Reproducibility of heart rate variability responses to lower body negative pressure

By

Dwayne Buchanan

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Exercise and Sport Science

Major Professor: Warren D. Franke

Iowa State University

Ames, Iowa

2000

Graduate College
Iowa State University

This is to certify that the Master's thesis of

Dwayne Bernard Buchanan

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

The cardiovascular system is an intricate mechanism that provides the body with nutrients and a means of gas exchange with the tissues and organelles. The heart is the organ responsible for pumping the blood throughout the body and providing pulmonary circulation for gas exchange in the lungs. The cardiovascular system is one of many systems in the body controlled by the Autonomic Nervous System (ANS). The latter is responsible for providing signals that cause either an increase in the heart rate, via increased sympathetic nervous system (SNS) activity, or a decrease by way of either an increase in parasympathetic nervous system (PNS) activity or a decrease in SNS activity.

The SNS and PNS regulate the heart rate with the use of baroreceptors that are located in the aortic arch, the carotid arteries and the heart. When the blood pressure in these receptor areas is low, there is a signal sent to the medulla oblongata (the portion of the brain responsible for autonomic function) which inhibits PNS activity to reduce vagal tone and stimulates the SNS to release epinephrine and norepinephrine (Sander-Jensen, Mehlsen, Stadeager, Christensen, Fahrenkrug, Schwartz, Warberg, and Bie, 1988). These catecholamines cause the heart rate to accelerate and elicit vasoconstriction thereby elevating blood pressure. If the blood pressure is elevated too much, the baroreceptors increase the amount of action potentials sent to the medulla; the SNS is inhibited and the PNS is stimulated, causing the release of acetylcholine (Ach) from the vagus nerve (Sander-Jensen et al., 1988). Peripheral vasodilation and a decline in heart rate result.

Heart rate variability (HRV) refers to the beat-to-beat temporal fluctuations that the heart undergoes. These occur because of the fluctuations in the neural outflow of the

SNS and the PNS. This beat-to-beat variability is difficult to determine by common measures such as measuring heart rate. Spectral analysis of HRV is a useful tool to assess the modulation of heart rate by PNS and SNS. It is begun by reducing the heart rate time signal into a frequency component and quantifying its relative power (Lipsitz, Mietus, Moody, and Goldberger, 1990). Spectral analysis uses frequencies between 0 to ~0 .4 Hz to assess the autonomic nervous system modulation of the cardiovascular system. A frequency component of 0.04 to 0.15 Hz reflects the effects of SNS activity; this is termed low frequency (LF). High frequency (HF) is found to be from 0.15 to 0.4 Hz and implies the presence of increased PNS activity.

For centuries, researchers have been trying to develop a better understanding of how the cardiovascular system is controlled. There is a link between the control of heart rate and the body's ability to resist a sudden change in the velocity of the body (Halliwill, Lawler, Eickoff, Joyner, and Mulvagh, 1998). For example, when a jet pilot pulls up from a dive, blood pools into the legs causing a decrease in the amount of blood circulating in the upper torso. This reduced cerebral blood flow causes a feeling of dizziness for a few moments until the cardiovascular system responds. The resultant increase in heart rate and peripheral constriction causes blood flow to the brain to normalize.

Older people, hypotensive populations and people with various cardiac diseases often experience this feeling of dizziness (presyncope) during orthostatic stress (Luutonen, Antila, Neuvonen, Raiha, Rajala, and Sourander, 1994). Astronauts also experience this feeling of near-syncope after prolonged exposure to microgravity (Verbanck, Larsson, Linnarsson, Prisk, West, and Paiva, 1997). This phenomenon is also seen in subjects

exposed to bed-rest for a lengthy period of time (Halliwill et al., 1998). Bed-rest can cause the baroreflex centers to become less reactive to an orthostatic stress (Halliwill et al., 1998). The population most susceptible to orthostatic intolerance is elderly people who are placed on bed rest because of a past ailment (Luutonen et al., 1994).

Lower body negative pressure testing has been used to determine the status of baroreceptor reflex function, assess the cardiovascular response to orthostatic stress, and assess tolerance. However, relatively few studies have involved the use of the lower body negative pressure with concurrent spectral reading of the heart. In order to use any of the heart rate variability indices for clinical purposes, the values must be shown to be reproducible. Repeatable results using spectral analysis of HRV and the table-tilt test as an orthostatic stress have been found (Kockiadakis, Kanoupakis, Rombola, Igoumenidis, Chlouverakis, and Vardas, 1998; Vardas, Kochiadakis, Orfanakis, Kalaitzakis, and Manios, 1994) while another study has shown repeatability of heart rate and blood pressure responses using lower body negative pressure to induce a physiological stress (Lightfoot, Hilton, and Fortney, 1991). However, there have been no published studies assessing the reproducibility of heart rate variability using lower body negative pressure.

Statement of the Problem

The tilt table test is a common method used to induce an orthostatic stress on patients in the clinical environment. LBNP is a tool used for inducing an orthostatic stress on individuals in a research environment. Heart rate variability responses are documented as being reproducible when assessed with the orthostatic stress of a tilt test. Hemodynamic

responses such as heart rate and blood pressure have also been shown to be reproducible using LBNP. However, there are no published data showing the reproducibility of heart rate variability responses using LBNP. Therefore, the purpose of this study was to determine if spectral analyses of the heart rate variability responses to lower body negative pressure are reproducible.

Research Hypothesis

Ho: The heart rate, heart rate variability, and spectral analysis values from two separate LBNP tests will be similar within subjects.

CHAPTER 2: REVIEW OF LITERATURE

This review of literature examines the components of the cardiovascular system which are responsible for the compensatory effects to changes in blood pressure. Lower body negative pressure testing is also discussed. The use of spectral analysis of heart rate variability as a tool for determining frequency differences is examined. The final segment of this review examines the reproducibility of heart rate variability indices and the importance of further studies.

Cardiovascular Control

The cardiovascular system is an important organ system because of its role in circulating blood, lymph, and nutrients throughout the extremities of the body. The cardiovascular system is also responsible for maintaining the temperature in the body by changing the flow of the blood within the body. Because of the need for blood, it is important to maintain adequate blood pressure to keep all the areas of the body appropriately perfused. Consequently, maintaining blood pressure homeostasis is a fundamental role of the cardiovascular control system. An important component of this system is the baroreceptors.

Baroreceptors are pressure-sensitive stretch receptors that help to maintain the blood pressure within the body. The baroreceptors are afferently connected to the medulla oblongata of the brain via the glossopharyngeal and vagus nerves. When these receptors are not stretched due to a decrease in blood pressure, there is a decrease in action potentials and a decrease in the inhibitory signals sent to the control centers of the medulla

oblongata. This results in an increase in sympathetic activity and a decrease in parasympathetic nerve activity. The result is an increase in heart rate and peripheral vasoconstriction that increases blood pressure (Fox, 1991). When the blood pressure is elevated, the receptors are stretched and the frequency of action potentials, sent to the cardiac control center, are increased. This increase in the inhibitory signals to the control center causes a decrease in heart rate and an increased vasodilation (Fox, 1991).

There are two types of baroreceptors: cardiopulmonary and arterial receptors. Cardiopulmonary baroreceptors are low-pressure receptors that are located within the atria and the lungs. These receptors are responsible for detecting changes in central venous pressure and central venous return. By this mechanism, forearm vascular resistance can be increased without significant changes in blood pressure, heart rate, or mean arterial pressure (Desai, Collins, Snell, and Mosqueda-Garcia, 1997). Lower body negative pressure testing has been used to determine the negative pressure that effectively decreased the blood pressure detected by the cardiopulmonary receptors. Cardiopulmonary baroreceptors are affected with graded LBNP from –5 to –20 mmHg (Johnson, Rowell, Niederberger, and Eisman, 1974). Zoller, Mark, Abboud, Schmid, and Heistad (1972) concluded that a lower body negative pressure of –10 mmHg could successfully unload the cardiopulmonary baroreceptors without unloading the arterial receptors. This process results in a decrease in central blood volume and central venous pressure. This also causes an increase in vascular resistance without the significant increase in blood pressure or heart rate commonly observed during arterial unloading (LaColley, Pannier, Slama, Cuche, Hoeks, Laurent, London, and Safar, 1992).

Cardiopulmonary receptors are also involved in the vasoconstrictor response to venous pooling (Zoller, 1972). Zoller and colleagues found vasoconstriction within the forearms of the subjects during LBNP without any remarkable changes in the arterial pressure. Rowell, Wyss, and Brengelmann (1973) noted a contribution of both skin and muscle vascular beds to the compensatory vasoconstriction in the forearm during LBNP; 50% of the decrease in total forearm blood flow is due to vasoconstriction in the skin.

Arterial baroreceptors are high-pressure receptors that reside in the carotid sinus and aortic arch. These receptors are affected by a change in the systemic arterial pressure and carotid arterial pulse pressure. LBNP of –40mmHg decreases the amount of blood at the carotid sinus baroreceptor and increases pooling of blood in the lower extremities which causes vasoconstriction of the splanchnic vessels, increased forearm resistance and tachycardia (Melchior, Srinivasan, Thullier, and Clere, 1994). Abboud, Eckberg, Johannsen, and Mark (1979) found a decrease in splanchnic blood flow during –40 mmHg accompanied with an increase in heart rate. This finding suggest that there is increased vascular resistance and an increase in heart rate when the arterial baroreceptors are unloaded.

There is evidence of an interaction between the cardiopulmonary and carotid baroreceptors in cardiovascular hemodynamics. Victor and Mark (1985) demonstrated that an inhibitory interaction between cardiopulmonary and carotid baroreflex control of vascular resistance exists in humans. By selectively unloading the cardiopulmonary receptors with non-hypotensive LBNP and using neck pressure to stimulate the carotid baroreceptors, the vasoconstrictor response was sustained indicating an inhibition of the

carotid baroreflex by the cardiac vagal afferents. By removing this inhibition, during upright posture, the carotid baroreflex stimulates the reflex vasoconstriction (Victor et al., 1985). Pawelczyk and Raven (1989) suggested that the reductions in central venous pressure and/or central blood volume augment heart rate and blood pressure by reducing an inhibitory influence from the cardiopulmonary receptors on the arterial baroreceptors.

Spectral Analysis of Heart Rate Variability

Heart rate variability refers to the beat-to-beat fluctuations in heart rate caused by the autonomic nervous system. These fluctuations are known to be caused when there is a change in the modulation of the efferent output of the parasympathetic or sympathetic nervous systems on the cardiovascular system (Pomeranz, 1985). When analyzed appropriately, these fluctuations in HRV can reveal various characteristics indicative of autonomic nervous system abnormalities. Another method of analyzing HRV, spectral analysis, has been used as a non-invasive technique for examining the functions of the autonomic nervous system. Spectral analysis has been useful in detecting sympathetic and parasympathetic failure (Luutonen, 1994).

Spectral analysis of HRV uses the time domain of the HRV signal and converts it to a frequency signal, producing a more coherent signal to be analyzed. The frequency signal read in the spectrum is typically between 0.01 to 0.50 Hz and is referred to as the total power spectrum. A power spectrum of 0.15 to 0.4 Hz is called the high frequency (HF) band spectrum and is associated with parasympathetic activity. The spectral reading between 0.01 to 0.15 Hz is referred to as the low frequency band and is associated with

respiratory and sympathetic activity (Luutonen et al., 1994). There is still some uncertainty about the amount of sympathetic activity that occurs in the low-frequency spectral band (Pomeranz et al., 1985). Because of this uncertainty, a computation of the low-frequency/ high-frequency ratio (LF/HF ratio) has been used as an index of sympathovagal interaction (Luutonen et al., 1994) or sympathetic activity. Another spectral band, very low frequency (VLF) which is less than 0.01 Hz, is much less defined and does not have coherent properties. VLF assessed from short-term recordings are dubious and should be avoided when power spectral densities of short-term ECGs are interpreted (Task force, 1996).

The influence of respiration has been an important factor frequently overlooked in the study of HRV. Novak, P. Novak, De Champlain, Le Blanc, Martin, and Nadeau (1993) studied the effects of respiration on heart rate and blood pressures fluctuations. Sixteen subjects (14 males and 2 females) inhaled and exhaled in synchrony with a tone to regulate the frequency of breathing. The LF/HF ratio from a global spectra were elevated (P=0.05) over the non-respiratory/respiratory frequency content indexes; this leads to false positive global spectral indexes (Novak et al., 1993). Breath-to-breath slowing of respiration from 0.46 to 0.05 Hz continuously paced the respiratory fluctuations in the R-R intensity, systolic blood pressure (SBP) and diastolic blood pressure (DBP) over the entire period; this indicated the control of respiration frequency on the time-frequency distribution (Novak et al., 1993). Novak and colleagues (1993) concluded that respiration can override the hemodynamic fluctuations in both the time and frequency domains and the

0.012 to 0.017 Hz rhythm reflects the brain stem network common to cardiovascular and respiratory systems.

Brown, Beightol, Koh, and Eckberg (1993) also studied the influence of respiration on R-R interval power spectra. Nine subjects were subjected to an array of breathing protocols at two tidal volumes (1,000 and 1,500 ml). They found a significant decrease in power at the respiratory frequency as the respiratory rate increased ($P< 0.001$). Major reductions occurred between the respiratory rates of 0.125 to 0.25 Hz (7.5 -15 breaths/minute) at both tidal volumes of 1,000 and 1,500 ml. They concluded that breathing frequencies strongly influence low-frequency power spectra and should be accounted or controlled for when evaluating HRV (Brown et al., 1993).

Schmitz, Claus, Neundorfer, and Handwerker (1995) compared different algorithms for evaluating respiratory sinus arrhythmia using 11 subjects. They found, by regression analysis, that the heart rate increase is more closely coupled to inspiration than the heart rate decrease to expiration (Schmitz et al., 1995).

Cooke, Cox, Diedrich, Taylor, Beightol, Ames, Hoag, Seidel, and Eckberg (1998) studied breathing protocols and cardiovascular rhythms and determined that the most efficient assessment can be made using a step-wise protocol without stringent control of inspired volume. Ten subjects followed 5 breathing protocols while R-R intervals and arterial pressure spectral power were measured. Control of inspired volume reduced R-R interval spectral power during 0.1 Hz breathing ($P < 0.05$). During stepwise breathing, the arterial pressure and the R-R interval spectral power increased as breathing frequency decreased. They concluded that a fixed and random-rate breathing protocol can decrease

CO_2 chemoreceptor stimulation. They also noted that a stepwise protocol without stringent control of inspired volume allows for the most efficient assessment (Cooke et al, 1998). Breathing at a frequency greater than 0.10 Hz does not influence low-frequency R-R interval or arterial pressure rhythms.

Reproducibility

Reproducibility of the HRV responses is important in identifying and interpreting true meaningful values for the spectral band readings. Such values allow clinicians to make inferential diagnoses of autonomic dysfunction such as predicting risk for sudden cardiac death. The reproducibility of HRV measures were observed by Kleiger, Bigger, Bosner, Chung, Cook, Rolnitzky, Steinman, and Fleiss (1991) using normal healthy subjects. Fourteen male subjects were studied for reproducibility of time and frequency domain measurements using 24-hour Holter monitoring. A Holter monitor is a portable ECG machine used to record ambulatory measurements. The baseline and placebo recordings were reproducible, showing a correlation >0.9 between the root mean square successive difference (r-MSSD), night/day difference (NDDiff) and high frequency power. Low frequency power was correlated with r-MSSD ($r = 0.9$), the proportion of adjacent normal R-R intervals differing by > 50ms [pNN50 ($r = 0.8$)], and high frequency power ($r = 0.9$) indicating a strong dependency of low frequency power on vagal tone in these normal individuals (Kleiger et al., 1991). They concluded that intraclass correlation ($r = 0.8$) as well as group measurements in normal subjects are remarkably stable (Kleiger et al., 1991).

A repeatability experiment was performed by Cloarec-Blanchard, Funck-Brentano, Lipski, Jaillon, and Macquin-Mavier (1997) on healthy young male subjects using nitroglycerin (NTG) and 60° of head-up tilt to stimulate tachycardia. This study used 10 volunteers aged 21-29 years for the nitroglycerin study and 13 volunteers aged 22-40 years for the tilt table study. Volunteers for the NTG study were placed in a supine position with an indwelling catheter placed in the left antecubital vein. Sodium chloride (0.9%) was infused while baseline recordings were taken for 30 minutes. After baseline recordings were taken, 0.45mg of NTG was infused in 1 minute and recordings were taken for 10 minutes. These subjects were brought back to the laboratory 24 hours later to repeat the protocol.

For the head-up-tilt tests each subject was strapped onto a table tilt in a resting supine position for 30 minutes. After the rest period, each subject was brought to a tilt of 60° head up over a 20-second period and was left in this position for 10 minutes. The subjects returned 1 week later and repeated the protocol. They found no difference in heart rate or blood pressure between the two test days. The LF, HF, and LF/HF ratio spectral measures were reproducible. The LF component had a change of 438 +/- 98 for trial 1 and 436 +/- 110 for trial 2. The LF/HF component had a change of 2.84 +/- 0.50 for trial 1 and 2.30 +/- 0.30 for trial 2. These changes were calculated as the difference of the values observed at 60 degrees of tilt minus the values observed at rest. The spectral components were found to be reproducible for both head-up tilt and nitroglycerin intervention (Cloarec-Blanchard et al., 1997).

Pardo, Merz, Paul-Labrador, Velasquez, Gottdiener, Kop, Krantz, Rozanski, and Peter (1996) studied the stability of heart rate variability in subjects with coronary artery disease (CAD). Thirty patients with stable CAD were studied using two consecutive 24 hour Holter monitoring recordings. They had the subjects withhold any anti-ischemic medications or beta-blockers during the study. They found the correlation for HRV measures between days remained high (range 0.871 to 0.983; $p < 0.0001$) despite the stratification by the magnitude of daily life ischemia (Pardo et al., 1996)

Ponikowski, Piepoli, Amadi, Chua, Harrington, Volterrani, Columbo, Mazzuero, Giordano, and Coats (1996) studied the reproducibility of HRV in subjects with chronic heart failure (CHF). They did not use any medical intervention to stimulate tachycardia. Sixteen patients (mean age: 61 +/- 9 years) were used in the study. The subjects reported to the lab on two separate occasions for 60 minutes of recording time. The mean of all normal RR intervals (mean RR), standard deviation of all normal RR intervals (SDRR) and the percentage of adjacent normal RR intervals more than 50 ms different (pNN50) were calculated for the time-domain measures. Frequency domain was expressed in ms^2/Hz. The reproducibility of time-domain and spectral readings were low compared to other studies. Reproducibility of spectral measures was very low for all of the indexes with a variation coefficient range of 45-111%. They recalculated the values under log and square root transformations. Ponikowski and colleagues concluded that the reproducibility of HRV in patients with moderate to severe CHF is low and recordings of 20 minutes in stable, controlled conditions should be taken to optimize signal acquisition

in subjects with CHF so that very low frequency power can be studied (Ponikowsky et al., 1996).

Sloan, Shapiro, Bagiella, Gorman, and Bigger (1995) used psychological challenge to stimulate tachycardia to study the stability of heart period variability. Subjects were exposed to a mental arithmetic task and a reaction time task within a 9-month period. The study found a reproducibility of low-frequency and total power measures but poor reproducibility of the high-frequency measures. This was believed to be different from other psychological studies because of the longevity of the experiment and the difference in methodology.

Vardas and colleagues (1994) examined the intraindividual reproducibility of HRV before and during a postural tilt in patients with syncope of unknown origin. Twenty patients with a history of syncope were tested and placed in two groups. Group A consisted of 11 patients who experienced syncope on one or both of the tilt tests. Group B consisted of 9 patients who did not experience any syncopal episodes during the tests. Spectral indexes (SI) were computed using the Fast Fourier analysis (FFT) of 2-minute segments of Holter recordings. They found that the SI was reproducible between the two test trials (6.43 +/-0.97 vs 6.80 +/- 1.30; P=NS).

Another study by Kochiadakis, Orfanakis, Rombola, Chrysostomakis, Chlouverakis, and Vardas (1997) examined the reproducibility of time-domain indexes of HRV in patients with vasovagal syncope. Nineteen patients with syncopal episodes were used as the experimental group and 15 healthy individuals were used as the control group. Each subject underwent a tilt table test on two separate occasions to determine the differences

in time-domain indexes. The control group showed good reproducibility of all of the HRV indexes (slope of 0.86 to 0.97) (Kochiadakis et al., 1997). The syncope group showed significantly less reproducibility of the rMSSD measure(slope 0.78) and a lack of reproducibility of pNN50 (slope 0.52). The HR, standard deviation of 5-minute mean of all coupling intervals between normal beats (SDANN), and standard deviation about the mean of all coupling intervals between normal beats (SDNN) were highly reproducible. The cause for this difference was due to the difference in HRV indexes between the group that had a negative second tilt test (P-N group) and the group that had a positive second tilt test (P-P group).

Another study by Kochiadakis and colleagues (1998) evaluated the reproducibility of tilt table testing in patients with vasovagal syncope and its relation to variations in autonomic nervous system activity. Thirty-five patients with vasovagal syncope underwent two tilt table tests to derive both time-domain and frequency domain indexes of HRV. Fifteen healthy volunteers also underwent the two tilt tests and served as the control group. The study reported HRV indexes between the patients that experienced syncope during both days of the tilt test (P-P), the group that experienced syncope during one of the days of testing (P-N) and the control group that showed no signs of syncope. The control group did not show any significant change in the mean LF/HF ratio between the two days (.01 +/- .05) and the P-P group also retained stable LF/HF values (0.002 +/- .05). The P-N group experienced a significant difference in the LH/HF ratio (0.10 +/- .06). The study concluded that patients with vasovagal syncope show variations in vagal

autonomic tone and these patients are prone to syncope during times when they have increased parasympathetic tone (Kochiadakis et al., 1998).

There are limited data on the stability of HRV indexes induced by lower body negative pressures (LBNP). Lightfoot and colleagues (1991) examined the repeatability of physiological factors, such as tolerance and heart rate, using LBNP instead of the tilt table. Four males and seven females were used for this study and the modified NASA LBNP protocol was implemented. Each subject was exposed to 4 LBNP tests with the exposures separated by 72 hours. They found similarity in the HR and BP measures between the 4 LBNP tests. The mean difference in peak LBNP minus pre LBNP HR (ΔHR) was 37 +/- 4 bpm for the first and last test. The ΔHR for the second and third tests were 45 +/- 5 bpm and 46 +/- 4 bpm ($p = 0.31$). The mean differences in peak LBNP minus pre LBNP systolic blood pressure (ΔSBP) for the first and second test were –19 +/- 2 mmHg and –24 +/- 2 mmHg. The ΔSBP for the third and last tests were –22 +/- 3 mmHg and –22 +/- 4 mmHg ($p = .70$). The ΔDBP was –1 +/- 2 mmHg for the all the LBNP tests. The correlation coefficients of the repeated tests were between 0.64 to 0.71. These results concluded that HR and BP measures are reproducible.

To date, many studies have been done to show the uses of heart rate variability in the clinical setting. Such studies involved resting undisturbed HRV measures as well as HRV measures perturbed by the induction of a stress. Most of these studies have been repeated to confirm the stability of HRV measures. The tilt test has been performed and spectral components have been reproduced. However, the question still remains unanswered: are spectral components of HRV during LBNP reproducible? LBNP is a direct means of

stimulating orthostatic stress and is commonly used in the experimental environment. Subjects under the LBNP testing protocol reach a definite ending point and this allows for a more accurate assessment of autonomic function. There are no published studies assessing the reproducibility of HRV during LBNP. This area of research is still fertile and needs to be explored in order to use HRV as a tool for accurate assessment of the ANS.

CHAPTER 3: METHODS

General Experiment Design

Sixteen college age students were used for this experiment. Subjects with previous vasovagal reactions were not included. All subjects were made aware of the risks and benefits of the experiment and provided written informed consent prior to data collection. Subjects came to the lab three times: once for orientation and twice for LBNP tolerance tests. Each subject completed a questionnaire to estimate physical activity and VO_2 max (George, Stone, and Burkett, 1996). Height and weight were recorded and body composition was estimated from the sum of three skinfold thickness readings (Jackson and Pollock, 1985).

The lower body negative pressure chamber was used to induce orthostatic stress. An automated sphygmomanometer was used to monitor blood pressure and an electrocardiograph (ECG) was used to monitor heart rate. The ECG data were used to perform the spectral analysis of heart rate variability. Forearm blood flow was measured every 20 seconds using a venous occlusion plethysmograph while cardiac output was determined using impedance cardiography. Breathing was controlled by a timed breathing protocol using a clock.

Orientation Session

Each subject reported to the Hemodynamics Laboratory one week before the first LBNP test to become acquainted with the breathing protocol and the LBNP chamber. After verbal explanation of the procedure to be used, the subject was placed into the LBNP testing chamber in the supine position. The LBNP testing chamber was not activated. Each subject had a laboratory timer placed within view so the display could be easily seen. The display had the time (in seconds) marked with a white and red marker. Each subject inhaled each time the second hand reached a white marker and exhaled when it approaches the red marker. The clock provided a consistent breathing pattern for all the subjects and prevented respiration-related disturbances in cardiac rhythms. Each subject breathed at a frequency of 0.2 Hz (12 respirations per minute) for 6 minutes with no negative pressure imposed (0 mmHg). After completing this timed procedure, the subject was exposed to graded LBNP. The subject underwent decreases in LBNP of -10 mmHg for 2 minute durations up to a maximum of -50 mmHg. The subject continued to breathe at the prescribed rate. This protocol was done 1 week before the true LBNP trials were performed.

LBNP Testing

Each subject reported to the Hemodynamics Laboratory and was instrumented for stroke volume, ECG, and forearm blood flow measurements before assuming the supine position inside the LBNP testing chamber. Once they were inside the chamber, they were sealed at the waist and told to relax for 15 minutes at ambient barometric pressure (0

mmHg). This allowed time for the subject to achieve a homeostatic state. Each subject's breathing frequency was controlled in the aforementioned manner.

The LBNP test consisted of graded LBNP to -100 mmHg, presyncopal symptoms or by subject request. LBNP was increased in 10 mmHg increments every 6 minutes. LBNP was monitored using an electronic barometer. Symptoms of impending presyncope include dizziness, nausea, profuse sweating or a change in blood pressure (decrease in SBP by 25mmHg or a decrease in DBP by 15mmHg within 1 minute). Each subject was monitored for presyncopal symptoms by close observation and questions directed toward the physical condition during each stage. Each subject inspired and expired at the appropriate time points as indicated by the markings throughout the test. The cumulative stress index (CSI) was calculated as the sum of pressures achieved times the amount of time at each pressure. This protocol was repeated one week later.

All subjects were asked to refrain from any exercise, alcohol, and caffeine ingestion 12 hours before their LBNP testing and from any food 3 hours before testing.

Assessment of Cardiovascular Responses

Forearm blood flow was measured using mercury-in-silastic strain gauge plethysmography (D. E. Hokanson, Bellvue, WA; Whitney, 1953). The left forearm was elevated with the wrist suspended slightly higher. The strain gauge was placed around the proximal portion of the forearm about one-third the distance from the olecranon to the ulna styloid and a blood pressure cuff was placed around the biceps. A wrist cuff was also used to occlude circulation to the hand during FBF measurements. The cuff around the

bicep was inflated to 45 mmHg for 10 seconds and was released for 10 seconds. Forearm blood flow was consequently assessed every 20 seconds.

Blood pressure was assessed continuously using a finger photoplethysmograph (Finepres, Critikon, Tampa, FL) on the upper right arm. Heart rate was assessed using standard electrocardiography (ECG) and 5 electrodes. Stroke volume was determined every 30 seconds using a Minnesota Impedance Cardiograph (Model 304B, Surcom, Minneapolis, MN). Two band electrodes were placed around the subject's neck and chest and ECG electrodes were placed on the forehead and the abdomen. This tetrapolar lead configuration has been validated (Gotshall and Sexson, 1994). The analog heart rate, blood pressure, forearm blood flow and LBNP level signal were relayed into an on-line microcomputer system using commercially available software (BIOPAC). The heart rate and impedance cardiograph signals were also analyzed using commercially available software (Microtronics Corp) to determine stroke volume and calculate cardiac output.

Heart Rate Variability Analysis

The analog heart rate signal was entered into a personal computer using an analog to digital converter. Amplification and anti-aliasing filters were used to ensure accurate data digitization from problems arising due to poor signal-to-noise ratios. R waves were detected and processed with an algorithm using the modified threshold method. The power spectrum was calculated using the Welch's averaged periodogram method. The last 256 R-R intervals of the rest period and the last 256 intervals of each level of LBNP were used in the power spectrum calculation. Total power (TP) for each subject was

defined as the area under the HRV spectrum. The HRV spectrum was between the frequencies of 0.0001 to 0.5 Hz. The magnitude of the low frequency (LF) component of the spectrum was based on the spectral amplitudes between 0.04 to 0.15 Hz and the high frequency (HF) component was set at 0.15 to 0.40 Hz (Task Force, 1996). The LF/HF ratio was calculated to serve as an indicator of sympathetic nervous system activation. Normalization represents the relative value of LF and HF in proportion to the total power minus the very-low frequency (VLF) component (Task Force, 1996). Normalized units of spectral components were reported to reduce the effect of the changes in total power on the values of LF and HF and it also emphasizes the controlled and balanced behavior of the autonomic nervous system (Task Force, 1996).

Statistical Analysis

Mean cardiovascular responses for the minutes 2-6 of each stage of LBNP common to all subjects were determined. If the subject did not complete a stage of LBNP, the minutes of the final stage (excluding the first minute) that was tolerated by the subject were used for data analysis. Cardiac output (Q) was calculated every 30 seconds using the stroke volume (SV), which was assessed by the impedance cardiograph, and the concurrent HR (CO = SV * HR). Pulse pressure was calculated as the diastolic blood pressure (DBP) subtracted from the systolic blood pressure (SBP). Mean arterial pressure (MAP) was calculated as the sum of DBP and 1/3 pulse pressure. Forearm vascular conductance (FVC) was calculated as forearm blood flow (FBF)/MAP. Total peripheral conductance (TPC) was calculated as Q/MAP. Cronbach's alpha was determined for each

variable at each stage to assess reliability. Statistical significance was set at $p < 0.05$ and data were presented as means ± SEM.

CHAPTER 4: RESULTS

Response to LBNP

Sixteen subjects completed both trials of LBNP testing. Data for 1 subject were excluded because of ectopic beats. Table 1 summarizes the physical characteristics of the subjects (n=15).

Table 1- Anthropometric data for all subjects (n=15). BMI, body mass index.

Age (years)	23.6±3.0
Height (cm)	69.4±2.4
Weight (kg)	83.1±17.1
BMI (kg/m^2)	26.7±4.9
Percent fat (%)	10.7±3.6
Estimated VO_2(ml/kg/min)	50.4±6.3

The cumulative stress index (CSI) was 1586 ± 622 for trial 1 and 1864 ± 605 for trial 2 ($P<0.05$). LTI was 400 ± 81 and 438 ± 77 for trials 1 and 2, respectively ($p<0.05$). These values equate to the subjects tolerating 4 minutes of –60 mmHg in trial 1 and 2 minutes of –70 mmHg in trial 2.

The qualitative cardiovascular responses were similar for all the subjects in both trials. Because of the differing stages at which people became presyncopal, data on 15 subjects were plotted through –60 mmHg. Seven subjects were plotted at –70 mmHg and 3 subjects were plotted at –80 mmHg. Heart rate increased for all of the subjects as the

level of LBNP increased (Figure 1). Cardiac output decreased as the level of LBNP increased (Figure 2). The mean arterial pressure was maintained throughout the protocol for all subjects (Figure 3). Forearm vascular conductance and total peripheral conductance decreased slightly throughout the LBNP protocol (Figures 4 and 5). Using qualitative analyses (means +/- SEM) there were no changes in the cardiovascular responses observed between trials.

High frequency (HF) values of HRV decreased with increased negative pressure (Figure 6). Low frequency (LF) values of HRV remained constant throughout the LBNP protocol (Figure 7). HF, in normalized units (Hfnu) also decreased throughout the LBNP protocol (Figure 8). LF in normalized units (Lfnu) increased during the LBNP protocol (Figure 9). The LF/HF ratio values increased throughout the LBNP protocol (Figure 10). Total power values decreased with increased negative pressure (Figure 11).

Reliability of LBNP Testing

Table 2 summarizes the Chronbach's alpha values for reliability of each stage of LBNP. Heart rate (HR) showed consistently high reliability ($\alpha > .70$) for each stage of LBNP. High frequency in normalized units (Hfnu) and low frequency in the normalized units (Lfnu) were all greater than 0.7 for each stage of LBNP with the exception of the resting stage (0mmHg). E-1 and E are not reported for the spectral data because spectral data can't be determined for these short (1 minute) durations.

Table 2- Chronbach's Alpha values for intraclass reliability (n=14).

	0	-10	-20	-30	-40	-50	-60	E-1	E
Lfabs	.81	.91	.54	.71	.81	.60	.68	--	--
Lfnu	.43	.78	.77	.84	.80	.78	.19	--	--
Hfabs	.93	.80	.81	.49	.90	.82	.70	--	--
Hfnu	.41	.77	.78	.84	.80	.84	.47	--	--
LF/HF	.19	.69	.78	.61	.56	.42	.36	--	--
Tot power	.90	.87	.60	.54	.74	.68	.51	--	--
HR	.87	.89	.88	.87	.88	.93	.92	.88	.68
CO	.65	.79	.69	.88	.37	.56	.51	.33	.44
SV	.85	.82	.85	.88	.56	.83	.44	.52	.31
FBF	.77	.74	.70	.66	.58	.61	.37	-.27	.66
FVC	.58	.65	.60	.45	.49	.60	.21	-.60	.26
TPC	.54	.59	.50	.58	.47	.42	.44	.14	.24
SBP	.04	.35	.68	-.43	.16	-.11	.48	-.13	.50
DBP	-.45	.41	.60	-.19	.12	.64	.19	-.71	.60
MAP	-.50	.35	.47	-.08	.16	.01	.23	-.75	.48
PP	.51	.41	.35	-.46	.29	-.32	.63	.51	.44

E-1: next to last minute of the final stage of LBNP achieved. E: last minute of LBNP achieved.

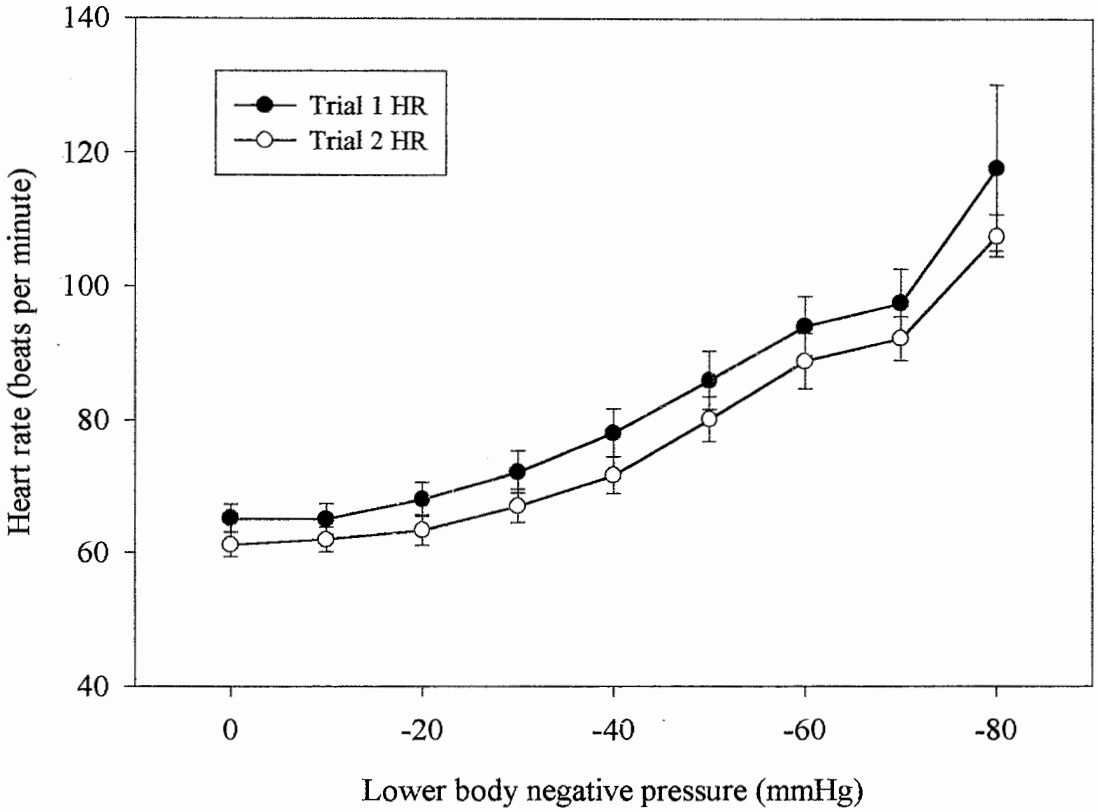

Figure 1. Heart rate responses to lower body negative pressure.

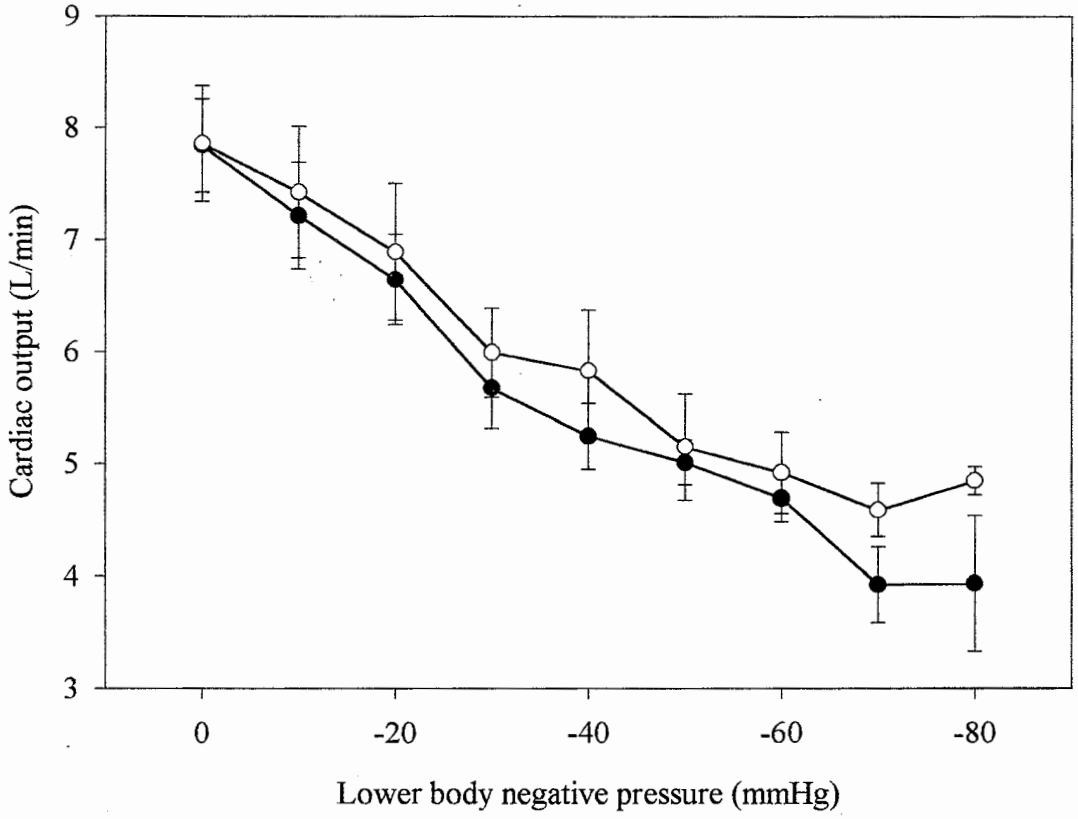

Figure 2. Cardiac output responses to lower body negative pressure.

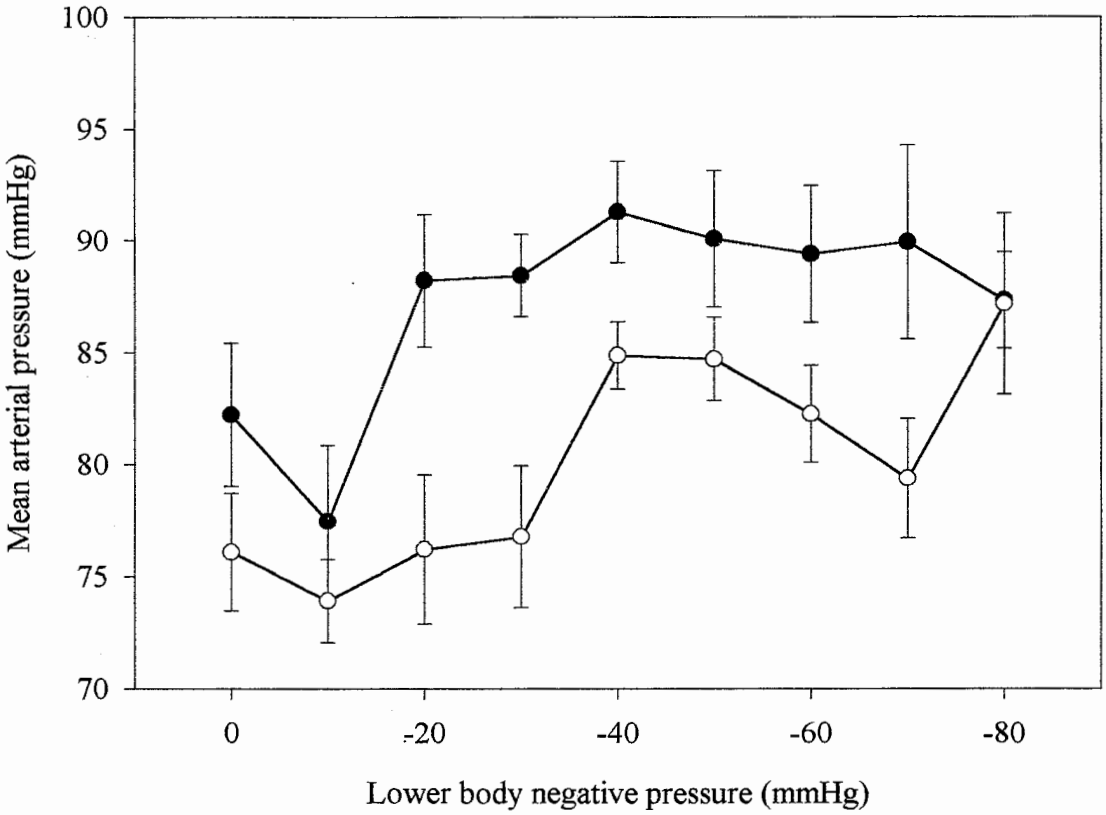

Figure 3. Mean arterial pressure responses to lower body negative pressure.

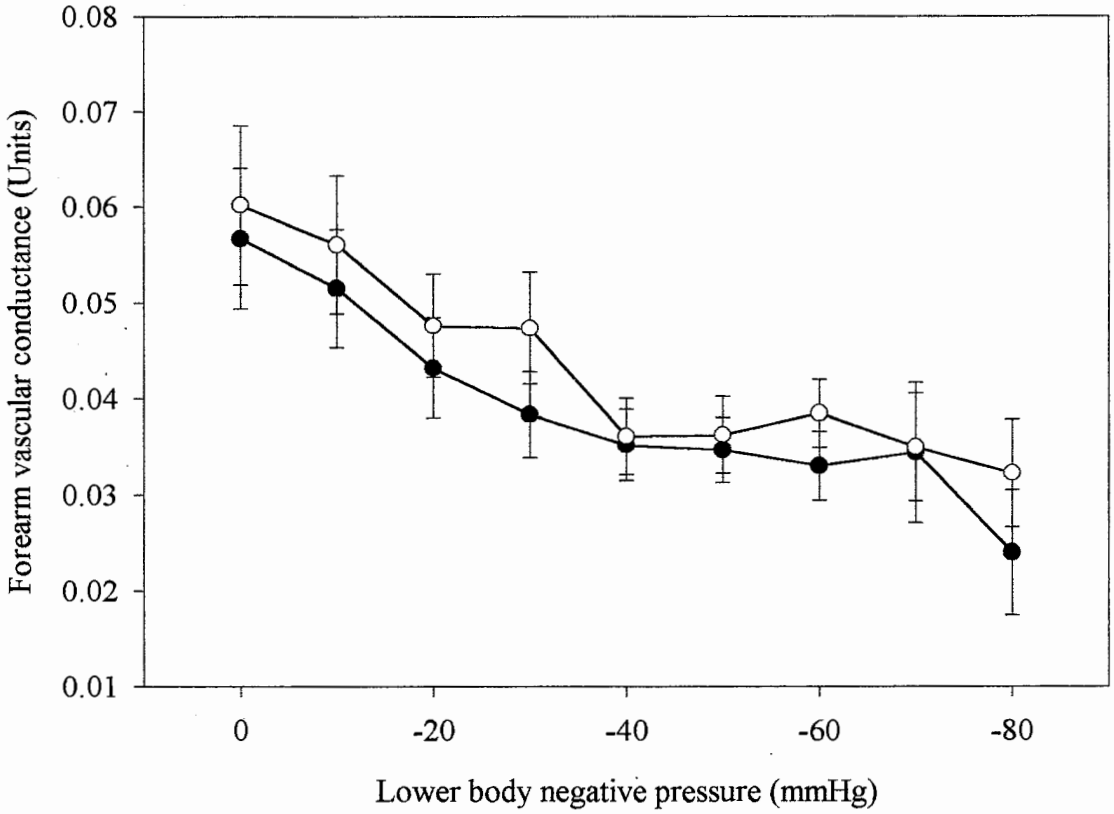

Figure 4. Forearm vascular conductance responses to lower body negative pressure.

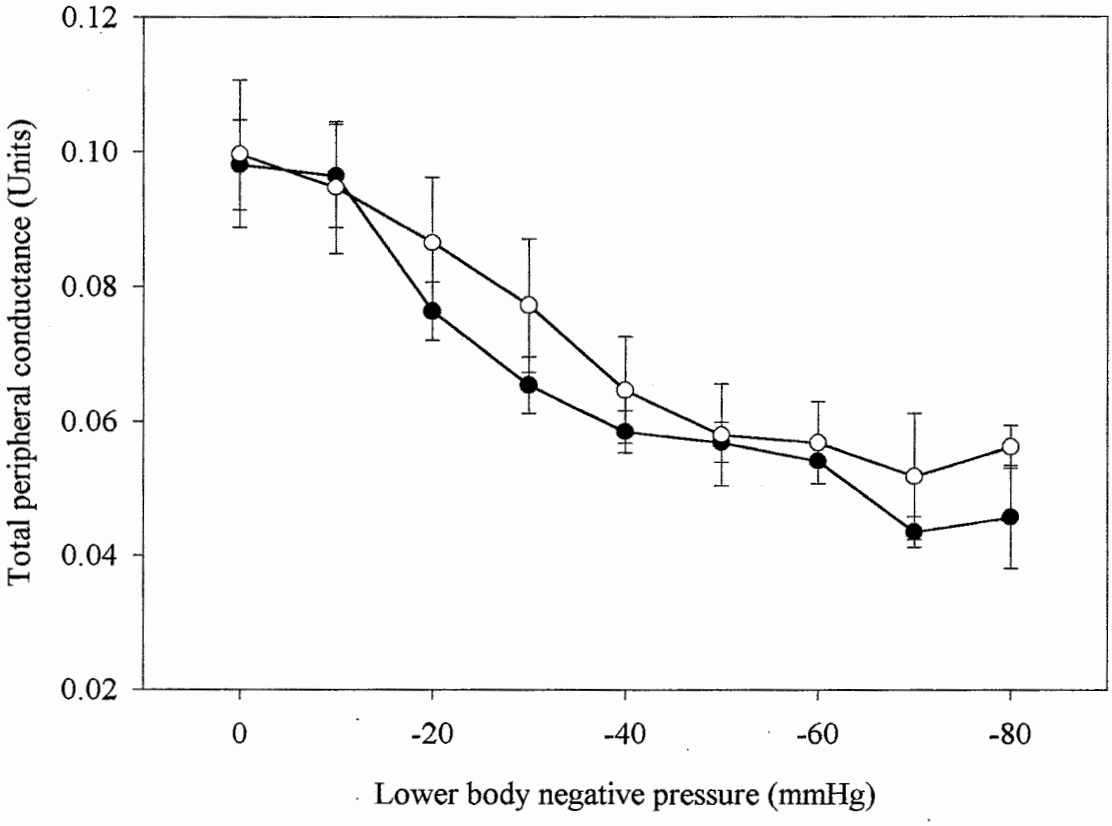

Figure 5. Total peripheral conductance response to lower body negative pressure.

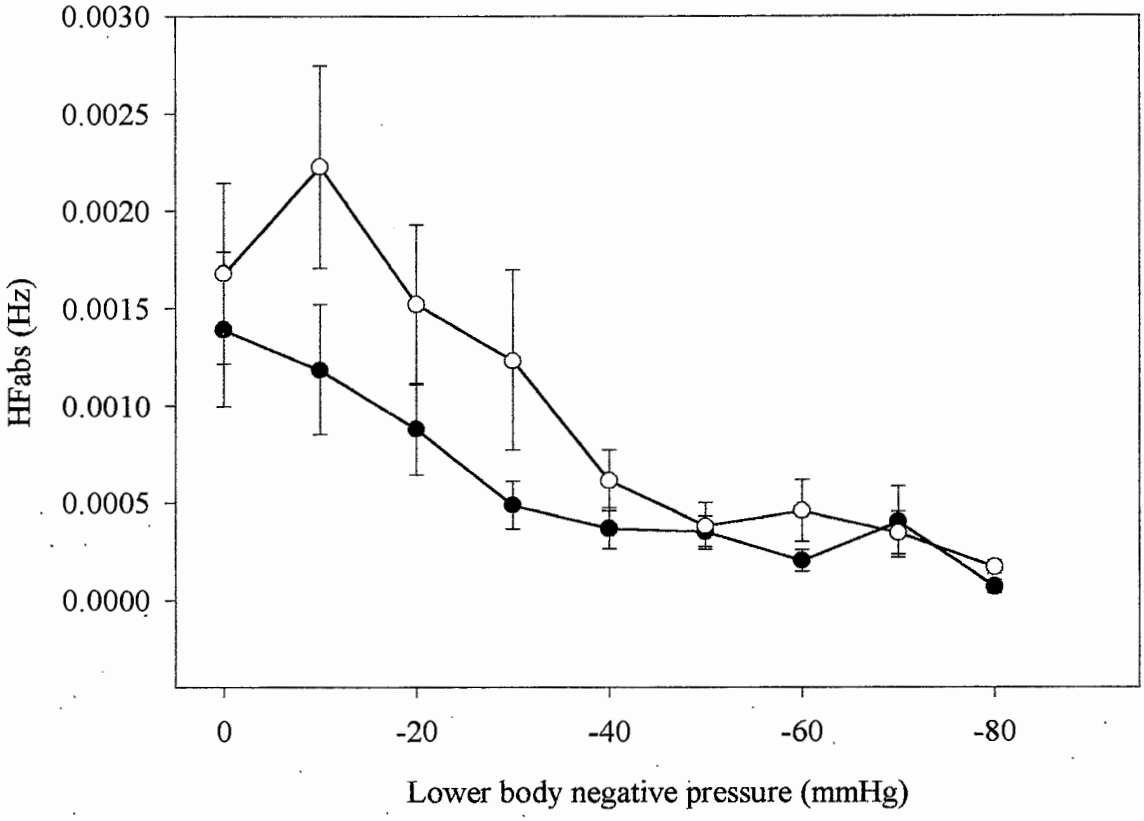

Figure 6: High frequency spectral response to lower body negative pressure.

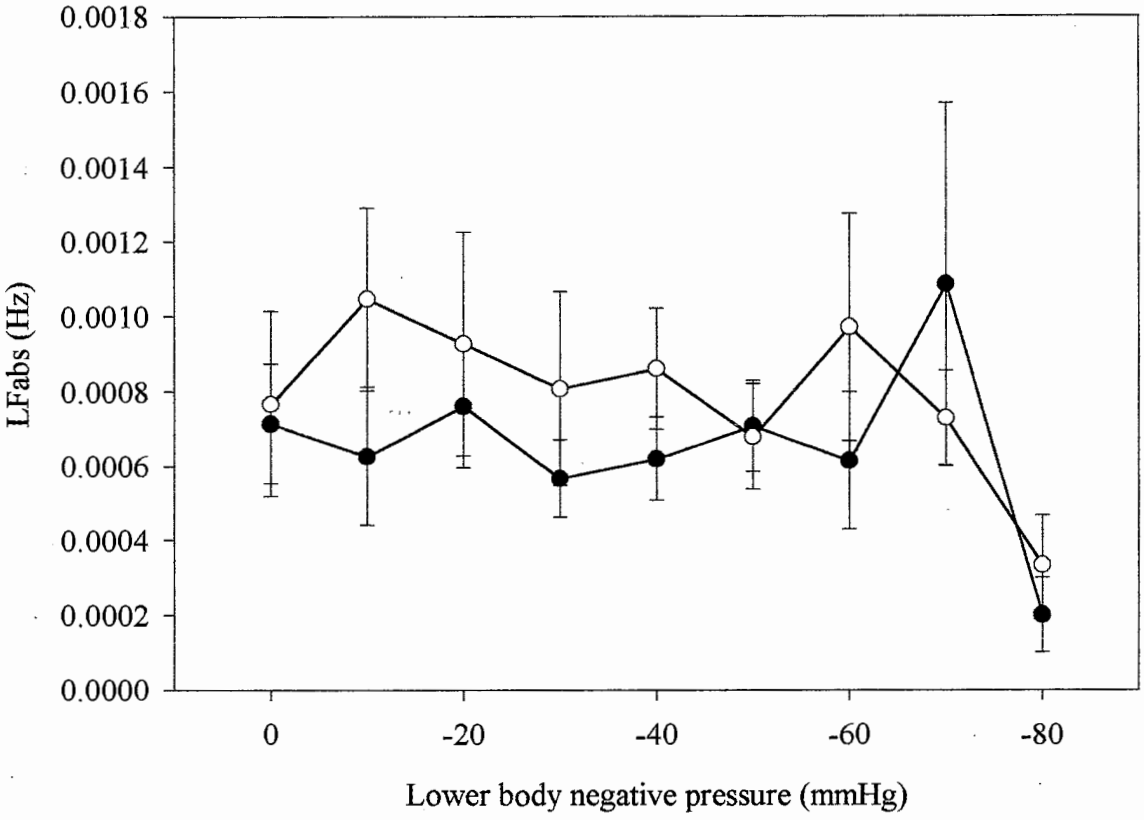

Figure 7. Low frequency spectral response to lower body negative pressure.

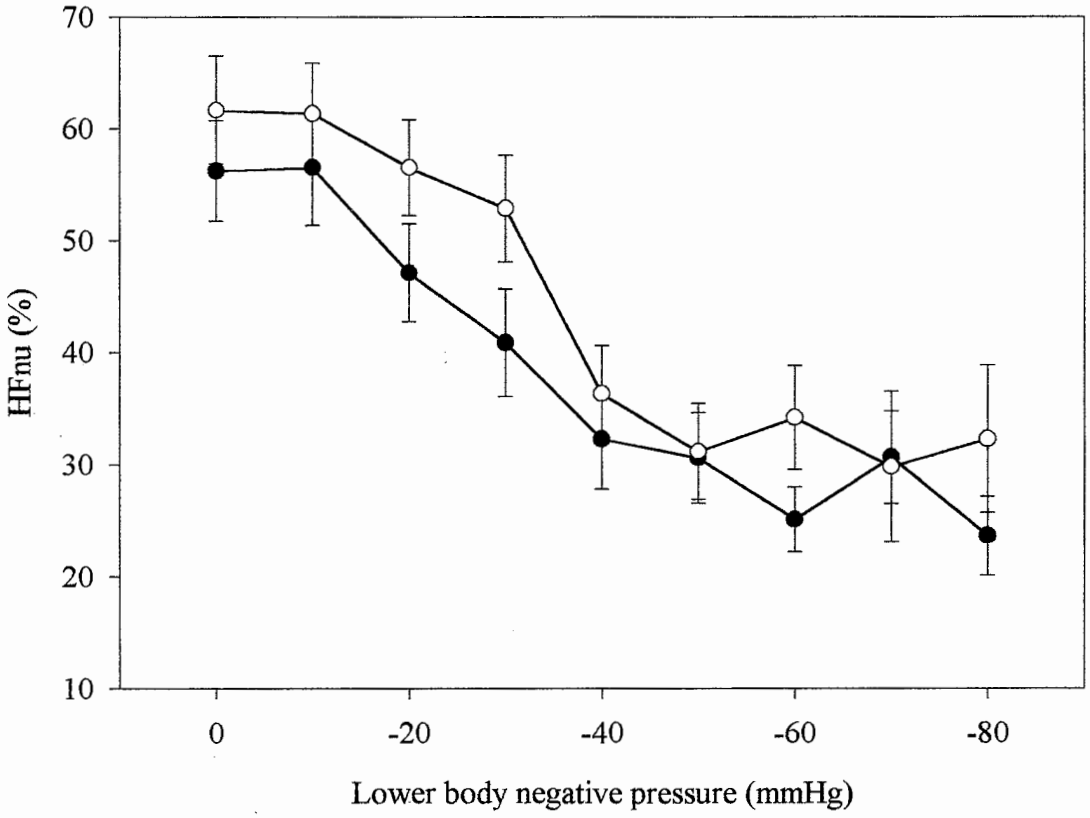

Figure 8. High frequency (in normalized units) response to LBNP.

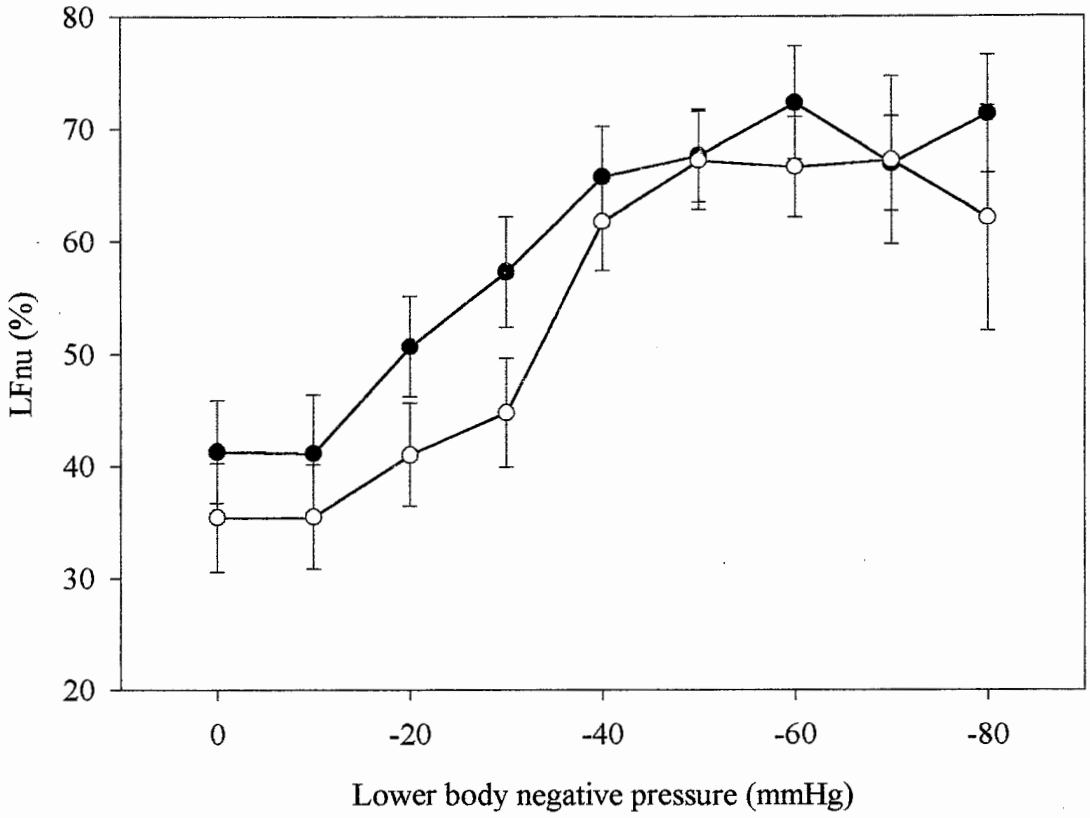

Figure 9. Low frequency (in normalized units) response to LBNP.

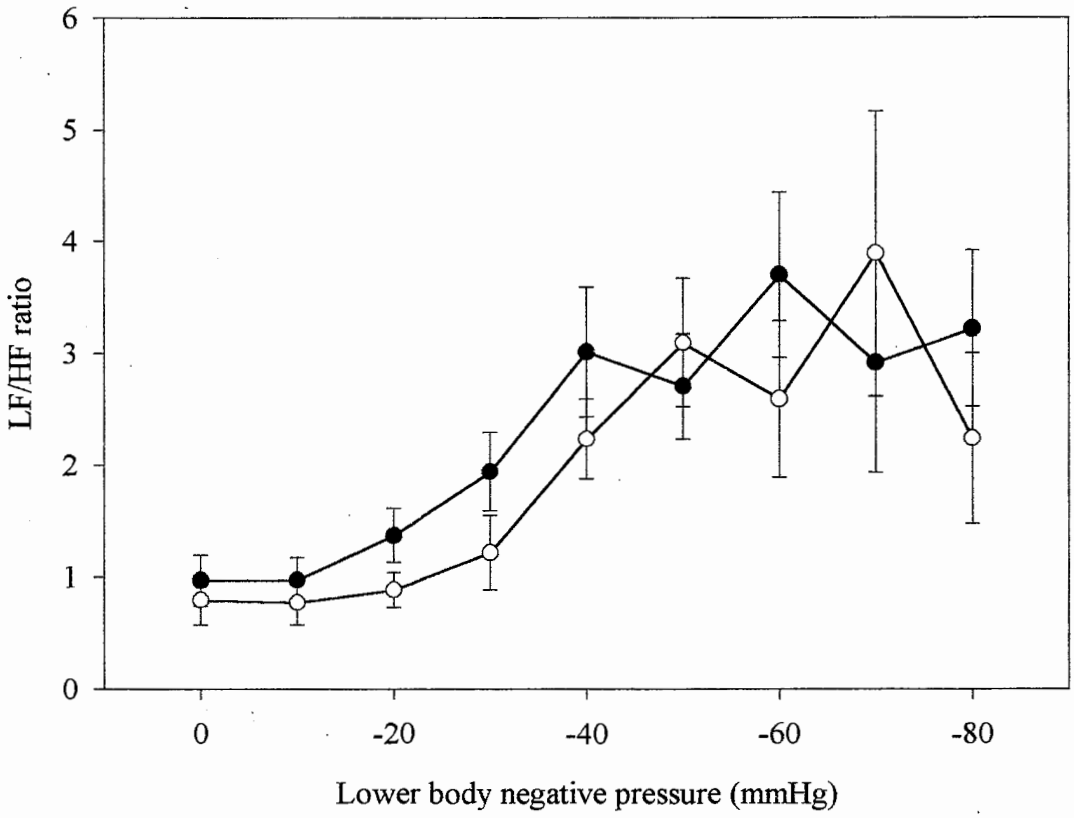

Figure 10. Low frequency to high frequency ratio to lower body negative pressure.

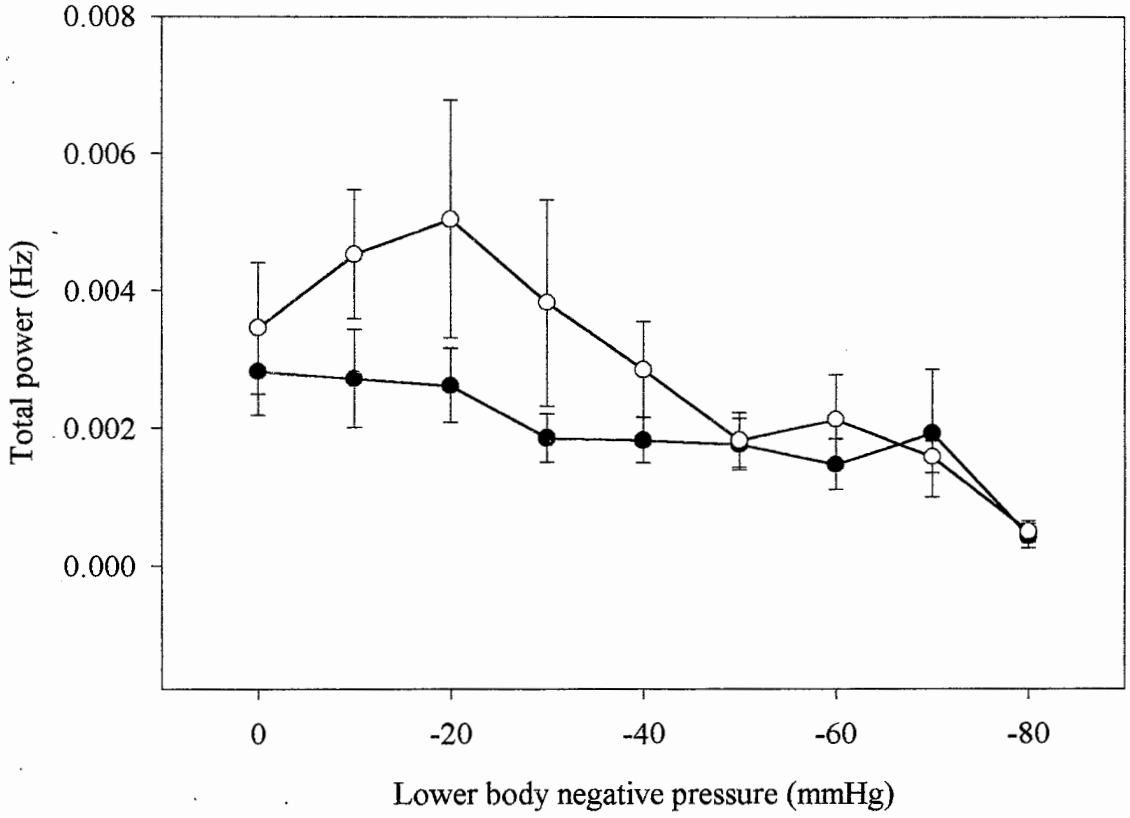

Figure 11. Total power response to lower body negative pressure.

CHAPTER 5: DISCUSSION

Reproducibility of heart rate variability, the beat-to-beat temporal fluctuations of the heart, has been previously assessed using the technique of the table tilt test. In these studies, the cardiovascular responses (heart rate, cardiac output, mean arterial pressure) to LBNP have also been examined for reproducibility. In this study, reproducibility of heart rate variability was examined using lower body negative pressure (LBNP). For comparison, reproducibility of the cardiovascular responses were also examined.

The results of this study show that spectral components of heart rate are reproducible in males during LBNP to tolerance. The qualitative response of HF, LF, and total power were identical in the test-retest protocol. The Chronbachs alpha data for HRV shows a good correlation ($\alpha > 0.7$) for LF and HF spectral components. No other similar studies have been published using LBNP as the technique for inducing an orthostatic stress. The subjects exhibited reproducibility of hemodynamic responses to the LBNP tests similar to that seen by others (Lightfoot et al., 1991).

Vardas and colleagues (1994) obtained results similar to the present study. Twenty patients with a history of syncope participated in two table tilt tests 1 to 6 weeks apart. Subjects were grouped according to the results of the test: Group A for subjects who had a positive test for 1 or both trial and Group B for the subjects that with 2 negative tests. In both groups, there was no significant difference in the spectral index for HF, LF and total power. Postural tilt test was performed in their study on a population with syncopal episodes. The current study involved the use of LBNP to induce the orthostatic stress and used a healthy population.

Sloan and colleagues (1995) demonstrated reproducibility of HRV using psychological challenges such as arithmetic and reaction time tasks to induces changes in cardiovascular activity. They reported an intraclass correlation coefficient (ICC) of 0.17 to 0.73 for HR and HF power. The investigators noted that the length of the experiment had some influence over their results. The present study, however, reported Chronbach's alpha coefficients between 0.87 to 0.93 and between 0.49 to 0.93 for HR and HF, respectively.

The LF/HF ratio data of the present study exhibited the stable features reported by Kochiadakis and colleagues (1998). Table tilt tests were used on 35 subjects with vaso-vagal syncope and 15 healthy volunteers. The spectral data from the subjects that completed both tests without syncope and the subjects that showed signs of syncope for both trials had reproducible LF/HF values. This was similar to the present study with the exception of the decrease in the LF/HF values for the subjects that demonstrated 2 positive tilt tests. The decrease in LF/HF values was due to the increase in parasympathetic activity creating the syncopal episode for those subjects. The present study used healthy subjects with no vaso-vagal syncope. Alpha coefficients were also computed; the reliability of the spectral indexes at each stage of LBNP for both trials were recorded.

Pardo and colleagues (1996) assessed HRV in patients with myocardial ischemia while ambulatory for two 24-hour periods. Holter monitors were used to record the heart rate and were worn throughout the experiment. They used intraclass correlation coefficients to assess the correlation between the two 24-hour periods. They reported very high intra-observer reproducibility of HRV measures with ICC ranging from 0.990 to 0.999

($p<0.0001$). The present study observed the intraclass correlation of HRV on healthy subjects with no history of ischemia. LBNP was also used as a method of inducing an orthostatic stress.

Lightfoot and colleagues (1991) examined the reproducibility of the cardiovascular responses to LBNP testing. Eleven subjects were exposed to 4 trails of LBNP with 72 hours of separation between each exposure. The subjects tested in their study had similar HR and change in systolic and distolic blood pressures between the 4 trials. They assessed the test-retest data using repeated measures ANOVA. There were no data presented for the reproducibility of either cardiac output or for the spectral components of HRV. The present study reports reproducibility in HR and MAP. Cardiac output showed a similar descending pattern in both trials, indicating reproducibility of cardiac output. The present study also reported decreases in the forearm vascular conductance and total peripheral conductance responses and reported reproducibility for these responses. The responses of the FVC and TPC are indicators of reproducible autonomic responses to an orthostatic stress. Spectral components (LF, HF, and LF/HF) were also analyzed in the present study and all values were assessed for intra-class correlation using the Cronbach reliability test.

The results of the current experiment demonstrate reproducibility of both cardiovascular and spectral component responses to LBNP. Heart rate for both trials increased while cardiac output decreased for both trials during the LBNP protocol. This increase in heart rate was due to an increased baroreceptor activation. HF spectral components decrease due to the decrease in parasympathetic activity. LF/HF ratio also increased during the LBNP protocol.

In summary, college-age men were able to increase heart rate during the LBNP exposures and decrease the HF spectral component of heart rate. These results were replicated within each subject during the second LBNP test. From the results of this study it can be concluded that spectral components of heart rate can be reproduced using LBNP.

Recommendations for Future Research

The subjects in the current study were moderately physically active. Comparing the reproducibility of HRV for subjects with low physical activity to subjects with high physical activity may uncover differences in the response of autonomic activity between subjects. The current study examined the reproducibility within individual subjects. The present study did not examine the effects of catecholamines during LBNP. It may be possible for catecholamines to build up to different levels for each trial. Testing the level of catecholamines in the blood for each trial of LBNP could uncover more secrets to the inner-workings of the cardiovascular system. Demonstrating reproducibility of HRV values can increase the integrity of spectral analysis as a tool in the clinical environment.

BIBLIOGRAPHY

Abboud, F. M., D. L. Eckberg, J. U. Johannsen, and A. L. Mark. Carotid and cardiopulmonary baroreceptor control of splanchnic and forearm vascular resistance during venous pooling in man. *J. Physiol.* 286: 173-184, 1979.

Brown, T. E., L. A. Beightol, J. Koh, and D. Eckberg. Important influence of respiration on human R-R interval power spectra is largely ignored. *J. Appl. Physiol.* 75: 2310-2317, 1993.

Cloarec-Blanchard, L., C. Funck-Brentano, M. Lipski, P. Jaillon, and I. Macquin-Mavier. Repeatability of spectral components of short –term blood pressure and heart rate variability during acute sympathetic activation in healthy young male subjects. *Clinical Science*. 93: 21-28, 1997.

Cooke, W. H., J. F. Cox, A. M. Diedrich, J. A. Taylor, L. A. Beightol, J. E. Ames IV, J. B. Hoag, H. Seidel, and D. L. Eckberg. Controlled breathing protocols probe human autonomic cardiovascular rhythms. *Am J. Physiol.* 274: H709-H718, 1998.

Desai, T. H., J. C. Collins, M. Snell, and R. Mosqueda-Garcia. Modeling of arterial and cardiopulmonary baroreflex control of heart rate. *Am. J. Physiol.* 272: H2343-H2352, 1997.

Fox, S. *Human Physiology* 5th edition. Dubuque: Wm. C. Brown Publisher, 1996, 410-411.

George, J. D., W. J. Stone, and L. N. Burkett. Non-exercise VO2max estimation for physically active college students. *Med. Sci. Sports Exerc.* 29: 415-423, 1997.

Goldberger, A. L., J. E. Mietus, D. R. Rigney, M. L. Wood, and S. M. Fortney. Effects of head-down bed rest on complex heart rate variability: response to LBNP testing. *J. Appl. Physiol.* 77: 2863-2869, 1994.

Gotshall, R. W., and D. S. Miles. Use of impedance cardiograph for assessing cardiovascular control in humans: Orthostatic and Valsalva tests. *Biological Psychology* 36: 107-117, 1993.

Gotshall, R. W. and W. R. Sexson. Comparison of band and spot electrodes for the measurement of stroke volume by the bioelectric impedance technique. *Critical Care Medicine*. 22: 420-425, 1994.

Halliwill, J.R., L. A. Lawlew, T. J. Eickhoff, M. J. Joyner, and S. L. Mulvagh. Reflex responses to regional venous pooling during lower body negative pressure in humans. *J. Appl. Physiol.* Vol 84: 454-458, 1998.

Hayano, J., S. Mukai, M. Sakakibara, A. Okada, K. Takata, and T. Fujinami. Effects of respiratory interval on vagal modulation of heart rate. *Am. J. Physiol.* 267: H33-H40, 1994.

Hughson, R. L., Y. Yamamoto, R. E. McCullough, J. R. Sutton, and J. T. Reeves. Sympathetic and parasympathetic indicators of heart rate control at altitude studied by spectral analysis. *J. Appl. Physiol.* 77: 2537-2542, 1994.

Johnson, J. M., L. B. Rowell, M. Niederberger, and M. M. Eisman. Human splanchnic and forearm vasoconstrictor responses to reductions of right atrial and aortic pressures. *Circulation Res.* 34: 515-524, 1974

Kleiger, R. E., J. T. Bigger, M. S. Bosner, M. K. Chung, J. R. Cook, L. M. Rolnitzky, R. Steinman, and J. L. Fleiss. Stability over time of variables measuring heart rate variability in normal subjects. *Am J Cardiol.* 68: 626-630, 1991

Kochiadakis, G. E., A. E. Orfanakis, A. T. Rombola, S. I. Chrysostomakis, G. I. Chlouverakis, and P. E. Vardas. Reproducibility of time-domain indexes of heart rate variability in patients with vasovagal syncope. *Am J Cardiol.* 79: 160-165, 1997.

Kochiadakis, G. E., E. M. Kanoupakis, A. T. Rombola, N. E. Igoumenidis, G. I. Chlouverakis, and P. E. Vardas. Reproducibility of tilt testing in patients with vasovagal syncope and its relation to variations in autonomic nervous system activity. *Pacing Clin Electrophysiol.* 21: 1069-76, 1998

La Colley, P. J., B. M. Pannier, M. A. Slama, J. L. Cuche, A. P. G. Hoeks, S. Laurent, G. M. London, and M. E. Safar. Carotid arterial hemodynamics after mild degrees of lower-body negative pressure in man. *Clinical Science.* 83: 535-540, 1992.

Lightfoot, J. T., F. Hilton, Jr., and S. M. Fortney. Repeatability and protocol comparability of presyncopal symptom limited lower body negative pressure exposures. *Aviat. Space Environ. Med.* 62: 19-25, 1991.

Lightfoot, J. T., and K. M. Tsintgiras. Quantification of tolerance to lower body negative pressure in a healthy population. *Med. Sci. Sports Exerc.* 27: 697-706, 1995.

Lipsitz, L. A., J. Mietus, G. B. Moody, and A. L. Goldberger. Spectral Characteristics of heart rate variability before and during postural tilt. *Circulation.* 81: 1803-1810, 1990

Luutonen, S., K. Antila, P. Neuvonen, I. Raiha, T. Rajala, and L. Sourander. Spectral analysis of heart rate variability in evaluation of sympathetic function in elderly subjects. *Age Aging*. 23: 474-77, 1994.

Melchior, F. M., R. S. Srinivasan, P. H. Thullier, and J. M. Clere. Simulation of cardiovascular response to lower body negative pressure from 0 to -40 mmHg. *J Appl. Physiol*. 77: 630-40, 1994

Novak, V., P. Novak, J. De Champlain, A. R. Le Blanc, R. Martin, and R. Nadeau. Influence of respiration on heart rate and blood pressure fluctuations. *J. Appl. Physiol.* 74: 617-626, 1993.

Pardo, Y., C. N. B. Merz, M. Paul-Labrador, I. Velasquez, J. S. Gottdiener, W. J. Kop, D. S. Krantz, A. Rozanski, and T. Peter. Heart rate variability reproducibility and stability using commercially available equipment in coronary artery disease with daily life myocardial ischemia. *Am. J. Cardiol.* 78: 866-870, 1996.

Pawelczyk, J. A., and P. B. Raven. Reductions in central venous pressure improve carotid baroreflex responses in conscious men. *Am. J. Physiol.* 257: H1389-H1395, 1989.

Pomeranz, B., R.J. Macaulay, M. A. Caudill, I. Kutz, D. Adam, D. Gordon, K. M. Kilborn, A. C. Barger, D. C. Shannon, and R. J. Cohen. Assessment of autonomic function in humans by heart rate spectral analysis. *Am. J. Physiology*. 248: H151-3, 1985

Ponikowski, P., M. Piepoli, A. A. Amadi, T. P. Chua, D. Harrington, M. Volterrani, R. Colombo, G. Mazzuero, A. Giordano, and A. J. S. Coats. Reproducibility of heart rate variability measures in patients with chronic heart failure. *Clinical Science*. 91: 391-398, 1996.

Rowell, L. B., C. R. Wyss, and G. L. Brengelmann. Sustained human skin and muscle vasoconstriction with reduced baroreceptor activity. *J. Appl. Physiol.* 56: 138-144, 1984.

Schmitz, J. M., D. Claus, B. Neundorfer, and H. O. Handwerker. Comparison of different algorithms for evaluation of respiratory sinus arrhytmia: Cross-correlation function histogram analysis and regression analysis. *Physiol Res*. 44: 197-203, 1995

Sander-Jensen, K., J. Mehlsen, C. Stadeager, N. J. Christensen, J. Fahrenkrug, T. W. Schwartz, J. Warberg, and P. Bie. Increase in vagal activity during hypotensive lower-body negative pressure in humans. *Am. J. Physiol.* 255: R149-R156, 1988.

Sloan, R. P., P. A. Shapiro, E. Bagiella, J. M. Gorman, and J. T. Bigger, Jr. Temporal stability of heart period variability during a resting baseline and in response to psychological challenge. *Pyschophysiology*. 32: 191-196, 1995.

Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart Rate Variability: Standards of Measurement, Physiological Interpretation, and Clinical Use (Special Report). *Circulation*. 93: 1043-1065, 1996

Vardas, P., G. Kochiadakis, A. Orfanakis, M. Kalaitzakis, and E. Manios. Intraindividual reproducibility of heart rate variability before and during postural tilt in patients with syncope of unknown origin. *Pacing Clin Electrophysiol*. 17: 2207-2210, 1994

Verbanck, S., H. Larsson, D. Linnarsson, G. K. Prisk, J. B. West, and M. Paiva. Pulmonary tissue volume, cardiac output, and diffusing capacity in sustained microgravity. *J Appl Physiol*. 83: 810-16, 1997

Victor, R. G., and A. L. Mark. Interaction of cardiopulmonary and carotid baroreflex control of vascular resistance in humans. *J. Clin. Invest*. 76: 1592-1598, 1985.

Zoller, R. P., A. L. Mark, F. M. Abboud, P. G. Schmid, and D. D. Heistad. The role of low pressure baroreceptors in reflex vasoconstrictor responses in man. *J. Clinical Invest*. 51: 2967-2972, 1972.