0.959</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>77.802</td>
<td>30.139</td>
<td>28.004</td>
<td>19.658</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10.538</td>
<td>7.156</td>
<td>2.743</td>
<td>0.638</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>13.882</td>
<td>9.356</td>
<td>3.668</td>
<td>0.858</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>17.542</td>
<td>10.912</td>
<td>4.510</td>
<td>2.119</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>19.869</td>
<td>13.609</td>
<td>5.110</td>
<td>1.150</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>12.930</td>
<td>8.597</td>
<td>3.486</td>
<td>0.847</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>14.372</td>
<td>9.841</td>
<td>3.606</td>
<td>0.926</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>9.821</td>
<td>6.615</td>
<td>2.580</td>
<td>0.626</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>9.177</td>
<td>6.228</td>
<td>2.392</td>
<td>0.557</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>10.323</td>
<td>7.062</td>
<td>2.593</td>
<td>0.668</td>
<td></td>
</tr>
</tbody>
</table>
Heart rate variability data included in statistical analysis continued

<table>
<thead>
<tr>
<th>Younger group</th>
<th>subject #</th>
<th>LFnu (nu)</th>
<th>HFnu (nu)</th>
<th>LF/HF</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pre</td>
<td>post</td>
<td>pre</td>
<td>post</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.794</td>
<td>0.812</td>
<td>0.206</td>
<td>0.188</td>
<td>3.843</td>
<td>0.977</td>
</tr>
<tr>
<td>3</td>
<td>0.818</td>
<td>0.759</td>
<td>0.182</td>
<td>0.241</td>
<td>4.499</td>
<td>1.078</td>
</tr>
<tr>
<td>4</td>
<td>0.785</td>
<td>0.751</td>
<td>0.215</td>
<td>0.249</td>
<td>3.662</td>
<td>1.046</td>
</tr>
<tr>
<td>5</td>
<td>0.773</td>
<td>0.748</td>
<td>0.227</td>
<td>0.252</td>
<td>3.407</td>
<td>1.033</td>
</tr>
<tr>
<td>7</td>
<td>0.759</td>
<td>0.772</td>
<td>0.241</td>
<td>0.228</td>
<td>3.154</td>
<td>0.983</td>
</tr>
<tr>
<td>9</td>
<td>0.809</td>
<td>0.791</td>
<td>0.191</td>
<td>0.209</td>
<td>4.227</td>
<td>1.022</td>
</tr>
<tr>
<td>12</td>
<td>0.816</td>
<td>0.704</td>
<td>0.184</td>
<td>0.296</td>
<td>4.431</td>
<td>1.158</td>
</tr>
<tr>
<td>13</td>
<td>0.716</td>
<td>0.798</td>
<td>0.284</td>
<td>0.202</td>
<td>2.520</td>
<td>0.897</td>
</tr>
<tr>
<td>14</td>
<td>0.818</td>
<td>0.776</td>
<td>0.182</td>
<td>0.224</td>
<td>4.506</td>
<td>1.055</td>
</tr>
<tr>
<td>15</td>
<td>0.701</td>
<td>0.590</td>
<td>0.299</td>
<td>0.410</td>
<td>2.348</td>
<td>1.188</td>
</tr>
<tr>
<td>16</td>
<td>0.754</td>
<td>0.774</td>
<td>0.246</td>
<td>0.226</td>
<td>3.072</td>
<td>0.975</td>
</tr>
<tr>
<td>17</td>
<td>0.806</td>
<td>0.802</td>
<td>0.194</td>
<td>0.198</td>
<td>4.149</td>
<td>1.005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Older group</th>
<th></th>
<th>pre</th>
<th>post</th>
<th>pre</th>
<th>post</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.811</td>
<td>0.821</td>
<td>0.189</td>
<td>0.179</td>
<td>4.293</td>
<td>4.597</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.637</td>
<td>0.777</td>
<td>0.363</td>
<td>0.223</td>
<td>1.752</td>
<td>3.491</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.714</td>
<td>0.784</td>
<td>0.286</td>
<td>0.216</td>
<td>2.495</td>
<td>3.621</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.812</td>
<td>0.815</td>
<td>0.188</td>
<td>0.185</td>
<td>4.308</td>
<td>4.398</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.588</td>
<td>0.699</td>
<td>0.412</td>
<td>0.301</td>
<td>1.425</td>
<td>2.320</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.811</td>
<td>0.809</td>
<td>0.189</td>
<td>0.191</td>
<td>4.296</td>
<td>4.246</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.810</td>
<td>0.811</td>
<td>0.190</td>
<td>0.189</td>
<td>4.276</td>
<td>4.290</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.680</td>
<td>0.799</td>
<td>0.320</td>
<td>0.201</td>
<td>2.128</td>
<td>3.971</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.816</td>
<td>0.803</td>
<td>0.184</td>
<td>0.197</td>
<td>4.442</td>
<td>4.074</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.805</td>
<td>0.799</td>
<td>0.195</td>
<td>0.201</td>
<td>4.118</td>
<td>3.982</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.796</td>
<td>0.806</td>
<td>0.204</td>
<td>0.194</td>
<td>3.896</td>
<td>4.151</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.805</td>
<td>0.807</td>
<td>0.195</td>
<td>0.193</td>
<td>4.121</td>
<td>4.171</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.811</td>
<td>0.814</td>
<td>0.189</td>
<td>0.186</td>
<td>4.294</td>
<td>4.366</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.795</td>
<td>0.777</td>
<td>0.205</td>
<td>0.223</td>
<td>3.882</td>
<td>3.492</td>
<td></td>
</tr>
</tbody>
</table>
## Pulmonary function measures and MIP data

<table>
<thead>
<tr>
<th>Young group</th>
<th>subject #</th>
<th>VC (liter)</th>
<th>FEV1 (liter)</th>
<th>MVV (liter/min)</th>
<th>MIP (cmH₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pre</td>
<td>post</td>
<td>pre</td>
<td>post</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3.5</td>
<td>3.7</td>
<td>3.2</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>6.5</td>
<td>6.5</td>
<td>5.1</td>
<td>5.3</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>3.3</td>
<td>5.9</td>
<td>3.2</td>
<td>3.9</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>4.3</td>
<td>4.5</td>
<td>3.8</td>
<td>3.7</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>3.6</td>
<td>3.7</td>
<td>1.8</td>
<td>2.8</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>4.5</td>
<td>4.2</td>
<td>3.3</td>
<td>3.5</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>4.5</td>
<td>4.6</td>
<td>4.2</td>
<td>4.4</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>3.5</td>
<td>3.5</td>
<td>3</td>
<td>3.2</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>4.2</td>
<td>4.4</td>
<td>3.9</td>
<td>4.2</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>3.5</td>
<td>3.8</td>
<td>2.6</td>
<td>2.7</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>5.2</td>
<td>5.3</td>
<td>4.2</td>
<td>4.3</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>2.5</td>
<td>2.6</td>
<td>2</td>
<td>2.3</td>
</tr>
</tbody>
</table>

| Older group |           |           |               |                 |             | pre          | post          |
|-------------|-----------|------------|--------------|-----------------|--------------|
| 2           |           | 3.2        | 2.8          | 2.6             | 2.3          | 114          | 109           | 0.44          | 0.76          |
| 3           |           | 2.7        | 2.9          | 2.3             | 2.4          | 103          | 98            | 0.46          | 0.86          |
| 4           |           | 2.1        | 1.9          | 1.7             | 1.6          | 66           | 84            | 0.56          | 0.99          |
| 5           |           | 2.1        | 1.8          | 1.6             | 1.4          | 77           | 76            | 0.83          | 1.07          |
| 6           |           | 2.4        | 2.8          | 2               | 2            | 90           | 96            | 1.14          | 1.31          |
| 7           |           | 2          | 2.1          | 1.6             | 1.5          | 53           | 68            | 0.53          | 0.81          |
| 8           |           | 2.3        | 2.3          | 1.8             | 1.8          | 85           | 90            | 0.99          | 1.11          |
| 9           |           | 1.2        | 2.1          | 1.1             | 1.7          | 71           | 61            | 0.43          | 0.84          |
| 10          |           | 2.4        | 2.5          | 2               | 2            | 98           | 100           | 0.82          | 0.98          |
| 11          |           | 2.3        | 2.6          | 2.1             | 2.5          | 94           | 110           | 0.54          | 0.97          |
| 12          |           | 2.6        | 3            | 2.2             | 2.5          | 98           | 106           | 0.68          | 0.97          |
| 13          |           | 2.7        | 3            | 2.2             | 2.4          | 101          | 110           | 0.93          | 1.04          |
| 14          |           | 3          | 3            | 2.5             | 2.5          | 118          | 118           | 0.6           | 1.15          |
| 15          |           | 3.1        | 3.3          | 2.6             | 2.6          | 126          | 124           | 1.12          | 0.97          |