The Reproducibility of Short verses Long-Duration Heart Rate Variability Methods and Relations to Aerobic Fitness in Normal Adults

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The Reproducibility of Short verses Long-Duration Heart Rate Variability Methods and Relations to Aerobic Fitness in Normal Adults

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Heart rate variability (HRV) has been used to evaluate cardiac autonomic function by measuring variations in electrocardiographic R-R intervals between cardiac cycles. HRV was first used to associate decreases in autonomic nervous system (ANS) control with an increased risk of mortality in coronary heart disease and in the diagnosis of diabetes (1). Current clinical research interest has extended to investigate uses of HRV to evaluate changes in the cardiovascular system due to disease, aging, physical activity, and cardiac rehabilitation treatment (2, 5). HRV scores are derivatives of R-R intervals and these may be represented as a function of either time or frequency domain parameters. Time domain analysis is the simplest and includes: the standard deviation of R-R intervals and the number of adjacent RR intervals that differ by $\geq$50ms (dRR50). Frequency domain measures involve more elaborate calculation and have been applied in studies to evaluate sympathetic and parasympathetic autonomic balance. The latter include: Low Frequency Power (LF), High Frequency Power (HF), and LF/HF ratio. HRV has been measured in a variety of ways, the most common being a continuous 24-hour collection of R-R data. In recent years, several investigators have sought to assess HRV by utilizing brief collection periods. Controversy exists about the potential of these

short-term sampling intervals to yield reproducible and meaningful measurements of HRV. Many confounders such as respiration, stress, and body positioning can influence HRV, which is why a longer collection period has been accepted as the standard for providing a stable index of ANS function. However, short sampling periods would be useful to evaluate HRV when faced with time constraints. The purpose of the current study was to evaluate the reproducibility of HRV using 8-hour daytime measures with the Polar R-R Recorder^{TM} (Polar Electro Oy, Kempele, Finland) and with short sampling duration of 512 cardiac cycles, using the Schiller AT-10^{TM} device (Schiller AG, Baar, Switzerland). Methods: 10 apparently healthy adult volunteers participated in the study, which was conducted at the Sleep Disorders Clinic in Christiansburg, VA. Each subject performed two HRV trials with the Cardiovit AT-10^{TM} device using recordings of 512 cardiac cycles. Within one or two days following the Schiller, the same subjects wore a Polar R-R Recorder^{TM} device to obtain an 8-hour recording of HRV during waking hours; 24-hour urine samples were collected on the same day. Urine was analyzed for catecholamine levels, including norepinephrine and epinephrine in order to evaluate sympathetic nervous system globally. Each subject recorded their personal impressions of unavoidable physical activity and daytime stress demands on the day of the 8-hour recording and urine collection. This entire protocol was repeated one week later. On one of the days of the short sampling recording, VO_{2pk} also was evaluated for each subject using a ramp protocol on the cycle ergometer and a metabolic cart. Results: The correlation analysis for the HRV response variables using the Schiller method

indicated a high-to-very high correlation between trials within a day for the time domain measures (r = 0.75-0.99). The frequency domain measures, however, were low-to-moderately correlated (r = 0.24-0.66) between trials within a day for the Schiller method. Correlations between days for HRV response variables using the Schiller method were similarly low for both time (r < 0.5) and (r < 0.4) frequency domain measures. Correlation coefficients between days for the HRV response variables using the Polar method were moderate (r = 0.59-0.67) for the time domain and only low-moderate for the frequency domain measures (r = 0.37-0.69). However, an important finding was that Polar R-R data for two of the subjects contained excessive signal artifact, which affected the fidelity of the HRV scores. When these two cases were excluded from the group analyses, the resulting correlations were high-very high for all time and frequency domain measures (r = 0.70-0.93). The means for each response HRV time and frequency domain variable between the Polar method and Schiller method were significantly different ($P \leq 0.05$). Additional correlational analyses did not reveal any systematic associations between HRV measures and simple markers of sympathetic activity (urinary NE or E) and aerobic fitness (VO_{2pk}) in this small sample of subjects. Conclusions: Due to this important change in reproducibility with the Polar method, the consequence of artifact-free recordings is unmistakable. Within the limitations of this small study sample it is concluded that, while HRV in apparently healthy adults may not be measured reliably with brief data collection periods, longer daytime sampling periods of 8 hours (e.g.

Polar device) yields acceptable reliability for both time and frequency domain parameters of HRV.

CHAPTER 1

INTRODUCTION

Heart Rate Variability (HRV) is a marker used to evaluate the autonomic nervous system (ANS); this marker has been used for many years due to the significant relationship between the ANS and cardiovascular mortality (1-3). HRV is defined as the fluctuation in heart rate, as assessed by evaluation of consecutive cardiac cycles. Therefore the oscillations are of greater concern than the actual rates (1). In 1996 the European Society of Cardiology and North American Society of Pacing and Electrophysiology published a special report concerning the standards of measuring HRV. Hon and Lee first recognized the use of HRV in 1965 when they observed that fetal distress was preceded by alterations in inter-beat intervals before any noticeable change occurred in the heart itself (1). Since then, HRV has been used to detect neuropathy in diabetics and the risk of mortality with cardiovascular disease. Currently clinical researchers use it to evaluate changes in the cardiovascular system due to disease, age, exercise, pharmacological intervention, and cardiac rehabilitation (2).

Numerous studies support the hypothesis that electrocardiographic R-R intervals vary depending on the degree of sympathetic and parasympathetic systems (1-3). It has been established, through pharmacological blocking studies that selectively inhibit either sympathetic or parasympathetic activation, that a reduced HRV is a reflection of elevated sympathetic activity or reduced parasympathetic activity (3). A large body of evidence indicates that the

sympathetic nervous system promotes cardiac arrhythmias, especially malignant ventricular arrhythmias, such as ventricular tachycardia and ventricular fibrillation. The parasympathetic nervous system plays a protective role, decreasing the likelihood of malignant ventricular arrhythmias (4). Therefore in a situation with reduced HRV from decreased parasympathetic and increased sympathetic influence, there is a predisposition to increased incidence of a cardiac event and overall increased risk of mortality (4,5).

The ANS can be assessed using many techniques including hemodynamic, pharmacological, biochemical, neurophysiological, neurochemical, and neural imaging. Many of these techniques are regarded as unreliable or almost obsolete while some are more valuable tools; however, even the valuable techniques have limitations due to their invasiveness and their being extremely demanding of the individual being evaluated (6). For example, norepinephrine (NE) measurement in plasma is one of the most commonly used indices in humans. It has limitations because it provides only a static picture of the sympathetic function and does not give information about regional sympathetic activity. Plasma NE is a reflection of accumulation in the blood pool over a finite period rather than a reflection of momentary changes acting at the effector organ; therefore, it is not the ideal measure for cardiac sympathetic activity. The reproducibility and sensitivity values of NE measurements are lower than other methods such as muscle sympathetic neural activity (MSNA) measured by microneurography as shown by Grassi et al, 1997 ($P<0.001$). The authors concluded that it is likely that the limited reproducibility measuring

plasma NE makes it a less ideal measure than microneurography due to effects of external confounding factors like environmental temperature and time of the day. Another drawback is that circulating NE represents only a minute fraction (5-10%) of the amount of adrenergic neurotransmitter secreted from the nerve terminals. MSNA, although an extremely valuable technique, it too, has limitations these include: 1.) Although this technique allows both a static and dynamic evaluation of adrenergic activity, the technique is mainly restricted to laboratory environment 2.) The technique is invasive, complex, and questionable of usefulness in resting conditions where sympathetic tone may be low. 3) MSNA is hard to compare between individuals because of the difficulty comparing burst amplitudes (6). Due to complications with existing measures, HRV may be a promising marker. It allows for a noninvasive and easily administered measurement of the ANS.

AUTONOMIC NERVOUS SYSTEM

The ANS helps regulate activities of the cardiac muscle, smooth muscle, and glands, all of which function under involuntary control. Impulses are conducted from the Central Nervous System (CNS) to the ANS via two neurons in the efferent pathway. The first neuron, the preganglionic neuron, does not directly innervate the effector organ, but instead, synapses with the second neuron, the postganglionic autonomic ganglion. There are two divisions of the ANS, the parasympathetic system and sympathetic system. The origin of the

preganglionic fibers and the location of the autonomic ganglia help differentiate between the two divisions.

The involuntary effectors innervated by the ANS are different from skeletal muscle in terms of the effect of denervation. Skeletal muscles atrophy and possibly paralyze when their motor nerves are severed unlike involuntary effectors, which are somewhat independent of their innervation. For example, the cardiac muscle has an intrinsic tone, which allows it to contract on its own even in the absence of innervation. Autonomic innervation will increase or decrease the activity of the cardiac muscle. Ironically, a damaged autonomic nerve actually makes the cardiac muscle, or any other effector tissue, hypersensitive to stimulating agents. The actions of both divisions of the ANS must be balanced to maintain homeostasis (7,8).

SYMPATHETIC NERVOUS SYSTEM

Within the sympathetic division, the preganglionic neurons are located in the thoracic and lumbar region of the spinal cord and the sympathetic ganglia parallel the spinal cord traveling as part of the spinal nerve to their effector organs. Because the impulses converge from the spinal cord to the ganglia and from within the ganglia, the result is usually a mass activation of all the effector organs at the same time. The sympathetic system stimulates the heart by a release norepinephrine (NE) from the sympathetic nerve, and the adrenal medulla secretes epinephrine (E) and NE into the blood. Because of complementary release of E and NE during mass activation, they are often grouped together as

the sympathetic catecholamines. NE and E bind to the adrenergic receptors alpha$_1$ and beta$_1$ increasing Gprotein$_S$ subsequently stimulating the production of cAMP. The overall result is an increased force of contraction, increased rate of relaxation, and increased peak force in the cardiac muscle contraction. This is known as the "fight or flight" response (7,8). NE causes vasoconstriction, which increases TPR (total peripheral resistance) resulting in an increase in arterial blood pressure. NE also causes an increase of contraction at the cardiac muscle. However, due to the increase in TPR there is no significant change in cardiac output.

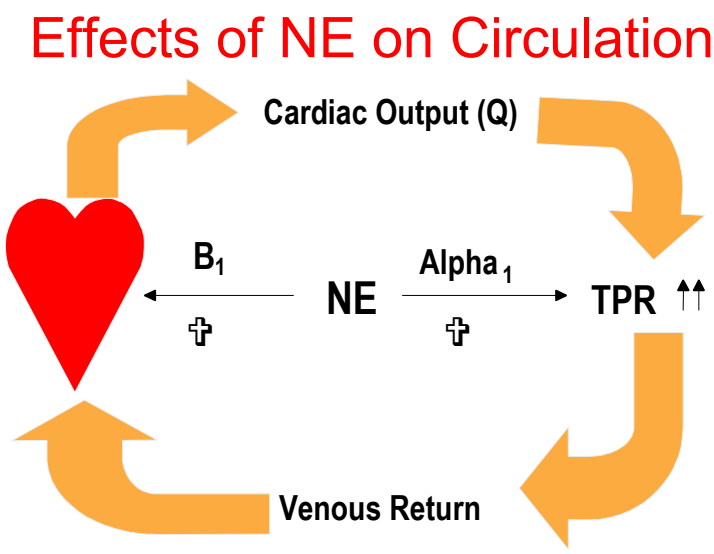


Figure 1. Effects of Norepinephrine (NE) on the Cardiovascular System

B1, beta1 receptors; TPR, total peripheral resistance; +, positive effect; ▲, increased effect

PARASYMPATHETIC NERVOUS SYSTEM

Within the parasympathetic system, the preganglionic neurons originate in the brain while the postganglionic, parasympathetic ganglia, are located in the effector organs. Specifically for cardiac muscle, the preganglionic neuron is located in the medulla oblongata travels along the vagal nerves and provides innervation to the heart at the SA node and AV node. When the neurotransmitter, achetylcholine, is released it binds with muscarinic receptors of the cardiac muscle and increases $G\text{protein}_{I.}$ This decreases the concentration of cyclic AMP. The overall result is a decrease in heart rate. Because it utilizes different neurotransmitters and receptor proteins, the parasympathetic system lacks a mass activation and differs from the sympathetic system. Table 1 describes the specific effects of the ANS on the heart. The regulation of heart rate depends on the net affect of these influences. In addition to ANS, HRV is affected by other accelerator and inhibitory controls of heart rate in the medulla oblongata that are affected by higher brain areas and sensory feedback reflex control like: pressure receptors, i.e. baroreceptors (7,8).

Table 1. Effects of the Autonomic Nervous System on the Heart

Region Affected	**Sympathetic Nerve Effects**	**Parasympathetic Nerve Effects**
SA node	Increased cardiac rate	Decreased cardiac rate
AV node	Increased conduction rate	Decreased conduction rate
Atrial muscle	Increased strength of contraction	Decreased strength of contraction
Ventricle muscle	Increased strength of contraction	No significant effect

HEART RATE VARIABILITY

HRV is a valuable measurement because it records the modulations of heart rate, which provides researchers with a better understanding of autonomic functioning. Reduced variability has been shown to reflect an increased risk for the incident of cardiac events or even cardiac mortality. Healthy humans may have strong fluctuations of muscle sympathetic nerve activity about every 10 seconds, a period sufficient enough for the SA node to respond to released NE and modulate heart rate (1,9). Respiration also contributes to the rhythmic changes in heart rate, which are exaggerated by changes in vagal activity (7,8).

HRV is measured statistically in time domains and frequency domains. Because it appears to resolve parasympathetic and sympathetic influences better than time domain measures, the frequency domain measures of R-R variability are more commonly used for mechanistic studies. However, for prediction of mortality, time and frequency domain measures provide equivalent information (4,10). R-R variability is influenced by factors other than the ANS such as respiration, blood pressure, emotional stress, and body position. The endocrine system works to adapt the body to change by sensing the change then secreting hormones in effort to maintain homeostasis. The endocrine and nervous system are often integrated because the two signaling systems have so much in common. The endocrine system responds more often to chemical stimuli while the nervous system responds to physical or mechanical stimuli (7,8). An example of respiration affecting ANS is, during inspiration heart rate increases causing a decrease in R-R interval and during expiration, the R-R interval

increases. This variation is referred to as respiratory arrhythmia, which is often observed to be higher in children and decreases with age. Changes in intrathoracic pressure, lung volume, venous return, arterial blood pressure, and baroreceptor reflex all contribute to respiration influencing changes in heart rate. Thus, respiration is important to monitor in order to assess HRV properly.

The time domain refers to the following measurements: standard deviation of RR intervals and the difference of the RR intervals between adjacent heartbeats (RR50). RR50 counts the number of beats with a beat-to-beat difference exceeding 50ms. A lower value would reflect a lower variability. The frequency domain is computed by the fast Fourier Transformation (FFT) or Autoregressive (AR) using the time domain measures to represent the distribution of RR intervals as a frequency spectral analysis allowing the divisions of the ANS to be distinguished. High frequency HRV (>0.15 Hz) is believed to represent cardiac parasympathetic activity. Low frequency HRV (0.05-0.15 Hz) reflects both cardiac parasympathetic and sympathetic activity. A ratio of LF/HF may be a valid indicator of interaction between the two divisions of the ANS. The frequency measures are expressed in normalized units (nu), which means powers at frequencies of interest, LF and HF, divided by total power minus power at very low frequencies. This results in LF defined as LF/LF+HF and HF defined as HF/HF+LF expressed as nu. Normalization is necessary because burst heights differ among individuals; if sympathetic activity is not normalized, subjects with large sympathetic bursts will dominate average results (1,3,4,9). In the past, 24-hour ECG was required to determine a reduced R-R variability,

however new methods may make it possible to determine RR variability by measuring only 512 cardiac cycles (11).

Table 2. Measurements of Heart Rate Variability

Measurement	**Description**	**Domain**
Standard Deviation	SD of all RR intervals	Time domain
dRR50	Number of RR intervals differing more than 50 ms	Time domain
High Frequency (HF)*	Frequency range of 0.15-0.4 Hz	Frequency domain
Low Frequency (LF)*	Frequency range of 0.04-0.15 Hz	Frequency domain
LF/HF ratio	Ratio of the divisions of the ANS	Frequency domain

* Normal units is HF/(total power-VLF)*100

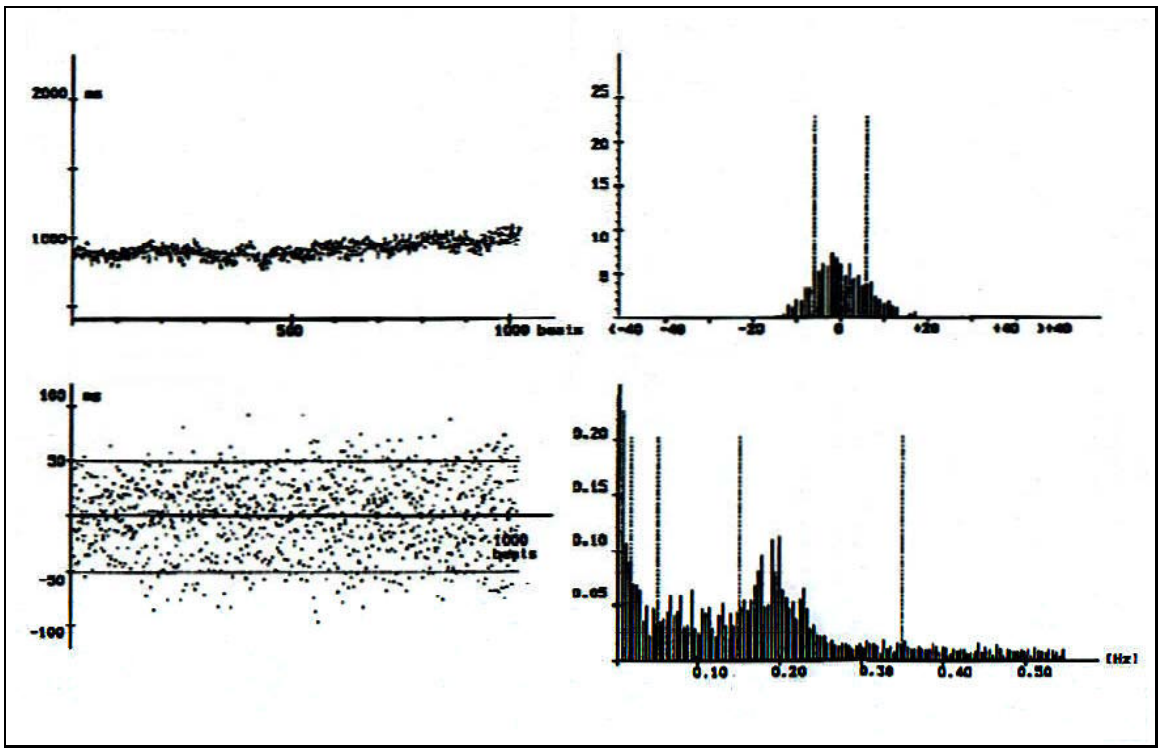


Figure 2. The Measurements of Heart Rate Variability
Top Left illustration: RR Tachogram, Bottom Left illustration: dRR Tachogram, Top Right illustration: RR distribution, and Bottom Right illustration: Spectrum.

This illustration is taken from the Schiller AT-10^{TM} Recorder Manual (Schiller AG, Baar, Switzerland).

STATEMENT OF PROBLEM

HRV analysis has the potential to be a useful marker to evaluate cardiac autonomic function in research as well as diagnostic settings. The use of short sampling periods may be an efficient and effective method of evaluating HRV. However, significant intra-individual variability of the results from different testing of HRV limits the use of HRV in settings that may benefit from using HRV as a noninvasive evaluation. Such variability may be due to some technical issues such as duration of recording, respiration, body position, or environmental factors (9-14). Controversy currently exists about the reproducibility of HRV measurements with short sampling durations (2,14,16,19). Variables need to be assessed within a set of normal, apparently healthy adults to evaluate reproducibility of HRV.

RESEARCH AIMS

- To evaluate intra-trial and inter-trial reliability for short sampling HRV and longer sampling HRV.
- To assess the agreement of short and long HRV recordings.
- To evaluate the degree of association for catecholamine levels and HRV in healthy adults.
- To evaluate the degree of association between aerobic fitness and HRV analysis.

RESEARCH HYPOTHESIS

- HRV, as assessed by an 8-hour continuous record of electrocardiographic R-R intervals and a short-duration HRV recordings of 512 cardiac cycles will agree.
- HRV will not be associated with catecholamine levels in normal, healthy subjects when catecholamine levels are normal which reflect global sympathetic activity rather than cardiac sympathetic modulations.
- HRV time domain measures will be associated with fitness levels using VO_{2pk}.
- HRV via the Polar device trial 1 will agree with HRV via Polar device trial 2.
- HRV via the Schiller device trial 1 will agree with HRV via Schiller device trial 2.

SIGNIFICANCE OF STUDY

The current study will investigate the reproducibility of short sampling periods (512 cardiac cycles) and 8-hour sampling periods of HRV in apparently healthy adults. It should successfully indicate that HRV short sampling periods are a quick, noninvasive method of assessing for the ANS cardiac regulation. The current study will also investigate the association of aerobic fitness and HRV

response variables. Also the results will provide a database of values for HRV in healthy adults that may be used by future researchers.

BASIC ASSUMPTIONS

The investigator made the following assumptions in conducting this study:

1. All subjects were truthful and correct in recording their answers to the questionnaires (i.e. VSAQ, ESS, and Daily Record)
2. All subjects did not take any over-the-counter medications, caffeine, and nicotine during testing periods that would skew their normal heart rates.
3. All subjects were honest about their medications, physical activity level, and health history as reported.
4. All measurements were performed and recorded accurately by R-R Polar Recorder ™, Schiller AT-10™, HPLC, and the technician.
5. All biochemical markers were collected properly.
6. Subjects served as a representative of the population as a whole.

DELIMITATIONS

The investigator delimited the study through the following methods:

1. All subjects were required to provide a medical release form from their PCP.
2. All HRV measurements with the Schiller device were performed in duplicate to ensure accuracy.

3. Each subject was supine and awake in the exact same position during the trials with the same technician present.
4. During the Schiller trials, subjects controlled their breathing using a metronome to pace the breathing rate.
5. During the Polar trials, a daily record of activity was used to ensure similar patient status.
6. All biochemical markers were measured in duplicate.

LIMITATIONS

Interpretation of the data was limited by the following:

1. The patients were unfamiliar with the testing procedures during the first trial and more familiar during the second trial, this may have resulted in a learning effect or less emotional anticipation.
2. The sample size may have been too small to ideally perform statistical analysis.
3. HRV via the Schiller and Polar device were recorded within a range of time apart (the day before or after) but where not recording at the exact same time.
4. The Polar Recorder ^{TM} did not store the ECG with the R-R data, which did not allow for cross checking results.

DEFINITION OF TERMS

Achetylcholine: A neurotransmitter located in somatic motor and the parasympathetic nerve fibers which carries information across a synaptic cleft to nerve cells. It is responsible for decreasing heart rate and decreasing force of contraction in the cardiac muscle fibers.

Autonomic Nervous System: Part of the nervous system in which the neurons are not under conscious control. It regulates key functions including the activity of the cardiac muscle, smooth muscles, and glands. It consists of two antagonistic components: the sympathetic division and parasympathetic division.

Alpha-adrenergic Receptor: Receptors for epinephrine or norepinephrine, such as those on effector organs innervated by postganglionic adrenergic fibers of the sympathetic nervous system.

B-adrenergic Receptor: A molecular structure within a cardiac muscle cell that when stimulated by neurotransmitters of the sympathetic nervous system causes an increase in the force of contraction of the cell.

Baroreceptors: Receptors for arterial blood pressure located in the aortic arch and the carotid sinuses.

C AMP: Cyclic adenosine monophosphate. A second messenger in the action of many hormones, such as catecholamines, serving to mediate the effects of these hormones on their target cells.

Cardiac Output: The volume of blood pumped from the ventricles each minutes. It is the product of heart rate and stroke volume.

Catecholamines: Chemical markers including norepinephrine, epinephrine, and dopamine, which indicate the degree of sympathetic activity.

Efferent: Efferent nerve fibers conducting impulses away from the CNS.

Epinephrine: A hormone produced and secreted by the adrenal medulla in response to low blood glucose, exercise, and stress. It causes a breakdown of glycogen to glucose and encourages a release of fatty acids from adipose tissue, vasodilation of small arteries within muscle, and an increase in cardiac output.

FFT: Fast Fourier Transformation-Algorhythm most commonly used to statistically manipulate and analyze the frequency domain of Heart Rate Variability.

Frequency Domain: A method of analysis of HRV through estimates of frequency and power using the FFT over time.

High-Frequency Component: Component of HRV in the frequency domain that is associated with influences from the parasympathetic nervous system.

HRV: Heart Rate Variability-A noninvasive technique measuring variations of instantaneous heart rates and R-R intervals in effort to

assess the autonomic nervous system. HRV uses time and frequency domains to analyze the variations.

Low-Frequency Component: Component of HRV in the frequency domain that is associated with influences from both the sympathetic and parasympathetic nervous system.

Muscarinic Receptor: Receptors for achetylcholine that are stimulated by postganglionic parasympathetic neurons.

Neurons: A nerve cell, consisting of a cell body with a nucleus, and short branching processes, called dendrites that carry electrical charges to the cell body.

Neurotransmitters: A chemical contained in synaptic vesicles in nerve endings that is released into the synaptic cleft, where it stimulates the production of either excitatory or inhibitory postsynaptic potentials.

Norepinephrine: A neurotransmitter released from postganglionic sympathetic nerve endings and as a hormone together with epinephrine.

Parasympathetic nervous system: Division of the autonomic nervous system.

R-R interval: Amount of time between consecutive ventricular depolarization.

Sinoatrial node: A mass of specialized cardiac tissue in the wall of the right atrium that initiates the cardiac cycle.

Spectral Analysis: Analysis of the heart rate variability in the frequency domain.

Sympathetic Nervous System: Division of the autonomic nervous system.

Time Domain: Basic assessment of HRV using simple statistical analysis.

Vagal Nerve: The tenth cranial nerve, composed of sensory dendrites from visceral organs and preganglionic parasympathetic nerve fibers.

LIST OF ABBREVIATIONS

Ach	Acetylcholine
ANS	Autonomic Nervous System
BMI	Body Mass Index
CAD	Coronary Artery Disease
CHF	Congestive Heart Failure
CNS	Central Nervous System
E	Epinephrine
ECG	Electrocardiogram
FFT	Fast Fourier Transformation
HF	High Frequency
HRV	Heart Rate Variability
HPLC	High Performance Liquid Chromatography
Hz	Hertz

LF	Low Frequency
MI	Myocardial Infarction
Ms	Milliseconds
MSNA	Muscle Sympathetic Nerve Activity
NE	Norepinephrine
RR Average	Average interval of heart beats measured during HRV.
RR Interval	Interval of heart beats measured during HRV.
RR50	Difference in consecutive RR intervals more than 50 ms
SA node	Sinoatrial node
SD	Standard Deviation
VLF	Very Low Frequency
VSAQ	Veterans Specific Activity Questionnaire

SUMMARY

HRV is a valuable measurement because of the information it provides about the ANS. Many pathological conditions result from alterations of the ANS and baroreflex systems. Researchers use HRV as a tool to assess pathological conditions such as: hypertension, chronic angina, CHF, MI, FAP, diabetes, multiple metabolic syndrome, and Obstructive Sleep Apnea (20). Although clinical implications of HRV appear promising, clinical determinants and normative values must be determined (1). HRV changes in normal, healthy humans with age and fitness level (20,21). These normal variations must be

determined before implicating this measurement tool to pathological conditions. HRV is limited due to significant intra-individual variability. Methods must be assessed before HRV measurement is used in further applications. The purpose of the proposed study is to primarily, evaluate the reproducibility of two time durations and separate devices of recording HRV and assess the correlation of HRV with VO_{2pk}. Secondarily, the aim is to provide normative values for 30-60 year old, sedentary adults.

CHAPTER TWO: LITERATURE REVIEW

INTRODUCTION

Because of the information it provides about the ANS, HRV is a valuable measurement. Currently researchers are using HRV as a tool to assess pathological conditions resulting from alterations of the ANS such as: hypertension, chronic angina, CHF, MI, Familial Amyloid Polyneuropathy (FAP), diabetes, multiple metabolic syndrome, and Obstructive Sleep Apnea (OSA) (10,15,18,23,24). HRV is influenced in normal, healthy humans by aging, obesity, and fitness levels (12,17,20-21,25-26). These normal variations must be determined before correctly implicating this measurement tool to pathological conditions. In addition, inconsistent findings with short-term measurement duration have caused controversy regarding the reproducibility of HRV (2,11). Although the clinical implications of HRV appear promising, clinical determinants and normative values must be determined.

This chapter will summarize 1) current research using HRV to understand pathologic alterations of the ANS. It is important to understand the value of HRV as a measurement tool. 2) the validity and reliability of measuring HRV, and 3) technical issues with the use of HRV regarding the length of recording time necessary to collect statistically relevant data.

CLINICAL APPLICATION OF HRV

HRV is has been used to assess the ANS in both healthy and diseased populations. HRV measurements have also been helpful to evaluate changes in the cardiovascular system due to treatments, exercise training, aging, and for risk

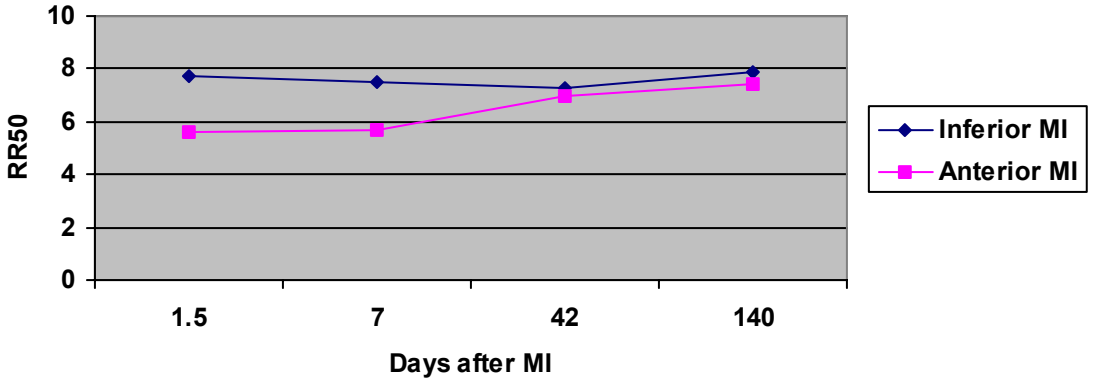
stratification. There is hope that measuring HRV could serve as a quick screening tool in many settings.

An American College of Cardiology position statement in 1993 acknowledged that because of the information it may provide about the cardiac autonomic function, HRV was a useful tool for risk stratification of life-threatening arrhythmias and possibly cardiac death. It is known that an enhanced vagal tone has a protective effect in preventing the development of life-threatening ventricular arrhythmias in many situations such as diabetic and post infarction patients (1). Measures of vagal tone may have prognostic clinical significance. For example, in many recent studies the 24-hour Holter ambulatory electrocardiograph was used to measure HRV. After a myocardial infarction, HRV better predicted future arrhythmic events in patients when compared to measures of baroreceptor sensitivity and left ventricular function. It was stated, however, that there is a need for standardization of the measurements of HRV and to quantify normal values under various circumstances (1,4).

Immediately following and even long after myocardial infarctions, spectral analysis of R-R variability has been used to predict arrhythmic events and even death. Flapan et al, 1993 used HRV to investigate the recovery of vagal tone after inferior and anterior MIs. Recovery in 20 carefully selected patients was evaluated at four time points: 1.5, 7, 42, and 140 days post MI. Each patient was treated with streptokinase and aspirin immediately after MI. During three-month follow up, they received atenolol and aspirin. Vagal recovery was defined by counting the number of times that successive R-R intervals differed by more

than 50 msec (RR50) from the adjacent interval within a 24-hour period. The RR50 strongly correlated with high frequency (HF) power, which represents parasympathetic influence. The difference in the pattern of recovery of RR50, depending on the site of the infarction, is shown in Figure 3. There is a significant increase in vagal recovery for anterior MI up to day 42. After day 42, there was no significant increase between the inferior and anterior MI groups. HRV was used to collect important information about pathological changes in patients after an MI. It was concluded that stimulation of vagal afferent nerves by ischemia and prostaglandins preserved the efferent vagal activity in inferior myocardial infarction. Not accounted for however, was the marked decrease in cardiac vagal activity in anterior myocardial infarction; the infarct may increase afferent sympathetic nerve traffic (4,27).

Figure 1. Recovery of Vagal tone after myocardial infarction (Flapan et al, 1993)



Another condition in which HRV measurements have proved valuable is Obstructive Sleep Apnea (OSA). OSA is a serious disorder caused by repetitive obstructions in the upper airway during sleep. If left untreated, patients with OSA

may be more prone to developing heart disease. Underlying mechanisms of this association are not known; however, because sympathetic drive is increased in OSA patients, alterations in the ANS may be somehow linked. Narkiewicz et al, 1998 tested the hypothesis that even in the absence of overt cardiovascular disease, OSA is accompanied by alterations in cardiovascular variability. Normal control subjects, patients with mild OSA, and patients with moderate-to-severe OSA were used. Based on apnea-hypopnea index (AHI) scores, patients were placed in either group. Those >20 were moderate-to-severe, and those <20 were categorized as mild OSA. It is known that there are higher mortality rates in mild-to-severe OSA patients who have an AHI>20. Patients were not receiving any medications or prior OSA treatment, they were newly diagnosed, normotensive, and were free of and cardiovascular complications. HRV, BP, and muscle sympathetic nerve activity (MSNA) was recorded during the daytime for 10 minutes while subjects were awake in a position of undisturbed supine rest. Using tungsten microelectrodes, MSNA was recorded in the nerve fascicle of the peroneal nerve in the lower leg. Sympathetic activity was calculated as burst/min and bursts per 100 heartbeats. Compared to the control subjects, MSNA was markedly elevated both OSA groups. Likewise in these groups the R-R intervals were significantly shorter than the control subjects (793+ 27 ms and 947+ 42 ms) ($p = 0.008$), and variance was reduced ($p = 0.02$, $p = 0.01$). The patients with moderate-to-severe OSA had an increased normalized LF variability of RR interval ($p = 0.045$) and a decreased normalized HF variability in the RR interval ($p = 0.04$). Ultimately it was determined that even in the absence of hypertension

and other disease states, cardiovascular variability is altered in patients with OSA. It may be that the altered variability is linked to the severity of the OSA. In patients with mild OSA, some of these abnormalities are less pronounced. HRV is useful in providing information about cardiovascular autonomic function (18).

HRV has also been used to gain insight into the pathogenesis of hypertension. In the Framingham Heart Study, 1998 time and frequency domains were used to evaluate HRV in hypertensive and normotensive subjects obtained from a pool of 931 men and 1111 women. The time and frequency domain measurement reported were: standard deviation of normal RR intervals (SDRR), the percentage of difference between adjacent normal RR intervals exceeding 50 ms (pRR50), total power (TP), high frequency power (HF), low frequency power (LF), and LF/HF ratio. The analysis revealed HRV measurements responded differently between genders when associated with hypertension. For example, SDNN, HF, and LF/HF were not associated with hypertension in either gender. But LF was associated with hypertension in men but not women. Among normotensive men, lower HRV was associated with a greater risk for developing hypertension. Using HRV analysis, it was concluded that autonomic dysregulation is present in the early stage of hypertension (15). HRV has the ability to provide valuable information about conditions with altered ANS function; however, the variables reported and technical issues, such as duration of recording, need to be evaluated.

HRV METHODOLOGY

Introduction

HRV is used as a quantitative marker of the ANS. New commercial devices can provide an automated measurement of HRV by measuring the oscillations between ECG cardiac cycles using the R-R interval (1). HRV is analyzed in two domains: time and frequency. The measures of the frequency domain measures are more commonly used in mechanistic studies because they distinguish the parasympathetic and sympathetic divisions of the ANS. For prediction of mortality the time and frequency domain are equally valuable (1,4). In an ECG recording, the R-R intervals are specifically identified and recorded then several variables are statistically calculated such as: mean RR interval, mean heart rate, the range, the difference between night and day heart rate, the standard deviation of the R-R intervals within the recording, and the number of interval differences of successive R-R intervals greater than 50ms (RR50).

Spectral methods for the analysis of the tachogram provide the basic information of how power (variance) distributes as a function of frequency. The most commonly used method for frequency domain measures is the fast Fourier Transformation algorithm (FFT). The FFT decomposes the variance of the input data into the variance attributable to each specific frequency. The frequency domain measures are: total power (TP), low frequency power (LF), high frequency power (HF), and a ratio of LF/HF, which are measured in absolute values of power (milliseconds squared). These measures are reported as percentages of total power minus the very low frequency power (VLF). The VLF

is not completely understood but is believed to reflect endocrine function therefore is omitted when evaluating the ANS. It is important to understand within the degree of autonomic modulations the frequency domain measures rather than the level of autonomic tone. The R-R interval spectra are inversely related to the log of the frequency. Therefore, lower frequency reflects greater power. Both the sympathetic and parasympathetic modulations contribute to LF (0.04-0.15 Hz) measures, while HF measures (0.15-0.40 Hz) represent only parasympathetic efferent signals modulated by respiration. Shown by the reciprocal relationship between the two divisions of the ANS, the LF/HF ratio is useful for assessing the sympathovagal balance. Here, the same relationship appears with the LF and HF components of HRV. Although these measures are indirect markers, HRV provides information about the ANS that would be otherwise impossible to reach without more invasive means (1,4,9).

Validity of HRV

In response to the controversy about the frequency domain of HRV and its implications, many studies have been conducted. Pharmacological blockades, neural lesions, and vagotomies are a few of the methods used in effort to validate the relationship between parasympathetic and sympathetic divisions of ANS with the HF and LF of HRV. Observations with administration of atropine clearly abolished the HF component of HRV, establishing HF is representing parasympathetic modulations (1). The LF component is not as established as is the HF component. In a study by Inoue et al, 1996 used tetraplegic patients who have a disorder that disrupts spinal sympathetic neurons

which leaves patients with deprived modulatory control. In these patients the LF component was absent and HF was well preserved. The absence of the LF component was most likely due to the interrupted spinal pathways connecting supraspinal centers with the peripheral sympathetic outflow. It was concluded that LF component represents sympathetic influences (1). In a second study by Pagani et al, 1991 the LF was significantly reduced when B-adrenergic receptors were pharmacologically blocked. Unlike the previous study, the LF component was not completely absent (28-29).

The effects of sympathetic influence on LF can also be seen during ANS stimulation. Rimoldi et al, 1990 observed a significant increase in LF during baroreceptor unloading with nitroglycerin infusion, transient coronary artery occlusion, and physical exercise. Because LF was increased with all three treatments despite differences in arterial blood pressure it is likely that LF reflects sympathetic excitation regardless of the mechanism (ie. baroreflex mechanism) (30).

Goldberger et al, 1999 used 14 normal male and female subjects, 22-38 years of age, to evaluate stimulation and blockade of the sympathetic and parasympathetic effects on HRV measures. After establishing baseline in a supine position, subjects were lifted into an upright tilt position (approximately 70º) to arouse a reflex stimulation of the sympathetic nervous system. After an intravenous infusion of epinephrine and a separate infusion of isoproterenol to achieve sympathetic stimulation, the same protocol was carried out with the subjects. A final measurement was recorded using atropine and propanolol to

achieve a double autonomic blockade. On the second day of testing, phenylephrine and atropine were used in subjects to stimulate sympathetic and block the parasympathetic nervous system. Goldberger defined the vagal-sympathetic effect (VSE) ratio as the R-R interval influenced by the ANS and circulating hormones over the intrinsic R-R interval. A balanced VSE ratio suggests that the effects are from the intrinsic rate. Therefore, if the ratio is <1 it reflects sympathetic predominance and a ratio >1 reflects parasympathetic dominance. Table 3 summarizes the results obtained from the data collected. Some meaningful conclusions were drawn about the responses of HRV measures in different autonomic conditions. Along with the VSE ratio, the R-R interval demonstrated the lowest inter-subject variability along with the VSE. However the VSE is not the most practical index to use. The table 3 illustrates how each treatment changes the HRV measures compared to baseline measurements. With the tilt and epinephrine, isoproterenol, and atropine infusion, there is a decrease in parasympathetic, increase in sympathetic compared, and an increased heart rate. The increase in heart rate is shown by the R-R interval decreasing. For all the parameters of HRV, the model produced significant results (3).

Table 1: Physiological Changes in Heart Rate Variability Measures after Autonomic Maneuvers, (Goldberger et al, 1999)

	Baseline	Tilt	Epi	Iso	B-Block	PE	Atropine
R-R ms	981	693	762	498	921	1335	542
SD ms	70.8	47.9	45.1	17.1	61.5	45.1	8.0
VSE	1.65	1.17	1.27	0.82	1.54	2.27	0.92
LF bt/min^2	3.24	7.41	3.77	2.37	2.94	0.35	0.07
HF bt/min^2	2.24	1.64	1.98	0.76	2.16	0.32	0.11
LF/HF	1.98	6.34	2.56	5.07	3.00	1.13	3.78

R-R, R-R interval; SD, Standard Deviation of R-R intervals; VSE, vagal-sympathetic effect; LF, low frequency power; HF, high frequency power; Epi, epinephrine; Iso, Isoproterenol; B-Block, B-Blockade; PE, phenylephrine.

Kingwell et al, 1994 compared three techniques to measure sympathetic activity. They included: microneurography, measuring norepinephrine spillover from the heart, and spectral analysis of LF domains in effort to validate HRV methods for measuring sympathetic nervous system. It was hoped that a noninvasive method such as HRV would be demonstrated to be just as valid as more invasive methods. Measuring NE spillover provided global sympathetic nerve firing rates in the heart. Measuring HRV provided information about the ANS at the SA node of the cardiac muscle. Finally, microneurography measured the firing of sympathetic nerves in skeletal muscle. The groups of subjects that were used for this study were: healthy volunteers, cardiac transplant recipients, and cardiac failure patients. HRV was recorded using the spectral analysis contributions to LF power defined as 0.07-0.14 Hz and were expressed in

absolute units and normalized units. NE spillover was measured using the radiotracer method. According to the Fick principle, the rate of NE spillover from the heart is defined as:

Cardiac NE spillover={(NE_{CS}-NE_A)+(NE_A*NE_{EX})}*CSPF

NE_{CS} represents the venous NE concentration while NE_A represents arterial NE concentration. NE_{EX} represents the extraction fraction of marked NE that was intravenously infused. And lastly, the CSPF represents coronary sinus plasma flow in ml/min. The results offered a comprehensive assessment of the cardiac neuroeffector response. NE spillover in the cardiac transplantation group, was very low early after the transplantation compared to normal levels (1.9$\pm$3.1 verses 23$\pm$3 ng/min, P<.05). Similarly HRV levels were low compared to normal immediately after transplantation as well (total power, 29$\pm$11 versus 1673$\pm$516 ms^2,P<0.05) while NE spillover returned to near-normal levels (17.8$\pm$1.9 ng/min). HRV levels remained low 2-years after transplantation (70$\pm$30 ms^2). NE spillover was 2-3 times greater in cardiac failure patients compared to the normal controls (59$\pm$14 versus 18$\pm$3 ng/min, P<0.05); however, total power was significantly reduced compared to the controls (P<0.05). There was no significant correlation between LF and the rate of NE spillover measurements. This is not surprising because sympathetic activity was being measured at different locations, which are different aspects of the sympathetic neuroeffector mechanism. MSNA recordings in efferent signals to skeletal muscle did not necessarily reflect cardiac sympathetic nerve firing. The NE spillover rate provided a cardiac specific measure of sympathetic nerve firing, while HRV represents an end-organ

response. The end-organ response is determined by factors such as nerve firing, electrochemical coupling, cardiac adrenergic receptor sensitivity, postsynaptic signal transduction, and neural reflexes. This is of particular interest because neuroeffector response is altered in many pathological conditions. For example, observed in the cardiac failure group was an elevation in sympathetic nerve firing, which was not shown in the HRV end-organ response. This may represent receptor desensitivity or postreceptor signal impairment (16).

Catecholamine levels in urine and plasma are often used to measure sympathetic nervous system. It is hypothesized that that levels of NE and E are altered in particular pathological conditions where HRV is also altered. A study by Fauvel et al, (1990) investigated the sympathetic nervous system in patients with essential hypertension and normotensive patients. Urinary norepinephrine excretion was significantly higher in the hypertensive group (31). However, Messerli et al, (1982) found differing results in a study using normotensive and hypertensive. Twenty-four-hour rate (HR) and 4-hourly excretion of epinephrine, norepinephrine, and dopamine were comparable between normotensive and hypertensive persons. No correlation between urinary catecholamines and arterial BP (systolic, diastolic, or mean), HR, or prevalence of ectopic beats was found in any of the groups or in the total study population. It was concluded that urinary catecholamine and variability of HR were not related. It should be noted that the urinary catecholamine sample was collected in only 4-hour segments (32). Dimsdale et al, 1995 investigated the effects of OSA on plasma and urinary

catecholamine levels. 4 groups were used: apneic hypertensive, apneic normotensive, nonapneic hypertensive, and nonapneic normotensive. Urinary catecholamines were measured using a radioenzymatic assay. The results revealed that 12-hour urinary norepinephrine was significantly higher in apneic hypertensive and apneic normotensive patients ($F_{1,32}$=11.55, p = 0.002). The urinary catecholamine levels suggest that OSA patients have increased sympathetic nervous system activity (33). Using a 24-hour biochemical measure may be beneficial to provide a better picture of the ANS. The usefulness of urinary catecholamine levels is limited by the uncertainty of what extent of the excreted urinary NE is derived from plasma or from renal sympathetic nerves (34).

HRV RELIABILITY

Due to changes that occur with the aging process, obesity, and physical fitness levels changing over time, HRV is difficult to reproduce in human studies. These factors all modify R-R variability in normal, healthy humans. Many studies have confirmed substantial decreases in HRV with increasing age. Pagani et al, 1986 studied 57 healthy normal adults who were divided into 3 age categories: 20-30 years, 30-45 years, and 45-60 years of age. Results revealed a decline in variability from age 20-60 with the steepest decline from age 20-40 years (28). Luutonen et al, 1994 used spectral analysis of HRV as an evaluation of sympathetic function in elderly subjects. Their purpose was to assess the usefulness LF as an evaluation of sympathetic modulation. LF spectral analysis measures were correlated with plasma NE levels after head-up tilt in elderly

hypertensive and diabetic subjects. Blood samples were drawn after the subjects had been lying for 30 minutes in supine position. The subjects were then lifted to a 70º tilt for 6 seconds then returned to supine position for the next blood sample. HRV data was collected for two trials of 2.5 minutes before and after the tilt. In hypertensive subjects, a significant correlation could be found between increases in plasma NE levels and increases in relative LF power. While the absolute LF power did not change in response to the head-up tilt protocol (supine 0.44 $\pm$ 0.16, tilt 0.45$\pm$ 0.15). But, the relative LF power did have a significant change (supine 35$\pm$ 4%, tilt 50$\pm$ 6%, $p = 0.015$). The plasma NE levels did increase with the tilt in the hypertensive group (supine 2.3$\pm$ 0.2 nmol/1, tilt 2.9$\pm$ 0.2 nmol/1, $p = 0.040$). The changes in the diabetic group were not significant in response to the head tilt, which was considered a sign of sympathetic failure in these subjects. The relative shifts to LF, which did not appear in absolute values, suggest this may have occurred due to decreases in HF power rather than increases in LF power. By examining healthy young, middle aged, and elderly subjects, it was found that elderly subjects have little to no increase in absolute LF power during tilt protocols. It was concluded that HRV is a useful tool in evaluating sympathetic nervous function in the elderly (35).

Because it is known that there is a decrease in overall HRV in the elderly, the influence of age on arterial baroreflex inhibition of sympathetic nerve activity in healthy adult subjects was investigated. Davy et al 1998 hypothesized that the arterial baroreflex exerts a strong tonic inhibitory influence on muscle

sympathetic nerve activity (MSNA). These reflexes are reduced in humans with age contributing to the age-associated rise in MSNA. Infusions of phenylephrine were used to increase blood pressure in a group of young and older male adults. After that MSNA was determined using the microneurographic technique with the peroneal nerve in the leg. The primary finding of the study was that reduction in arterial baroreflex tonic sympathoinhibition does not appear to contribute to the age-related increase in MSNA. It appears that the responsiveness of the reflex is well preserved with age in healthy adult humans. This means that the age-related increase in sympathetic influence may be due to some mechanism other than the baroreflex response. It is possible that it could be related to the increase in adiposity that occurs with adult aging or an age-related increase in preganglionic sympathetic drive (12).

Karason et al 1999 examined HRV in obese subjects and how it is effected by weight loss. Three subject groups were used: a nonobese group (BMI 17-27 kg/m^2), an obese group who were referred for weight reducing gastric surgery, and an obese group who were treated with dietary recommendations (BMI 31-52 kg/m^2). HRV measurements were recorded in 5-minute segments while NE was estimated by liquid chromatography in a 24-hour collection of urine. At baseline, both obese groups had higher 24-hour NE excretion. One year after operation, the first group of obese subjects had significantly lower NE excretion than did the obese group treated only with diet ($p = 0.01$). These levels almost matched the nonobese group. The HRV variable, standard deviation of R-R intervals (SDRR), was significantly lower in the obese groups at baseline (p

= 0.003) with an equal reduction of the HF and LF components. There was a significant increase in SDNN for the surgery group compared to the dietary obese group (p = 0.037). Similarly, there was a significant increase in both HF and LF during daytime measures in this group when compared to the obese group (p = 0.031 and p = 0.002). At baseline body weight correlated inversely with all HRV indexes, but the strongest association was seen at the 24-hour LF and HF amplitude, with correlation coefficients of -0.45 and -0.68. Because of associated alterations in HRV and NE excretion, it was concluded that increased sympathetic activity and reduced vagal control. These autonomic disturbances appear to be reversible after weight reduction -- they are likely a consequence of the obese state (36). Obesity has been shown to alter HRV although future research is needed in this area.

Changing the level of fitness can create changes in the ANS, which alters HRV measures. A study conducted by Lazoglu et al 1996 assessed HRV in three subject groups: 1) a sedentary control group, 2) a group who trained aerobically with cycling, and 3) a group who lifted weights. Each subject performed a maximal cycle ergometer stress test with ECG monitoring to determine VO_{2max} and left ventricular mass. While not participating in any physical exercise and restraining from beverages with cardiotonic effects, HRV was recorded for 24-hours. Results showed a significant correlation between VO_{2max} and the standard deviation of the R-R intervals (r = .41, p = 0.02). No significant correlations were found with the frequency domain of HRV and

VO_{2max}. Overall degree of physical activity, rather than the type of exercise, requires consideration when performing HRV analysis (12).

TECHNICAL ISSUES WITH HRV METHODOLOGY

In the past, a 24-hour period was used for HRV recording. HRV is currently easy to measure, but when time is limited, it may be advantageous to use a shorter sample duration of the recordings to provide a screening tool. It is important to have a proper recording duration in order to remain accurate. Many reports utilizing HRV analysis are often difficult to interpret due to the variations in study design. Some of the variability within in clinical research may be attributed to a wide spectrum of environmental, temporal, hemodynamic, and behavioral factors. Specific to the frequency domain, the recording duration should last for a time that is 10 times the wavelength of the lower frequency bound of the investigated component (1). For example, the LF component needs approximately two minutes of recording in order to be recognized.

In a study conducted by Marks and Lightfoot et al, 1999 the reproducibility of short-sampling periods of resting HRV was assessed in 8 college-aged, healthy physically active females. None of the subjects were using nicotine, or any medication that would skew heart rate. On the day of testing, subjects reported that they had refrained from physical exercise, caffeine, and nicotine, and were not feeling stressed at the time of their appointments. Each subject was prepped with a 12-lead ECG and rested in a supine position to establish a resting baseline. Breathing rate was set by a metronome at 10-12 breaths per

minute for the entire period including the 10-minutes of HRV recording. This procedure was repeated on a second day within the week at the same time of day. The time domain was assessed by using heart rate, mean RR, and the standard deviation of the RR intervals. During the 2.5-minute data set, an average of 164 RR intervals were collected. The 5-minute data set collected an average of 331 RR intervals, which exceeded the 256 intervals needed for the FFT. Because of the controversy that exists with VLF when using short sampling periods, the frequency domain used HF and LF power. There were no significant differences found between the 2 days for the time domain measures for 2.5 or 5 minutes ($p \geq 0.23$). No significant differences were found with the frequency domain between the days ($p \geq 0.16$). Table 5 shows the reproducibility of time and frequency domains. Although it has been suggested that it is inappropriate to compare time domain measures of different durations due to the potential increase in variance of HRV as the length of the recording increases, the comparison of 2.5 to 5-minute periods no significant differences were observed ($p \geq 0.35$). The significant differences between days and sampling periods, coupled with good intraclass correlation coefficients, confirmed previous reports that time domain variables are reproducible with short sampling periods. The frequency domain revealed higher values for HF rather than LF, which is representing more vagal influence. While there were no significant differences between days, the unexplained variance was far from optimal. It was concluded that time domain measures are more reliable for short sampling and may be a better screening tool (2).

Table 2: The Reproducibility of Heart Rate Variability with Short Sampling Duration

	Difference	P value	r	R^2
Mean RR 2.5	43.1	.229	.87	.76
SDNN 2.5	14.7	.278	.89	.79
LF 2.5	.058	.436	.78	.61
HF 2.5	.151	.351	.76	.58
LF:HF 2.5	-.004	.899	.96	.92
Mean RR 5	37.8	.336	.88	.77
SDNN 5	12.5	.318	.90	.81
LF 5.0	.087	.406	.82	.67
HF 5.0	.241	.385	.67	.45
LF:HF 5.0	-.012	.831	.86	.74

P, repeated measures ANOVA; r, intraclass correlation; R^2, variance explained; mean RR, mean of normal R-R intervals; SDNN, standard deviation of normal R-R intervals; LF, low frequency power; HF, high frequency power; LF:HF, ratio for sympathovagal balance. Values are reported in normalized units.

The results from a preliminary study by Chittenden and Kaleth et al 2000 demonstrated different results from the former study. The reproducibility of HRV measures was evaluated in 16 apparently healthy young women (mean age 19.8 years; BMI = 23.8). Each subject performed 2 trials of 512 cardiac cycles and 128 cardiac cycles on two separate days. The subjects used paced breathing at

10-12 breaths per minute. While there was moderately high reliability for mean RR interval for both 128 and 512 sampling periods (r=0.69-0.95), the frequency domain measures had low and inconsistent reliability for both sampling periods (LF=0.02-0.84, HF=0.2-.62, and LF/HF=0.13-0.93). It was concluded that, regardless of HRV frequency measures that were examined, no discernable intra- or inter-trial trends were observed to suggest any potential for improvement by increasing the number of cardiac cycles sampled (128 vs. 512). Further investigations are needed before meaningful conclusions can be drawn regarding the usefulness of this tool as a research or clinical measure (19). The reproducibility of short sampling periods of HRV has been questioned by some and supported by others. If it is to be used as a quick screening tool, it must be reliable. Careful and precise measurements must be standardized in an effort to properly make use of HRV methods. In addition, independent testing of all the devices is needed prior to implementing the HRV methods into research studies. These commercial devices will automatically perform ECG recordings, artifact identification, and RR interval rejections, which was previously performed manually. The testing of the equipment is extremely important and recommended (1). With more normal data representing the various populations becoming available, HRV could prove to be an important diagnostic tool for identifying potential risk for developing unexpected cardiac dysfunctions.

Summary

HRV has potential to assess the role of ANS fluctuations in various populations including normal healthy individuals and diseased populations. More

studies need to be conducted to enhance the understanding of standards, normal values, physiological interpretation, and uses of HRV. It has been shown that as age increases in humans there is a decrease in overall HRV. This inverse relationship has been observed although the mechanism for the decrease in HRV with age is still unknown. Obesity is another factor in humans that affects HRV. It has been shown that overall variance is decreased with obesity in humans. Studies have also detected favorable changes in HRV that accompany weight loss. Lastly, increased fitness levels also favorably affect HRV by increasing parasympathetic modulations. Studies have shown that regardless of the type of training, VO2pk has a strong degree of agreement with increased overall HRV. HRV analysis is specific to the length of recording and various technical issues; therefore, interpretation of data is limited. Because no comprehensive investigations of all HRV indices in large population have been conducted, normal values are not included in published articles. The small amount of information published has been obtained from studies involving small sample sizes. Therefore it is important to confirm which is used when using short sampling durations or long sampling durations. It is advantageous to use short sampling recordings of HRV in order to use this method as a quick screening tool for the ANS, and in situations when evaluating immediate effects of a treatment is of interest. Otherwise long duration recordings provide more stable robust means for calculating HRV indices.

Journal Manuscript
Chapter III
Title: The Reproducibility of Heart Rate Variability Methods and Relations to Aerobic Fitness

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ABSTRACT

The Reproducibility of Short verses Long-Duration Heart Rate Variability Methods and Relations to Aerobic Fitness in Normal Adults

Heart rate variability (HRV) has been used to evaluate cardiac autonomic function by measuring variations in electrocardiographic R-R intervals. Current clinical research interest has extended to investigate uses of HRV to evaluate changes in the cardiovascular system due to disease, aging, physical activity, and cardiac rehabilitation (2, 5). HRV scores are derivatives of R-R intervals and these may be represented as a function of either time or frequency domain parameters. HRV has been measured in a variety of ways, the most common being a continuous 24-hour collection of R-R data. In recent years, several investigators have sought to assess HRV by utilizing brief collection periods. Controversy exists about the potential of these short-term sampling intervals to yield reproducible and meaningful measurements of HRV. Many confounders such as respiration, stress, and body positioning can influence HRV, which is why a longer collection period has been accepted as the standard for providing a stable index of ANS function. However, short sampling periods would be useful to evaluate HRV when faced with time constraints. The purpose of the current study was to evaluate the reproducibility of HRV using 8-hour daytime measures and with short sampling duration of 512 cardiac cycles.

CHAPTER III-JOURNAL MANUSCRIPT

INTRODUCTION

Heart Rate Variability (HRV) is a marker used to evaluate functional attributes of the autonomic nervous system (ANS). Various HRV indicators have been used for many years due to the early demonstration of its association with cardiovascular mortality (1). HRV is defined as the fluctuation in heart rate, as assessed by evaluation of consecutive cardiac cycles using electrocardiographic R-R intervals. Currently clinical researchers use it to evaluate changes in the cardiovascular system due to disease, aging, exercise training, pharmacological intervention, and cardiac rehabilitation (1,2). The ANS can be assessed using many techniques including hemodynamic, pharmacological, biochemical, neurophysiological, neurochemical, and neural imaging. Many of these techniques are regarded as unreliable or almost obsolete while some are more valuable tools; however, even the valuable techniques have limitations due to invasiveness and being extremely demanding of the individual being evaluated (3). Due to complications with existing measures, HRV may be a promising marker. It allows for a noninvasive and easily administered measurement of the ANS. HRV is measured statistically in time domains and frequency domains. Because it appears to resolve parasympathetic and sympathetic influences better than time domain measures, the frequency domain measures of R-R variability are more commonly used for mechanistic studies. However, for prediction of mortality, time and frequency domain measures provide equivalent information (1,2). The time domain refers to the following measurements: mean R-R interval (RR), standard deviation of RR intervals (SDRR), and the percent of R-R

intervals that differ from the adjacent interval exceeding 50ms (pNN50). A lower value for SDRR and pNN50 would reflect a lower variability. The frequency domain is computed by the fast Fourier Transformation (FFT) to represent the distribution of RR intervals as a frequency spectral analysis allowing the divisions of the ANS to be distinguished. High frequency HRV (>0.15 Hz) is believed to represent cardiac parasympathetic activity. Low frequency HRV (0.05-0.15 Hz) reflects both cardiac parasympathetic and sympathetic activity. A ratio of LF/HF may be a valid indicator of interaction between the two divisions of the ANS (4). HRV analysis has the potential to be a useful marker to evaluate cardiac autonomic function in research as well as diagnostic settings. The use of short sampling periods may be an efficient and effective method of evaluating HRV. However, significant intra-individual variability of the results from different testing of HRV limits the use of HRV in settings that may benefit from using HRV as a noninvasive evaluation. Such variability may be due to some technical issues such as duration of recording, respiration, body position, or environmental factors (5-8). Controversy currently exists about the reproducibility of HRV measurements with short sampling durations. Thus, the purpose of the current study was to investigate the reproducibility of short sampling periods of HRV (512 cardiac cycles) and longer sampling periods (8-hours), and evaluate the agreement between the two methods of HRV recording in apparently healthy adults. A secondary aim was to clarify whether HRV as assessed with short sampling periods would afford a quick and convenient way to measure HRV responses in studies involving apparently healthy adults.

METHODS

***Subjects*:** Volunteers were recruited by means of a flyer, word of mouth, and email to participate in the study. The Institutional Review Board (IRB) at Virginia Tech approved the study protocol. Subjects were sedentary, healthy adults between ages 28-60 years. The criteria for participation was as follows: sedentary; no heart problems, including heart attack, chest pain that a physician has said may be related to heart problems, or surgery for your heart or its blood vessels (coronary revascularization), or congestive heart failure; no chronic lung diseases; no uncontrolled high blood pressure or use of blood pressure medications or antihistamines; no uncontrolled diabetes mellitus; no orthopedic (bone or joint) problems, musculo-skeletal conditions, and/or neuromuscular conditions that would prevent you from doing vigorous exercise; no use of tobacco products use (only non-smokers could participate). The subject characteristics (mean $\pm$ SD) were: age = 39.7$\pm$7.01 years, weight = 177.8$\pm$27.7 lbs, and Body Mass Index = 28.6$\pm$4.9.

Location of study: All testing was held at Southwest Virginia Sleep Disorders Center (SVSDC) in Christiansburg, Virginia. Both visits where HRV analysis was conducted was held at approximately the same time of day, in the same room, the number of technicians present was kept consistent, and the noise levels were minimized.

Questionnaires: Each subject completed: Informed consent form, medical and health history form, physician's referral form from their primary care physician, Epworth sleepiness scale to make screen for possible sleep disorders, Veteran's

specific activity questionnaire to screen for sedentary lifestyles, and Daily activity log used to ensure no significant changes between stress and activity levels on the days of testing.

***Experimental protocol*:** Each subject participated in 3 visits: 1.) An orientation visit, which included: an informed consent, medical history screening, questionnaires, pretest instructions, and scheduling the test dates 2.) Subjects performed 2 trials of Schiller AT-10^{TM} (Schiller AG, Baar, Switzerland) HRV protocol (512 cardiac cycles), 3.) Subjects performed 2 trials of Schiller AT-10^{TM} HRV protocol followed by a maximal cycle ergometer exercise test. Visit 2 and 3 were scheduled a week apart at the same time of day. Along with the 2nd and 3rd visits, all subjects performed an 8-hour HRV collection with the Polar R-R Recorder^{TM} (Polar Electro, Kempele, Finland) protocol and 24-hour urine collection. On the days of the 8-hour Polar HRV and urine collection, the subjects completed a daily activity log to identify stress and activity levels on a scale of 1-4 to report differences of stress and/or activity that may influence changes in the ANS.

Visit One: Schiller AT-10^{TM} HRV procedures. Prior to the day of the test each subject was instructed to: not participate in exercise on the day of testing, avoid alcohol, tobacco, over the counter meds that would change heart rate, and not eat for at least 2 hours prior to testing. On the day of the test, measurements for height, weight, and body circumference were recorded. First the subjects were prepped with the electrodes placed in a modified Mason-Likar 12-lead configuration, and the subject information was entered in code into the Schiller

device. The Blackman-Harris was the type of signal limitation selected and the Fast Fourier Transformation (FFT) was used for analysis of the frequency domain measures. The subjects rested in a supine position without any tilt, legs uncrossed lying flat, awake with eyes open throughout the test. They were taught the breathing technique with the metronome and headphones, which provided auditory cues to pace respiration at 10-12 breaths/min. The metronome was set at 40 bpm and the subject would inhale for two beats and exhale two beats. They were allowed to stay in this position and get accustomed to the breathing rate for to establish a resting baseline (heart rate). After the subject established resting baseline, data collection began for 512 cardiac cycles. HRV analysis recorded and excluded any artifact or complexes that were not normal. The recording of 512 cardiac cycles lasted about 8.5 minutes for an average resting heart rate (RHR) of 60bpm and if 90bpm is resting heart rate the test often was as short as 5.7 minutes. Two trials were administered to each subject with 3-5 minutes to move around between the trials: 1 minutes to sit up and move around then 3-4 minutes supine to reestablish baseline heart rate. The second trial was not started until baseline was established and they were accustom to the breathing pattern; this took anywhere from 3-5 minutes depending on subject. Each subject performed two trials on 2 separate days, 1 week apart at approximately the same time of day. The appointments were made at early morning times (ranged from 7:15-8:00am), noon, or late afternoon (ranged from 3:30-4:00pm); therefore, each subject's trial two was tested within 30 minutes range from the prior weeks trial one.

Visit two, Schiller and exercise test: The same procedures were followed as visit one for the HRV analysis. Following the HRV analysis, the subjects performed an exercise test on a cycle ergometer using a maximal ramp protocol. The initial workload started at 25 watts, which increased every 90 seconds. The amount of watts increased depended on their body weight. Blood pressure was measured every 2 minutes, heart rate was recorded every minute using the Schiller AT-10, and ratings of perceived exertion (RPE) were reported every minute. Exercise was terminated when the subject reached 17-20 RPE, voluntary termination, or other termination criteria as outlined in ACSM Guidelines for Testing and Prescription (9). A licensed physician was present during every exercise test.

8-hour Polar R-R Recorder^{TM} Procedure. Subjects were instructed how to properly use the Polar monitor as well as given an instruction sheet outlying procedures. The subjects were connected to a computer, which previously had the Polar R-R Software Version 1.1 (Polar Electro Oy, Kempele, Finland,1999) installed, in order to set the parameters. The parameters included: current time of day, beginning and ending time of recording, activating it by the button, ECG limits, and setting a filter for the ECG. After setting the subject for proper parameters in the Polar software, the subjects wore the Polar R-R Recorder for 8-hours of wakeful time. During the time of recording, all activity levels and stress levels were logged and rated on a scale of 1-4. After the recorder was returned, each recording was scanned thoroughly to filter any artifact. In effort to ensure proper filtering of the data, 5 separate filtering methods were performed

and compared for significant differences. After determination of a proper filtering method, each of the recordings was filtered. The manual filtering method included an excel spreadsheet with 6 steps: 1) determined the difference between the R-R interval from the adjacent interval, 2) excluded any intervals that exceeded 100ms difference from the adjacent interval, 3) determined the pNN50, 4) excluded any intervals that were less than 300ms in length, 5) excluded any intervals that exceeded 1500ms in length 6) determined the mean and standard deviation of the R-R intervals. In short, all of the time domain measurements were manually filtered and the frequency measures were determined from the polar software. The time domain results that were produced from the polar software were compared to the manually filtering method to determine the level of agreement.

Urine Collection & Catecholamine Assay Procedures: Each subject was given containers to collect 24-hour urine output on the same day of the 8-hour HRV recording. The urine was measured for total volume then 100 mL was extracted and stored in the freezer at -20C for later analysis. Each sample was labeled with date, subject number, name of the study, and time point of measurement. The urine was properly discarded after extracting 100mL. NE and E were determined using high performance liquid chromatography (HPLC). A Beckman "Gold" HPLC system (San Ramon, CA) was connected to Luna 5uC18 column (250 x 3 mm) (Phenomenex, Torrance, CA). On the day of determination, urine samples were thawed in cool water, 100µL aliquot was centrifuged for 30 minutes at 4000 RPM and filtered through Microcon YM-30

filters. The standard curve was determined prior to testing. The injection volume was 10μL at a flow rate of 1mL/min, and the mobile phase was 75mM NaH2PO4*H3O, 1.7mM octane sulfuric acid, 10μM EDTA, 10% Acetonitrile, pH 3.1 with H3PO4. NE and E were expressed as μg/g of creatinine. Creatinine clearance was determined with the Sigma Diagnostics Creatinine clearance kit (Sigma Diagnostics, INC St. Louis, MO) and total volume of urine in mL.

Statistical Analyses: All results were analyzed using SPSS Version 8.8 statistical software (Chicago, IL). To measure the degree of association within each method and response variable, Pearson Product Moment correlation coefficients were computed. To compare the means for within each method and response variable, t-tests for related samples were computed. Variances between the two HRV methods and between HRV responses were assessed by determining intraclass correlation coefficients. These statistics were expressed as percentage variation among and within groups. A high proportion of variance of an observation due to between subject variability suggests 'true' scores of a measuring instrument (10-11). Measurements from HRV with both methods, Schiller (512 cardiac cycles) and Polar (8-hour recording) were analyzed dependentt tests for comparison of mean differences. To estimate the degree of association between urinary catecholamine levels and HRV, as well as the aerobic fitness and HRV, Pearson-Product Moment correlation coefficients were calculated.

RESULTS

Schiller HRV results: HRV response variables using the Schiller method showed high-to-very high correlations between trials, within each day for the time domain measures (r=0.75-0.995). For the frequency domain measures, however, correlations were low-to-moderate (r=0.27-0.66) between trials within each day. The intraclass correlations (ICC) suggested that 6%-19% of the total variation was between trials for the time domain measures and 53%-80% of the total variation for the frequency domain measures were attributable to between trial variation. Correlations between days for HRV response variables using the Schiller method indicated low-to-moderate association for the time domain measures (r=0.40-0.50), with no significant difference in the means. The correlations for the frequency domain measures were low (r=.001-0.36). Tables 1-2 illustrate Schiller results between trials and between days.

Table 1A, 1B. Reproducibility of Heart Rate Variability Response Variables Between Trials within Days (1 and 2) using the Schiller AT-10^{TM} HRV protocol.

A. DAY 1	Trial 1	Trial 2	Mean Difference	Pearson r
RR (ms)	904.4	909.6	5.2	0.93*
RRSD (ms)	51.9	55.2	3.3	0.75*
pNN50 (%)	12.1	11.9	0.2	0.79*
LF (%)	18.1	19.8	1.6	-0.12
HF (%)	39.0	26.1	12.9*	0.65*
LF/HF	0.6	1.1	0.5	0.60

B. DAY 2	Trial 3	Trial 4	Mean Difference	Pearson r
RR (ms)	867.6	879.1	11.5	0.98*
RRSD (ms)	48.6	57.5	8.9*	0.95*
pNN50 (%)	11.2	11.9	0.7	0.99*
LF (%)	21.3	17.4	3.9	0.58
HF (%)	30.8	28.9	1.88	0.41
LF/HF	.88	.80	0.08	0.72*

Significant correlations (N=10; P < 0.05) are denoted by asterisk (*).

Table 2. Reproducibility of Heart Rate Variability Measures between Days using the Schiller AT-10^{TM} HRV protocol.

	Day 1	Day 2	Mean Difference	P Level	ICC	Pearson r
RR (ms)	907.0	873.4	33.7	0.42	0.52	0.44
RRSD (ms)	53.6	53.1	0.5	0.95	0.32	0.50
pNN50 (%)	12.0	11.6	0.5	0.83	0.44	0.40
LF (%)	19.0	19.4	0.4	0.90	0.17	0.00
HF (%)	32.5	29.9	2.7	0.63	0.16	0.36
LF/HF	0.8	0.8	0.0	0.97	0.22	0.30

Significant correlations (N=10; P < 0.05) are denoted by asterisk (*).

Results for Polar HRV: Table 3, presents the variations in results that were found with the Polar software filtering methods, when applying different criteria for automatically rejecting R-R time variations that suggest presence of artifact in the record, i.e. the standard default filter, a high default filter setting, and a low default filter setting. Column one in Table 3 shows the resulting HRV values when the R-R data were analyzed manually, without filtering, and within a computer spreadsheet program. The manual spreadsheet filtering consisted of 6 steps including: 1)-counted the number of intervals that differed from the adjacent interval by 50ms or more, 2)-excluded intervals that differed from the adjacent by more than 100ms due to artifact, 3)-calculated the percent of the intervals that differed by $\geq$50ms from the total number of intervals collected, 3)-excluded RR intervals that exceeded 1500ms, 4)- excluded RR intervals that were less than 300ms, 5)-calculated the mean RR interval, and 6)-calculated the standard deviation of RR intervals. The three different filtering methods correlated closely

with the manual filtering methods for the time domain measures of R-R interval and standard deviation of R-R. However, the pNN50 correlated poorly between these methods. The manual filter was developed for the time domain measures only. Therefore, all further comparisons in this study in which time domain measures are presented with the Polar system reflect data derived from the manual filtering method.

Table 3. Reproducibility of Heart Rate Variability Time Domain Measures with Different Filtering Methods

Manual:	Default	High	Low
RR	0.99*	0.99*	0.99*
SDRR	0.81	0.80	0.81
PNN50	0.28	0.29	0.27

Legend: Manual-excel spreadsheet 6 step filter; Default-the default filter within the Polar software, High-the high filtering power settings, and Low-the low filtering power settings. Significant correlations (N=4; $P < 0.05$) are denoted by asterisk (*).

Table 4 illustrates the reproducibility using the Polar device between days. Correlations coefficients between days for the HRV response variables using the Polar method were moderate (r=0.59-0.67) with the time domain and low-moderate correlations for the frequency domain measures (r=0.37-0.69). However, an important finding that should be considered is that 2 of the data samples were outliers due to excessive artifact in the recording. When performing correlations excluding the two data samples that were outliers, the correlations were high-very high for all time and frequency domain measures (r=

0.70-0.93). Due to this important change in reproducibility, the consequence of artifact-free recordings is unmistakable.

Chi squared tests were performed to determine if the subjects had significant differences in activity and stress level between days. The tests revealed no significant differences at the 0.05 level; therefore, stress and activity levels appear to not be contributors for variation between days.

Table 4. Reproducibility of Heart Rate Variability Measures between Days using the Polar R-R Recorder™ after manually filtering time domain variables and polar software filtering for frequency domain variables.

N=10	Day 1	Day 2	Mean Difference	Pearson r
RR (ms)	694.3	656.3	38.0	0.59
RRSD (ms)	94.6	112.7	18.1	0.67
pNN50 (%)	4.8	3.9	0.9	0.67
LF (%)	92.7	93.2	0.5	0.37
HF (%)	7.3	6.8	0.5	0.67
LF/HF	13.1	14.5	1.4	0.69

N=8	Day 1	Day 2	Mean Difference	Pearson r
RR (ms)	685.2	682.2	3.0	0.91**
RRSD (ms)	89.4	90.4	0.9	0.79*
pNN50 (%)	4.6	4.4	0.3	0.93**
LF (%)	92.7	93.7	1.0*	0.70*
HF (%)	7.3	6.3	1.0*	0.70*
LF/HF	13.1	15.4	2.3**	0.85**

Significant correlations (N=10; P <0.05 or <0.01) are denoted by single (*) or double asterisks (**), respectively.

Method comparison results: The means for each response variable between the Polar method and Schiller method on day 1 were significantly different as shown in Table 5. Correlations for day one scores between these two methods ranged from low-moderate (r= 0.63-0.16). It is clearly obvious that it is inappropriate to compare HRV measures that were obtained from recordings of

different durations. Figure 1-2 illustrates the obvious differences in means between trials and methods using the pNN50 and standard deviation.

Table 5. Reproducibility of Heart Rate Variability Response Variables on Day 1 between Methods using the Schiller AT-10^{TM} and Polar R-R Recorder^{TM} (n=10)

	Polar	Schiller	Difference	Pearson r
RR (ms)	694.3	907.0	212.7*	0.63
RRSD (ms)	94.6	53.6	41.0*	0.15
pNN50 (%)	4.8	12.0	7.2	0.43
LF (%)	92.9	19.0	74.0*	-0.23
HF (%)	7.3	32.5	25.3*	0.16
LF/HF	13.1	0.8	12.3*	-0.15

Significant differences between methods (N=10; P <0.01) are denoted by asterisk (*).

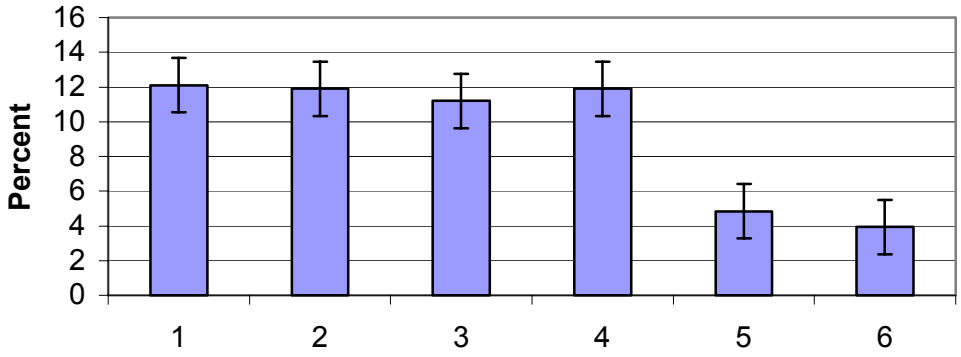

Figure 1. The percent of the total R-R intervals that exceed 50 ms to the adjacent R-R interval (pNN50) with the Schiller HRV method and Polar HRV method.

Legend: 1, Schiller Day 1, Trial1; 2, Schiller Day 1, Trial 2; 3, Schiller Day 2, Trial 1; 4, Schiller Day 2, Trial 2; 5, Polar Day 1; 6, Polar Day 2.

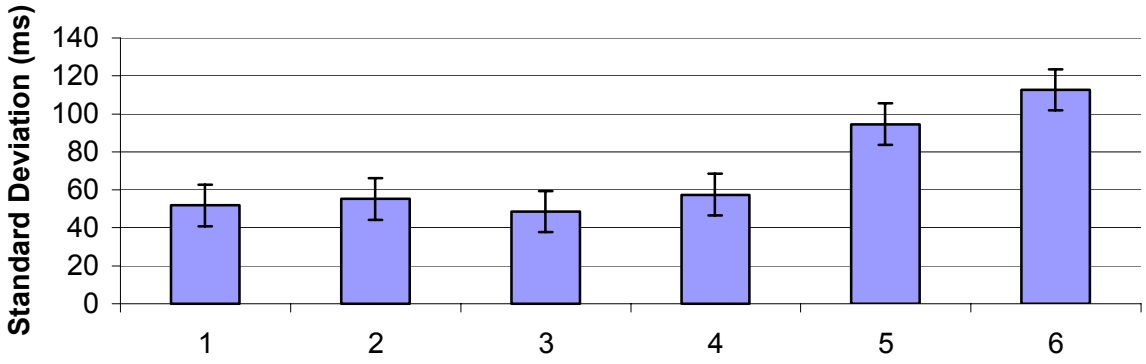

Figure 2. Standard deviation of R-R intervals (ms) with the Schiller HRV method and Polar HRV method.

Legend: 1, Schiller Day 1, Trial1; 2, Schiller Day1, Trial 2; 3, Schiller Day2, Trial 1; 4, Schiller Day 2, Trial 2; 5, Polar Day 1; 6, Polar Day 2.

Urinary catecholamine and HRV results: Correlations revealed no significant association between NE and E with any HRV response variable using the Polar method for day 1 ($r < 0.1$). However, one subject with an extremely high NE level had a lower R-R standard deviation, low HF power, high LF power, and high LF/HF ratio. These trends noticed in the single subject having high NE levels with a reduced HRV agree with results published previously (12-13). These urinary catecholamine and creatinine data are presented in Table 6 with the means, standard deviation, and ranges. Many of the values and ranges do not correspond to expected values for normal adult subjects (26) .

Table 6. Summary of Urinary Data for all subjects (n=10)

Variables	Mean	Standard Deviation	Range
Total volume urine (mL)	1526.3	773.6	560-3155
Creatinine (mg/dL)	79.6	35.5	15.73-134.73
Creatinine (g/24hours)	1.0	0.3	0.4-1.69
NE (ug/g of Creatinine)	3726.6	3452.6	980-15160
E (ug/g of creatinine)	1778.6	1257.9	31-4085

Exercise and HRV results: No statistically significant associations were found between Vo_{2pk} and any of the HRV response variables ($r < 0.5$).

DISCUSSION

The reproducibility of HRV from short-sampling periods has been questioned by some researchers (7) and supported by others (6). If HRV is to be used as a quick screening tool for insight into physiological and pathological conditions and to enhance risk stratification for important disease states, a reliable and stable data collection is needed. The current study has shown the reproducibility obtainable between days using short sampling methods arfe not sufficiently stable to utilize either in research or clinical settings. Because HRV is needed to evaluate changes in the cardiac autonomic system due to treatments, aging, exercise training programs, and other changes over time, it would seem that much larger samples must be obtained over longer monitoring intervals that may be obtained in a laboratory setting over a few to several minutes.

Marks and Lightfoot *et al*., 1999 (6) performed a study using two different short sampling periods (2.5 minutes and 5-minutes) to investigate the reproducibility of HRV time and frequency domain measures. It should be noted

that their (6) experimental design differed in several respects from that employed in current study. They continuously recorded a 10-minute ECG in resting subjects and the two separate sample periods were taken from this same recording. In the current study, each trial was separated by five minutes for the subjects to sit up and move around then reestablish resting baseline measures in supine position. Other differences between the two studies that may further explain differences include the HRV device, subject population, and the method used for controlling breathing rate was different. Marks and Lightfoot *et al. (*6*)* used auditory cues with a metronome while the current study used a headset with a metronome tone in effort to block out other noise. The mean SDRR for the current study using short sampling durations is 53.6$\pm$23 while the mean SDRR for the Lightfoot *et al*. (6) study was 74.2$\pm$54. Lightfoot *et al*. (6) study used 5-minute duration while the current study used 512 cardiac cycles, which lasted approximately 8 minutes. It is recommended that when using short sampling durations, the frequency domain primarily should be used for analysis, and the time domain measures should be used for analysis during long duration recordings (1). The time domain measures have shown to be predominantly more robust for short and longer sampling periods than the frequency domain measures in the current study, which is different from previous published literature (1). For the short sampling period there is a possibility that because the data was recorded while each subject was resting supine with controlled breathing the power spectrum may have revealed extremely low amplitudes for all spectral components making it difficult to separate signal from noise (2). The

results from the current study reveal low-to-moderate correlations suggesting poor reproducibility using short sampling periods, which differs from other studies (6).

Because the process for filtering data using the Polar device is complex and the Polar device does not store the actual ECG recordings, the frequency domain measures may not be exact. It has been recommended to primarily use time domain measurements when evaluating 24-hour recording durations (1). It is advised to use the frequency measures with short sampling duration. The time domain measurements, i.e. mean and standard deviation of R-R intervals and pNN50, were manually calculated to compare with the Polar software calculations to ensure fidelity of transformation of the basic R-R data. In a comparison of the results using software filtering methods to the manual filtering method for a small subset of four cases in this study, it was found that the pNN50 was significantly different (Table 3). This disparity suggests that application of the filtering method within the Polar software tends to remove more of the biological variance and hence, introduces a reporting error. Also, it should be noted that the pNN50 is a time domain measurement that is derived from the differences between R-R intervals instead of from direct measures as are the mean R-R interval and standard deviation. This may explain why only the pNN50 is significantly different from the two filtering methods while other time domain measures are not significantly different. The reproducibility of time domain measures for longer sampling duration using the Polar method with manual filtering are significant at the 0.05 level and moderate-to-high correlations (r=

0.59-0.67). It should also be noted that two of the data samples were outliers due to excessive artifact in the recording. When our data were re-evaluated after removal of results from two data outliers, the correlations were high-very high for all time and frequency domain measures (r= 0.70-0.93). Due to this important change in reproducibility, the consequence of artifact-free recordings is unmistakable. A procedure of some importance in our study was the request made of subjects to refrain from exercise during the wakeful measurement period while they wore the Polar device and to log their incidental physical activity and perceived stress levels. The log data were tested using a non-parametric procedure and no differences were noted between the two days in which Polar HRV measures were obtained.

It has been stated that it is inappropriate to compare time domain measures of different durations due to the potential increases in variance of HRV as the length of the recording increases (1). The current study did indicate significant differences between the two methods, which used 512 cardiac cycles and 8-hour recording. Aside from the time domain, the frequency domain uses a different model to interpret Schiller than does the Polar method. The Schiller device uses the FFT algorithm, which is parametric and simpler to use but has several disadvantages too. Some disadvantages of FFT algorithm are: number of data points must be an integer of two, FFT operations makes the implicit assumption, that the data are cyclic, which is not strictly fulfilled for biological data, and spectral resolution is dependent of the number RR intervals. The Polar method used autoregressive model which is nonparametric, a smoother spectral

estimation and is independent of the number of RR intervals. A possible means for comparison may be to take shorter selections from the 8-hour recording to compare to the Schiller method. However, in order to take a selection of the polar to match the duration of the schiller, it would be important to control the activity in the supine position with controlled breathing. Body position and breathing has been shown to alter HRV.

The Task Force for the Standards of HRV in 1996 stated that no comprehensive investigations of all HRV indices in large normal populations have yet been performed; therefore, normal limits for age, sex, and environment were not included in the special report and are limited to studies with small sample sizes (1). It is believed that the average value for the mean R-R interval for a sedentary adult is 678 ms (2). Results from the current study lie within that range. However, values lower than 50ms for standard deviation of R-R intervals is reported to be indicative of cardiovascular dysfunction (6). The standard deviation for the short sampling period of the current study average 53 ms for apparently healthy, sedentary adults. It has been stated that longer duration recordings are more sensitive than simple resting recordings. Beside their increased sensitivity, the time domain measures for the long duration recording are more stable and reproducible from day to day (1). The correlations for the longer duration method of the current study were very high when complete filtering was possible. This reinforces how essential proper filtering is to provide jitter-free results.

There were not significant associations between aerobic fitness and HRV response variables. However, it is possible that this is due to the small sample size. Because the exclusion criteria strictly selected non-exercisers, there may have been very little difference among them in parasympathetic tone preventing a range large enough to assess any associations. Also VO_{2pk} may reflect not only results from regular activity but also genetic endowment. This could be another reason for weak associations.

CONCLUSIONS

The HRV data obtained from apparently healthy, sedentary adults using short sampling durations were not reproducible in neither time nor frequency domain. However the data from the same group of subjects using longer, 8-hour, recordings with the Polar R-R Recorder was reproducible after excluding the data sets that were contaminated with errors and artifact. A limitation to the current study was using 8-hour recordings because it limits comparison to any previous literature. HRV has been measured for 24-hours duration in many studies and 5-minutes for the short sampling. Because the current study used 512 cardiac cycles and 8-hours, there are not normal values available. The normal values that are published are also limited however due to using small sample sizes. More studies need to be conducted to enhance the understanding of standards, normal values, physiological interpretation, and uses of HRV. HRV analysis is specific to the length of recording and various technical issues; therefore, interpretation of data is limited.

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CHAPTER IV

SUMMARY, CLINICAL IMPLICATION, AND FUTURE RESEARCH

SUMMARY

The purpose of the current study was to investigate the reproducibility of short and long duration sampling of HRV with differing methods. HRV is appealing because it uses recordings of electrocardiograms and created software for analyzing ECG signals to non-invasively evaluate cardiac autonomic function. Current researchers are using HRV to assess ANS after exercise training, aging, cardiac rehabilitation, and other treatments. HRV is expressed in time and frequency domain measures. The time domain measures include: mean RR interval (RR), standard deviation of RR intervals (SDRR), and the percentage of intervals that differ more than 50 ms from the adjacent interval (pRR50). The frequency domain measures, low frequency, high frequency, and the ratio, have been shown to represent the sympathetic and parasympathetic divisions of the ANS with studies using pharmacological blockades with HRV recordings. Similar to many methods used to test humans, HRV is hard to reproduce due to changes that occur over time like aging, body weight changes, and exercise training. There are many different sampling durations being used in research, which also make it hard to replicate prior studies. The task force for the standards of HRV in 1996 stated that no comprehensive investigations of all HRV indices in large normal populations have yet been performed; therefore, normal limits for age, sex, and environment were not included in the special report and are limited to studies with small sample sizes (1). The primary aim of

the current study was to test the reproducibility of different sampling durations and methods of HRV. Secondary aims of the current study were to investigate the level of agreement between urinary catecholamine levels and HRV and VO_{2pk} and HRV.

The current study has shown the reproducibility between days using short sampling methods ranges from low-to-moderate correlations. Because HRV is needed to evaluate changes in the cardiac autonomic system due to treatments, aging, exercise training programs, and other changes over time, the tool must be more reliable than shown in the current study. The time domain measures have shown predominantly higher correlations than the frequency domain measures using the short sampling periods in the current study. For the short sampling period there is a possibility that because the data was recorded while each subject was resting supine the power spectrum may have revealed extremely low amplitudes for all spectral components making it difficult to separate signal from noise.

The results from longer recording durations, 8-hour, of HRV using the Polar device presented more stable correlations for all HRV response variables. However, the process for filtering data using the Polar device is complex and the Polar device does not store the actual ECG recordings, which limits the certainty of the frequency domain measurements. However, the time domain measurements, mean and standard deviation of R-R intervals and pNN50, were manually calculated to compare with the Polar software calculations to ensure truthful answers. The data is not usable if it is improperly filtered or has

excessive artifact; all results will be inaccurate. The current study revealed very high correlations for 8-hour recordings of HRV using the Polar device while low-to-moderate correlations for short sampling durations using the Schiller methods.

CLINICAL IMPLICATIONS

HRV is a promising measurement because it is non-invasive, quick, and fairly easy to perform. Physician could use this screening tool to identify patients who may be at risk of sustained ventricular tachycardia and of sudden cardiac death. Patients suffering from certain disease show a significantly reduced RR variability. According to studies on long-term ECG examinations, the risk of death from sudden cardiac death after myocardial infarction is five times higher for patients with a R-R variability below 50 ms than for patients with higher variability. Using the Polar device, physicians can monitor patients up to 24-hours consecutively. The software allows for selections to be made for any portion of the recording to calculate time and frequency domain measures. The importance of testing each device for validity and reliability is extremely important as the results from the current study found. It is not appropriate to compare HRV response variables obtained from recordings of different durations. The results from differing sampling durations suggest that using short sampling durations does not significantly correlate between measurements taken on separate days. Few studies have been conducted on the stability of long-term measures on HRV. The current study revealed longer durations of recording were more stable and reproducible between days. Some technical issues such as filtering

methods, lead placement, missing data, and storing ECG complexes must be examined. Because the longer-duration indices appear to be stable and free of placebo effect, they may be ideal variables to assess intervention therapies.

FUTURE RESEARCH

Few studies have been conducted to evaluate stability and validity of long duration HRV measures. This study suggests that long duration recordings appear stable and reproducible in apparently healthy, sedentary adults. However, there is a need for large population studies to establish normal HRV standards for various age and sex subsets. As no comprehensive investigations of all HRV indices in large normal populations have yet been performed, we are limited to values obtained from small numbers of subjects. Therefore making clinical conclusions and determinations are limited. The adjustment of normal limits for age, sex, and body fat are another area for future research. New commercial devices designed to measure HRV need to be tested against other means for evaluating the ANS or other HRV recordings that have previously been validated. The technical requirements and analytical part of the device should be used to record a fully reproducible signal, which needs to be conducted at the testing site independent of the manufacturers of the tested device.

A second area for future research is with the short sampling duration of HRV. The reproducibility of HRV from short-sampling periods has been questioned by some researchers (7) and supported by others (6). The current

study has shown the reproducibility between days using short sampling methods ranges from low-to-moderate correlations. It has been stated that it is inappropriate to compare time domain measures of different durations due to the potential increases in variance of HRV as the length of the recording increases (1). Therefore, studies using short sampling durations will also need a set of normal values and variations for HRV time and frequency domain measures. It may be possible to take a shorter selection from the 8-hour recording to compare to the Schiller method. The conclusions for the current study suggest long sampling duration are more stable and will reproduce all variables while short sampling reveals no discernable intra- or inter-trial trends to suggest any potential for improvement by increasing the number of cardiac cycles sampled. Further investigations are needed before meaningful conclusions can be drawn regarding the usefulness of this tool as a research or clinical measure

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Appendix A

Informed Consent

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Control Subjects Participating in Investigative Project

Title of research Project: The short-term effects of exercise training in conjunction with CPAP therapy on cardiovascular function, exercise tolerance, and quality of life in obstructive sleep apnea patients

Principal Investigators: William G. Herbert, Ph.D., Don Zedalis, MD, John Gregg, DDS, Ph.D., William Huckle, Ph.D., Sharon Nickols-Richardson, RD, Ph.D., Thomas W. Chittenden, MA, Anthony S. Kaleth, MS, Brian J. Hawkins, MS, Lawrence Cross, Ph.D.

I. Purpose of This Research

The purpose of your participation is to provide information on how apparently healthy adults respond to a special set of fitness, cardiovascular, and biochemical tests which are being used in an investigation of patients with a sleep disorder. Thus, your results will help the investigators understand how the test results from patients with Obstructive Sleep Apnea (OSA) differ from those of people who do not have a diagnosis of this disorder. In the study, the OSA patients are taking the same tests, but doing so in conjunction with therapeutic treatment. You will not be asked to do tests to evaluate your sleep status, but most of your tests will be done at the Sleep Disorders Clinic in Christiansburg, Virginia. Before you may enter the study, you will be interviewed and complete questionnaires to be certain that you do not have any unusual sleep problems. The scientific purposes of this overall study are: 1) to determine how the OSA disorder affects the heart and circulation, physical fitness, and risk factors for heart disease; and 2) to see if treatment for the OSA patients can be improved by combining nighttime positive pressure-breathing (CPAP) with a moderate exercise program.

As part of determining your eligibility for this study, you and your doctor will be asked to verify that you do not have a known health history that includes any of the following:

Heart problems, including heart attack, chest pain that may be related to heart problems (this is called angina pectoris), surgery for your heart or its blood vessels (coronary revascularization), or congestive heart failure;

Chronic lung diseases;

Uncontrolled high blood pressure or use of blood pressure medications or antihistamines;

Uncontrolled diabetes mellitus;

Orthopedic (bone or joint) problems, musculoskeletal conditions, and/or neuromuscular conditions that would prevent you from doing vigorous exercise;

Use of tobacco products use (only non-smokers can participate)

In addition, to be eligible you must not have been following a program of regular moderate physical activity. If you have been exercising within the past 6 months at a moderate intensity or more, 30 minutes/day for 3 or more days a week, you are not eligible. Finally and in addition to the above, to be eligible you must have seen your personal physician at least once in the past 4 years and they must provide the investigators with written confirmation that they know of no health condition that would make vigorous exercise inadvisable for you.

II. Procedures

You will be asked to complete the following procedures at six (6) different time points over a 12-week period:

1) **Orientation Session**: A 45-minute orientation at the Sleep Center in which study procedures are explained and you complete interviews and study questionnaires;

2) **Baseline Exercise Test**: Within 1 week of the Orientation Session, you will be asked to do a resting heart rate and an exercise test at the Sleep Center; the day before this test, you will carry small devices throughout your normal day to assess your heart rate and blood pressure and you will collect all your urine output over a 24-hour period. The Sleep Center part of this will last ~90 minutes;

3) **Baseline Heart Rate Monitor Test**: Within 2 days after your Baseline Exercise Test, you will have another 30-minute resting heart rate test at the Sleep Center again. On the

day before or after this Sleep Center test, you will repeat the daytime measures for heart rate, blood pressure, and urine collection. No exercise test will be done during this period.
4) **Week 3 Exercise Test**: This test will be done at the Sleep Center, in the same way as the Baseline Exercise Test, but no resting heart rate test will be done. This procedure will last ~60 minutes;
5) **Week 6 Exercise Test:** Procedures performed exactly as for the Baseline Exercise Test. This will last ~90 minutes;
6) **Week 12 Exercise Test:** Procedures performed exactly as in Baseline Exercise Test. This will last ~90 minutes.

Information about the **specific test procedures** you will do is presented below:

- Three or four questionnaires that request your opinion about the quality of your sleep, your current quality of life, and daily physical activities;
- A questionnaire that asks about your eating habits;
- Measurements of height, weight, waist and hip circumference, blood pressure, heart rate;
- During your Orientation Session, you will be asked to practice breathing techniques while wearing a special mouthpiece and nose clip; this practice will help you perform the actual exercise tests which you will do at Baseline, and Weeks 3, 6, and 12;
- Collect a 24-hour urine sample in a jug that the investigators will provide, as indicated in the above schedule of test sessions;
- Collect a small venous blood samples (10mL) immediately before and after each exercise test (Baseline, 6 weeks, and 12 weeks) to measure the ability of your blood vessels to relax, as well as how your nervous and cardiovascular systems are adjusting. Part of the blood sample collected before the exercise tests also will be assayed for blood fats. Slight bruising may occur around the area of the needle stick;
- Measurements of your daytime heart rate and blood pressure that you will do with small portable monitoring devices that you carry with you throughout your normal day;
- Measurements of resting heart rate and blood pressure during test sessions at the Sleep Center in Christiansburg. This will involve having electrodes placed on your

chest and then lying down and breathing quietly for about 20 minutes. During this procedure, you will pace your breathing to match a signal that you hear through a stereo headset. During this period, your heart rate will be measured with a small recorder to evaluate the nervous control of your heart.

- Measurements of your maximal exercise performance on a stationary cycle, including evaluation of your heart's pumping ability and your body's oxygen requirements. The exercise test will last ~14 minutes and include assessments of oxygen consumption, heart rate, blood pressure, and cardiac output (pumping ability of your heart). To measure how much oxygen you use, we will ask you to breathe into a lightweight rubber mouthpiece. During the bicycle test, you will breathe only through the mouthpiece and may experience some dryness in your mouth. You will be asked to perform several exercise cardiac output measurements that require you to slowly exhale a special mixture of oxygen and tiny amounts of a harmless gas that doesn't interact with the body; the time you exhale for this measurement will last only ~5 seconds. You may experience more difficulty completing this procedure during higher intensities of exercise, but the investigators will only ask that you do your best to accomplish this.

The total time involved to complete all of the above procedures over the 12 weeks you are in the study will include ~7 hours of activities at the Sleep Center plus the time and some limited time for receiving and returning the portable monitoring devices and urine jugs required when you collect daytime heart and blood pressure and 24-hour urine volumes at three different days during the study.

After your Baseline Exercise Session, you must agree not to initiate any formal exercise programs. Your participation in this study will be deemed as non-exercise or sedentary control group. The initiation of vigorous exercise on your part would greatly compromise the integrity of the research data. By agreeing to participate in this study, you are also agreeing to not participate in vigorous exercise for 12 weeks.

III. Risks

It is my understanding and I have been informed that there exists the possibility during exercise of adverse changes during the actual test. I have been informed that these changes could include abnormal blood pressure, fainting, disorders of heart rhythm, and in very rare instances, heart attack or death (~1 death in 10,000 exercise tests). Every effort will be made to minimize these possibilities for you by preliminary examination and by precautions and observations taken during the test. The intensity of the cycling exercise will increase as you pedal, over about 14 minutes. At first it will be very easy and then become harder; during the last few minutes, the work will become very intense and will represent a maximal effort on your part. It may be as hard as any exercise that you remember doing.

Qualified medical personnel will be available to perform CPR and contact the community Emergency Medical Services by telephone to deal with unusual situations, should these occur during your exercise tests. Emergency equipment and defibrillation are available at this facility and a medical professional with training in advanced cardiac life support will be onsite for all exercise testing. However, a thorough screening for signs of active heart disease and a review of your medical records from your primary healthcare provider will be done before you are allowed to take the exercise test. This will further reduce the chance of heart problems during the exercise testing procedure.

I understand that there is a very small risk of injury, heart attack, or death as a result of my performance in these maximal exercise tests, but knowing these risks, it is my desire to proceed to participate and be a subject in this research project. I understand that the results of this test will be sent to my primary care physician, if I so request. These results may help to determine my ability to safely perform certain types of physical work or exercise.

I understand that my participation in this research project is voluntary and that I may withdraw at any time, without penalty of any kind. Furthermore, I also understand that there is no guarantee that I will benefit from this research project.

IV. Benefits of Your Participation in This Project

Both you and your physician will be provided with your individual results from your exercise test. This test is also be used for evaluating the condition of your heart and lungs. If your physician notes a concern after reviewing the results of this procedure, you and your physician may decide that you should consult with an appropriate healthcare specialist. However, any and all costs related to such a referral and medical care will by borne by you and not by Virginia Tech, nor any of its agents, including the investigators.

A licensed physician will be in the facility and available to assist with monitoring your status during all exercise tests.

A trained medical professional will act as a research coordinator stay in contact with you to monitor and manage your progress throughout the study and when you may request information related to your participation in the study.

A trained nutritionist or dietitian will evaluate and make general recommendations to you about the type and amount of foods that you are eating. This information may be beneficial for your health and controlling risk factors for chronic diseases, such as coronary heart disease. Were you not in the study, this type of analysis normally costs $50 per evaluation; you will receive three such evaluations, without charge.

V. Extent of Anonymity and Confidentiality

The results of this study will be kept strictly confidential. At no time will the researchers release my results of this study to anyone other than the individuals working on the project without your written consent. However, if the need arises, I give my permission for Dr. Zedalis' office to obtain my medical records from my primary healthcare physician. Furthermore, I understand that the information I provide will have my name removed and only a subject number (excluding social security numbers) will identify me during analyses and written reports of this research.

VI. Compensation

I can expect the following compensation for my participation in this 3-month study:

- I will receive three maximal exercise tests and resting and exercise cardiovascular assessments (blood pressure, blood flow, ECG, heart rate, etc), along with reports that I may provide to my personal physician. These tests typically cost from $250 to $300 per test in a healthcare facility. This equates to ~ $600; and
- I will receive three nutritional analyses over the 3-month study. This type of analysis normally costs $50 per session. This equates to ~$150.

VII. Freedom to Withdraw

I understand that, if I decline to participate in this research study or choose to discontinue my participation at anytime, there will be no penalties or loss of benefits that have been promised to me and described in this consent form.

VIII. Approval of Research

This research project has been approved, as required, by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic and State University and the Department of Human Nutrition, Foods, and Exercise.

IX. Subject's Responsibilities

I know of no reason why I cannot participate in this study. I accept that it is my responsibility to:

Accurately report my medical history or changes in my health during the 12-week study duration.

Refrain from regular participation in vigorous physical activity for the 12-week period I am enrolled in the study.

Consume no food during the 12-hour period before arriving at the testing lab for a scheduled exercise tests; and consume no foods during the 1-hour before an exercise training sessions;

Refrain from caffeine, and nicotine products for 24 hours prior to the exercise tests;

Remain in the testing and/or exercise area 30 minutes after each of the exercise testing periods.

Report any physical or medical problems that might occur outside the lab during the period of testing, even if I feel it is not related to the testing to: Carol Haskell (951-8814), Tony Kaleth (231-6469/951-1136), Tom Chittenden (231-6469/953-1941) or Dr. William Herbert (231-6565/951-0974).

X. Subject's Permission

I have read and understand the informed consent and conditions of this research study. I agree to undergo all screening procedures described above prior to acceptance into the study. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent for participation in this project.

If I participate, I may withdraw at any time without penalty. I agree to abide by all the rules of the project.

Questions/Response: _____

_____

_____

_____

_____

_____ _____

Signature Date

Witness (Research Coordinator) Date

[ ] Please check the box if you would like the information from these tests sent to your primary care physician.

Physician's Name and Telephone: _____

Should I have any questions about this research or its conduct, I will contact:

Carol Haskell, MD 951-8814
Research Coordinator

William G. Herbert, Ph.D. 231-6565
Principal Investigator
Human Nutrition, Foods, & Exercise

Tony Kaleth 231-6469
Investigator

Dr. David M. Moore. 231-4991
Chair, IRB, Research Division

Tom Chittenden 231-6469
Investigator

Appendix B

QUESIONNAIRES AND NECESSARY FORMS

If you are interested in being a

Volunteer

FOR A CLINICAL EXERCISE PHYSIOLOGY RESEARCH PROJECT

Contact Carol Haskell, M.D. Clinical Research Coordinator
email chaskell@vt.edu or telephone at 951-8814

PURPOSE OF THE PARTICIPATION AND THE STUDY

The purpose of your participation is to provide information on how apparently healthy adults respond to a special set of fitness, cardiovascular, and biochemical tests which are being used in an investigation of patients with a sleep disorder. You will serve as the control group for a study in which the scientific overall purpose is to: 1) to determine how this disorder affects the heart and circulation, physical fitness, and risk factors for heart disease; and 2) to see if treatment for those sleep disorder patients can be improved by combining nighttime use of a special breathing assist device with a moderate exercise program.

LOCATION OF THE STUDY

The majority of the procedures, including the maximal exercise tests will take place at the Sleep Disorders Clinic in Christiansburg, VA, a medical clinic for sleep patient care.

QUALIFICATIONS TO ENTER

To be a control subject, you must:

- Be between ages 30-60 years
- Be a non-smoker
- Exercise **not more than** 30 minutes/day and not more than 3 days/week in the last 6 months

Exclusion criteria will include:

- Heart problems, including heart attack, chest pain that a physician has said may be related to heart problems, or surgery for your heart or its blood vessels (coronary revascularization), or congestive heart failure;
- Chronic lung diseases;
- Uncontrolled high blood pressure or use of blood pressure medications, antihistamines, and cholesterol lowering medications;
- Uncontrolled diabetes mellitus;
- Orthopedic (bone or joint) problems, musculoskeletal conditions, and/or neuromuscular conditions that would prevent you from doing vigorous exercise;
- Use of tobacco products use (only non-smokers can participate)

BENEFITS FOR PARTICIPATING

You will receive free exercise testing that will include: your blood pressure and heart rate measurements; your maximal aerobic exercise capacity (aerobic fitness) including an ECG report and your blood cholesterol score that may be sent to your doctor, if you wish.

Principal Investigators: William G. Herbert, Ph.D., Don Zedalis, MD, John Gregg, DDS, Ph.D., William Huckle, Ph.D., Sharon Nickols-Richardson, RD, Ph.D., Thomas W. Chittenden, MA, Anthony S. Kaleth, MS, Brian J. Hawkins, MS, Lawrence Cross, Ph.D., Ali Arner, B.S., Christopher Holm, B.S.

Medical and Health History

Demographic Information:

Name: _____ _____ Age: _____ Date of Birth: _____
Address: _____
Phone number: Home: _____Work: _____
Occupation _____Place of employment _____
Education
(check highest level) Elementary ___ High School___ College ___ Post Graduate____
Person to contact in case of emergency: _____
Relationship: _____Phone: _____
Primary Care Physician: _____ Phone: _____
Marital Status: _____single _____divorced _____married _____widower
Have you ever had a stress test? Yes___ No ___ date_____

Medical History:

Please indicate any current or previous conditions or problems you have experienced or have been told by a physician you have had:

	YES	NO
Heart disease or any heart problems:	_____	_____
Rheumatic fever:	_____	_____
Respiratory disease or breathing problems:	_____	_____
Circulation problems:	_____	_____
Kidney disease or problems:	_____	_____
Urinary problems:	_____	_____
Reproductive problems:	_____	_____
Musculoskeletal problems:	_____	_____
Fainting or Dizziness:	_____	_____
High Cholesterol:	_____	_____
Diabetes:	_____	_____
Thyroid problems:	_____	_____
Allergies:	_____	_____

If "yes" to any of the above please indicate the date, explain and describe:
_____
_____

Other medical problems ?
_____

Please list any hospitalizations/operations/recent illnesses (Type/Date):

Type: _____ Date: _____
Type: _____ Date: _____
Type: _____ Date: _____

Have you ever been diagnosed as having high blood pressure? Yes _____ No _____

Are you currently being treated for high blood pressure? Yes _____ No _____
If "yes" please explain:
_____
_____

Are you currently being treated for high cholesterol or hyperlipidemia? Yes__ No __
If yes, Please list medication _____

Medications:

Please list all medications (prescription and over-the-counter) you are currently taking or have taken in the past week:

Drug	_____	Dose	_____	Reason Taking	_____
Drug	_____	Dose	_____	Reason Taking	_____
Drug	_____	Dose	_____	Reason Taking	_____
Drug	_____	Dose	_____	Reason Taking	_____
Drug	_____	Dose	_____	Reason Taking	_____
Drug	_____	Dose	_____	Reason Taking	_____
Drug	_____	Dose	_____	Reason Taking	_____

Health Habits:

	Yes	No
Do you add salt to your food?	_____	_____
Are you on any special type of diet?		
If yes, how long have you been dieting ____ months		
Who prescribed the diet? Physician____ Self_____		
Please describe diet:		

_____
_____

Do you drink caffeinated beverages?	_____	_____
How many cups per day? _____		
Do you drink alcoholic beverages?	_____	_____
How many drinks per week? _____		
Do you smoke cigarettes?	_____	_____
Packs per day: _____		

Exercise Habits

What is your occupational activity level? sedentary____ ; light_____ ; moderate____ ; heavy_____

	Yes	No
Do you engage in regular exercise?	_____	_____

If "yes" please list:

Activity	Frequency (times per week)	Duration (minutes)

Do you ever feel faint, short of breath, or chest discomfort with exertion? _____

If "yes", please explain:

_____
_____

Do you engage in any recreational or leisure-time physical activities?
No__ Yes___ What type_____
How often?_____ times/week For how long?_____

Are there any orthopedic limitations you have that may restrict your ability to perform exercise on a stationary cycle? _____Yes _____No
If "yes" please explain:

_____
_____

Family History:

Has anyone in your family been diagnosed or treated for any of the following?

	Yes	No	Relationship	Age
Heart attack				
Heart disease				
High blood				
Pressure				
Stroke				
Kidney disease				
Diabetes				

Please sign to indicate the above information is correct:

_____ _____
Print Name Signature

VSAQ

Draw one line Below the Activities You are Able To Do Routinely With Minimal or No Symptoms, Such As Shortness of Breath, Chest Discomfort, or Fatigue

1 MET:	• Bathing, getting dressed, working at a desk
2 METs:	• Taking a shower • Walking down eight steps
3 METs:	• Walking slowly on a flat surface for one or two blocks. • A moderate amount of work around the house, like Vacuuming, sweeping the floors or carrying groceries.
4 METs:	• Light yard work i.e., raking leaves, weeding or pushing a power mower. • Painting or light carpentry.
5 METs:	• Walking briskly, i.e., four miles in one hour. • Social dancing, washing the car.
6 METs:	• Play nine holes of golf carrying your own clubs. • Heavy carpentry, mow lawn with push mower.
7 METs:	• Perform heavy outdoor work, i.e., digging, spading soil, etc. • Play tennis (singles), carry 60 pounds.
8 METs:	• Move heavy furniture. • Jog slowly, climb stairs quickly, carry 20 pounds upstairs.
9 METs:	• Bicycling at a moderate pace, sawing wood, jumping rope (slowly).
10 METs:	• Brisk swimming, bicycle up a hill, walking briskly uphill, jog six miles per hour
11 METs:	• Cross country ski. • Play basketball (full court)
12 METs:	• Running briskly, continuously (level ground, eight minutes per mile).
13 METs:	• Any competitive activity, including those which involve intermittent sprinting. • Running competitively, rowing, backpacking

ResMed2 Clinical Trial

Epworth Sleepiness Scale

Subject ID ____ Name _____ Date Completed ___/___/___

This questionnaire asks you to indicate the chances of you becoming drowsy during hours of the day that you are not in bed sleeping. "How likely are you to doze off or fall asleep in the following situations?"

Use the following scale and indicate the most appropriate number for each situation.

0 = would never doze
1 = slight chance of dozing
2 = moderate chance of dozing
3 = high chance of dozing

	Situation	**Chances of Dozing**
1.	Sitting and reading	_____
2.	Watching T.V.	_____
3.	Sitting, inactive in a public place (ex. Theatre or meeting)	_____
4.	As a passenger in a car for an hour without a break	_____
5.	Lying down to rest in the afternoon when circumstances permit	_____
6.	Sitting and talking with someone	_____
7.	Sitting quietly after a lunch without alcohol	_____
8.	In a car, while stopped for a few minutes in the traffic	_____

Sum of Scores, items 1-8 (staff use only) ____/24

Johns MW. A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. Sleep. 1991;14:540-545.

POLAR RECORDER INSTRUCTIONS:

PRETEST INSTRUCTIONS:

No exercise on the day of testing
Avoid alcohol, tobacco, over the counter meds that are cardioactive
Charging prior to use: Polar device must be charged for 4-5 hours prior to use. (Red when charging and the light will go out when fully charged). Green blinks when parameters are set and ready to go.

TESTING INSTRUCTIONS:

1. You will have the Recorder (small box), wires, electrodes or chest strap, and battery charger. **Make sure to not leave this in extreme temperatures**.
2. The electrode chest belt should be secured right below the chest muscles against skin. The strap can adjust to the length needed to fit snug and secure. May need to wet the backside of the electrode belt (the part that is grooved and against the skin). A second option is to use the electrodes provided by the lab.
3. Connect the red lead wire to the left electrode and black to the right electrode. And the opposite ends should fit into the recorder to the appropriate color-coded slot. Make sure the connections are in properly.
4. Press the start button and hold 5 seconds until you hear a beep to begin recording. Watch for the red light to blink to make sure the recorder has started properly. You can check periodically to see that the red light is flashing meaning it is recording data.
5. Wear the recorder for 8-12 hours while awake.
6. REMEMBER TO PRESS STOP WHEN YOU TAKE IT OFF!!!!!!

Thanks!

Appendix C

RAW DATA

RAW DATA

Table 1. Physical Characteristics of subjects (n=10)

Subject	Gender	Weight (kg)	Age (years)	BMI (kg/m^2)	VO_{2pk} (ml/kg/min)	VSAQ
1	M	73	42	23	39.0	9
2	F	93	28	32	24.7	8
3	F	88	46	34	21.6	10
4	F	82	44	31	28.9	10
5	F	70	34	24	26.8	10
6	M	100	36	31	24.3	11
7	M	86	51	30	34.6	9
8	F	87	45	34	21.7	8
9	F	58	34	21	36.6	9
10	M	71	37	25	36.7	9

HEART RATE VARIABILITY DATA:

TIME DOMAIN MEASUREMENTS

Table 2. HRV Variable, R-R Interval (ms) where D, Day of recording, Schiller, Schiller HRV method, and Polar, Polar HRV method

Subject No.	Schiller D1 RR	Schiller D2 RR	Polar D1 RR	Polar D2 RR
1	856	852	803.4	800.4
2	737	826	575.0	545.2
3	809	862	645.8	664.3
4	854	851	675.0	590.8
5	900	932	679.1	679.1
6	1161	845	757.5	801.8
7	1123	1071	760.0	587.8
8	893	983	701.7	518.0
9	897	731	638.9	628.0
10	842	783	706.6	747.8

Table 3. HRV Variable, Standard Deviation of R-R Intervals (ms) where D, Day of recording, Schiller, Schiller HRV method, and Polar, Polar HRV method

Subject	Schiller D1 RRSD	Schiller D2 RRSD	Polar D1 RRSD	Polar D2 RRSD
1	34	26	91.5	83.1
2	37	89	95.2	71.6
3	29	30	70.8	81.8
4	69	82	79.5	81.8
5	91	63	70.0	70.0
6	86	80	120.0	111.0
7	49	37	123.4	200.2
8	30	47	106.6	203.6
9	44	33	58.9	87.2
10	68	45	129.9	136.5

HEART RATE VARIABILITY DATA:

FREQUENCY DOMAIN MEASUREMENTS

Table 4. HRV Variable, Low Frequency Percent of Total Power (%ms^2 of Total Power) where D, Day of recording, Schiller, Schiller HRV method, and Polar, Polar HRV method

Subject	Schiller D1 LF	Schiller D2 LF	Polar D1 LF	Polar D2 LF
1	32	20	92.1	93.2
2	20	11	93.6	94.2
3	24	10	93.4	94.7
4	14	11	94.5	95.8
5	14	17	92.5	93.7
6	15	19	92.8	92.3
7	21	18	93.1	93.2
8	20	35	92.9	89.3
9	8	24	92.5	92.7
10	22	30	90.1	92.9

Table 5. HRV Variable, High Frequency Percent of Total Power (%ms^2 of Total Power) where D, Day of recording, Schiller, Schiller HRV method, and Polar, Polar HRV method

Subject	Schiller D1 HF	Schiller D2 HF	Polar D1 HF	Polar D2 HF
1	17	22	7.9	6.8
2	35	64	6.4	5.8
3	23	34	6.6	5.3
4	31	31	5.5	4.2
5	49	38	7.6	6.3
6	36	19	7.3	7.7
7	19	15	6.9	6.8
8	18	19	7.1	10.7
9	65	33	7.5	7.4
10	35	23	9.9	7.1

Table 6. HRV Variable, Low frequency/High Frequency Ratio (ms^2) where D, Day of recording, Schiller, Schiller HRV method, and Polar, Polar HRV method

Subject	Schiller1 LF/HF	Schiller2 HF/LF	Polar1 LF/HF	Polar2 LF/HF
1	1.95	0.89	11.66	13.71
2	0.54	0.17	14.63	16.25
3	1.33	0.41	14.20	18.01
4	0.44	0.35	17.80	22.75
5	0.47	0.51	12.09	14.95
6	0.43	1.04	12.79	11.92
7	1.20	1.19	13.43	13.64
8	1.18	1.83	13.10	8.37
9	0.12	0.71	12.30	12.61
10	0.71	1.34	9.12	13.06

URINARY DATA

Table 7. Creatinine, NE, and E data

Subject	Day	Total volume	mg/dL	g/24hour	NE (ng/mL)	NE (ug/g	E (ng/mL)	E (ug/g
		urine (mL/24hr)	creatinine	creatinine		of creatinine)		of creatinine)
1	1	1620	64.33	1.04	3541.8	5505.99	2628.2	4085.73
1	2	1605	60.35	0.97	2020.11	3347.47	1319.4	2186.34
2	1	1000	92.94	0.93	1025.39	1103.32	456.18	490.85
2	2	560	105.15	0.59	1693.89	1610.89	1699.84	1616.55
3	1	3155	15.73	0.4	1613	10195.28	363.8	2299.47
3	2	2540	15.82	0.5	2384.2	15160.58	401	2549.87
4	1	1540	92.94	1.43	1580.5	1700.62	1190.4	1280.87
4	2	920	120.51	1.11	1602.7	1329.98	2303.6	1911.62
5	1	840	125.24	1.05	2266.6	1809.77	4631.1	3697.72
5	2	907	112.17	1.02	2750.3	2451.94	2104.4	1876.11
6	1	878	134.73	1.18	2512.4	1864.82	797.9	592.24
6	2	1838	59.21	1.09	1457.4	2461.39	1230.6	2078.35
7	1	1633	70.48	1.15	2460.8	3491.28	1825.2	2589.52
7	2	1882	49.17	0.93	2463.6	5010.53	1918.3	3901.49
8	1	926	107.24	0.99	3551.6	3311.76	2817	2626.77
8	2	2112	46.23	0.98	2451.9	5303.52	454.9	983.96
9	1	772	84.89	0.66	3453.4	4068.16	26.4	31.1
9	2	697	122.13	0.85	1197.2	980.27	395	323.43
10	1	3064	55.14	1.69	1305	2366.82	213.6	387.4
10	2	2037	56.84	1.16	828.1	1456.84	35	61.57

Appendix D

Frequency/Descriptive Summary of Data

Table 1. Descriptive Physical Characteristics for all subjects (n=10)

Variables	Mean	Standard Deviation	Range
Age (years)	39.7	7.01	28.51
Weight (lbs)	177.76	27.7	128-220
BMI (kg/m2)	28.58	4.9	20.34
VSAQ	9.3	0.95	8.00-11
VO2pk (ml/kg/min)	29.49	6.66	21.36

Table 2. Summary of Urinary Data for all subjects (n=10)

Variables	Mean	Standard Deviation	Range
Total volume urine (mL)	1526.3	773.55	560-3155
Creatinine (mg/dL)	79.56	35.45	15.73-134-73
Creatinine (g/24hours)	0.99	0.3	.4-1.69
NE (ug/g of Creatinine)	3726.56	3452.57	980-15160
E (ug/g of creatinine)	1778.55	1257.92	31-4085

Table 3. Summary of HRV Time domain variables where S1, Schiller Day 1; S2, Schiller Day 2; P1, Polar Day 1; P2, Polar Day2; RR, the average R-R interval (ms); and RRSD, the standard deviation of R-R intervals (ms).

Variables	Mean	Standard Deviation	Range
S1 RR	907	133.25	737-1161
S2 RR	873.35	98.3	730.5-1070.5
P1 RR	694.30	67.23	575-803
P2 RR	656.33	101.17	545-801
S1 RRSD	53.55	23.16	29-91
S2 RRSD	53.05	23.54	25.5-89
P1 RRSD	94.56	24.85	58-129
P2 RRSD	112.67	51.02	69-203

Table 4. Summary of HRV Frequency domain variables where S1, Schiller Day 1; S2, Schiller Day 2; P1, Polar Day 1; P2, Polar Day2; LF, low frequency power (ms^2); HF, high frequency power (ms^2); and LFHF, the ratio of LF/HF power.

Variables	Mean	Standard Deviation	Range
S1 LF	18.95	6.74	7.65-32.2
S2 LF	19.39	8.15	10.25-34.95
P1 LF	92.73	1.15	90.12-94.5
P2 LF	93.2	1.73	89.3-95.78
S1 HF	32.53	15.15	16.7-64.5
S2 HF	29.89	14.4	14.5-64.45
P1 HF	7.27	1.15	5.5-9.88
P2 HF	6.8	1.73	4.21-10.7
S1 LFHF	0.84	0.56	.12-1.95
S2 LFHF	0.84	0.52	.17-1.83
P1 LFHF	13.11	2.25	9.12-17.80
P2 LFHF	14.53	3.87	8.37-22.75

APPENDIX E

CALUCULATIONS

FOR

URINARY CATECHOLAMINE AND CREATININE CLEARANCE

1. Determination of creatinine levels (mg/dL):

Equation 1: {{((Initial sample absorbency)-(Final sample absorbency))/((Initial creatinine standard absorbency)-(Final creatinine standard absorbency))}*3}*Dilution Factor= creatinine (mg/dL)

The initial sample absorbance was the diluted sample with alkaline picrate solution, final sample absorbance was the diluted sample, alkaline picrate solution, and acid reagent. The initial creatinine standard absorbance was the creatinine standard and alkaline picrate, and the final creatinine standard absorbance was the creatinine standard, alkaline picrate, and acid reagent. All absorbency levels were read at a wavelength of 500nm.

2. Conversion of creatinine into g/24 hours from mg/dL:

Equation 2: {(mg/dL)(1dL/10mL)(mL/24hours)(1g/1000mg)}= g/24hours

Creatinine began in mg/dL from equation 1. Using total volume of urine collected measured in mL/24 hours, the result is expressed as gram of creatinine per 24 hours.

3. **Determination of catecholamine levels in micrograms per gram of creatinine:**

Equation 3: {(catecholamine ng/mL)(total volume mL/24hours)(1000µg/ng)}/creatinine g/24 hours= µg/g of creatinine

Catecholamines were determined from the HPLC in ng/mL, total volume of urine was measured in mL/24 hours, and creatinine was converted to µg/24hours by equation 2.

APPENDIX F

CORRELATION TABLES

Table 1. Intraclass correlation coefficients (ICC) for HRV response variables with the Schiller method

Effect	R-R interval	RRSD	pNN50	LF	HF	LF/HF ratio
Subject	0.423	0.491	0.446	0.032	0.29	0.252
Day	0.519	0.319	0.437	0.172	0.16	0.217
Trial	0.06	0.191	0.117	0.796	0.56	0.531

Table 2. Correlations between trials (within days) with the Schiller method (r).

	R-R interval	RRSD	pNN50	LF	HF	LF/HF ratio
Day 1(T1-T2)	.927*	.752**	.790*	-0.11	.653**	0.596
Day 2(T1-T2)	.980*	.954*	.995*	0.579	0.41	.724**

*correlation is significant at the 0.01 level (2-tailed)

**correlation is significant at the 0.05 level (2-tailed)

Table 3. Correlations of HRV response variables between days (average trials on Day 1 and average trials on Day 2) using the Schiller method (r).

	R-R interval	RRSD	pNN50	LF	HF	LF/HF ratio
Avg D1-Avg D2	0.443	0.504	0.4	0.001	0.36	0.298

Table 4. Intraclass correlation coefficients for HRV response variables using the Polar method.

Effect	**R-R interval**	**RRSD**	**pNN50**	**LF**	**HF**	**LF/HF ratio**
Subject	0.213	0.373	0.729	0.279	0.28	0.521
Day	0.787	0.627	0.271	0.721	0.72	0.479

Table 5. Correlations of HRV response variables between days using the Polar method (r)

N=10	**R-R interval**	**RRSD**	**pNN50**	**LF**	**HF**	**LF/HF ratio**
D1-D2	0.586	.670**	.670**	0.368	0.37	.687**
N=8	**R-R interval**	**RRSD**	**pNN50**	**LF**	**HF**	**LF/HF ratio**
D1-D2	0.91*	0.79**	0.93*	0.7**	0.7**	0.85*

*correlation is significant at the 0.01 level (2-tailed)

**correlation is significant at the 0.05 level (2-tailed)

Table 6. Correlations of HRV response variables between methods, Schiller method (Day 1 is the average trials on day 1 while Day 2 is the average trials on day 2) and Polar method (r).

	R-R interval	**RRSD**	**pNN50**	**LF**	**HF**	**LF/HF ratio**
Day 1	0.63**	0.15	0.43	-0.23	0.16	-0.15
Day 2	-0.36	-0.27	-0.00	-0.88	-0.4	-0.79

Table 7. Correlations of HRV response variables with the Polar method (average of day 1 and 2) and urinary catecholamine levels, ug/g of creatinine (average of day 1 and 2), n=10 (r).

	R-R interval	**RRSD**	**pNN50**	**LF**	**HF**	**LF/HF ratio**
NE	0.03	-0.08	-0.32	0.15	-0.15	0.10
E	0.25	0.09	-0.20	0.22	-0.22	0.14

Table 8. Correlations of HRV response variables with the Polar method (average day 1 and 2) and VO_{2pk} (ml/kg/min) (r).

	R-R interval	**RRSD**	**pNN50**	**LF**	**HF**	**LF/HF ratio**
VO_{2pk}	0.44	0.04	0.22	-0.19	0.19	-0.23

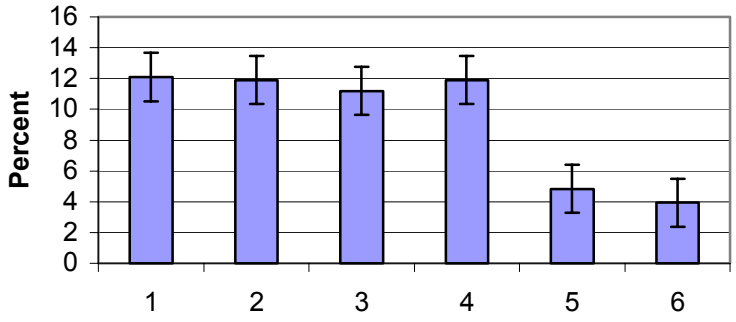
APPENDIX G

RESULTS

IN

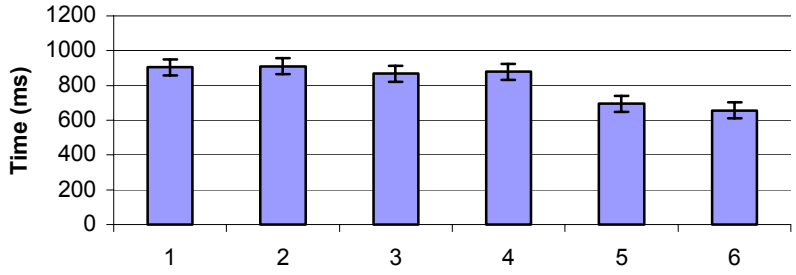
SCATTERPLOTS AND BAR CHARTS

Figure 1. The percent of the total R-R intervals that exceed 50 ms to the adjacent R-R interval (pNN50) with the Schiller HRV method and Polar HRV method



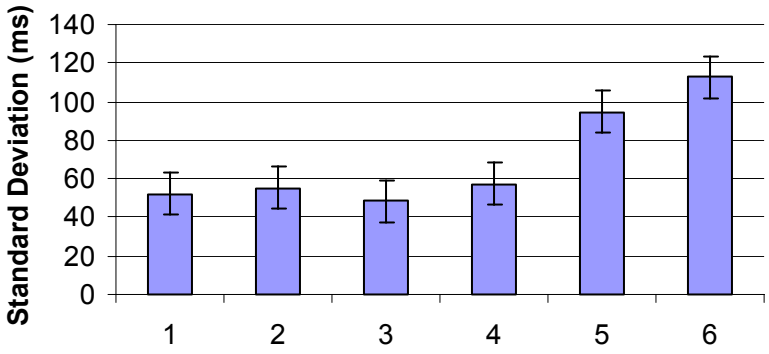
. Legend: 1, Schiller Day 1, Trial1; 2, Schiller Day1, Trial 2; 3, Schiller Day2, Trial 1; 4, Schiller Day 2, Trial 2; 5, Polar Day 1; 6, Polar Day 2.

Figure 2. R-R intervals (ms) with the Schiller HRV method and Polar HRV method.



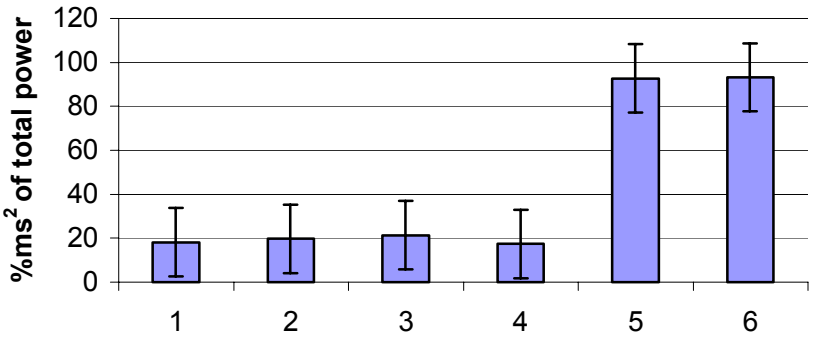
Legend: 1, Schiller Day 1, Trial1; 2, Schiller Day1, Trial 2; 3, Schiller Day2, Trial 1; 4, Schiller Day 2, Trial 2; 5, Polar Day 1; 6, Polar Day 2.

Figure 3. Standard deviation of R-R intervals (ms) with the Schiller HRV method and Polar HRV method.



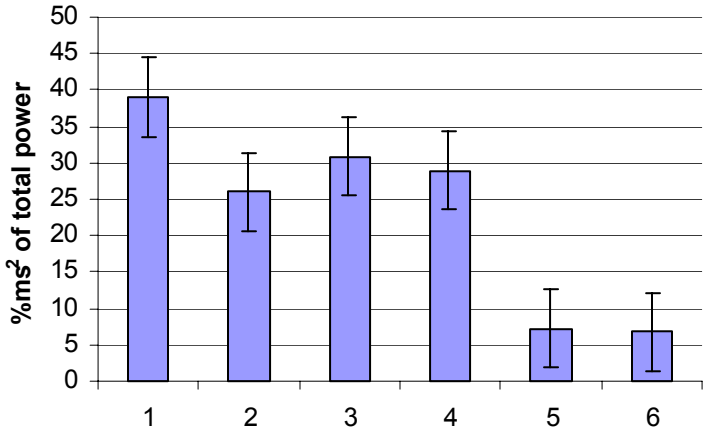
Legend: 1, Schiller Day 1, Trial1; 2, Schiller Day1, Trial 2; 3, Schiller Day2, Trial 1; 4, Schiller Day 2, Trial 2; 5, Polar Day 1; 6, Polar Day 2.

Figure 4. Low Frequency Power (ms^2) with the Schiller HRV method and Polar HRV method.



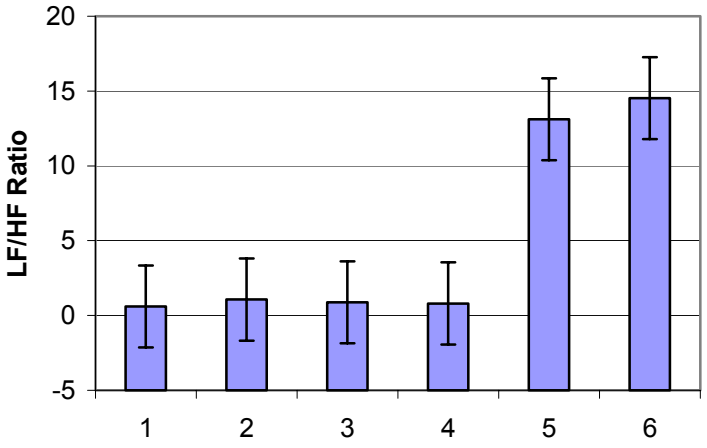
Legend: 1, Schiller Day 1, Trial1; 2, Schiller Day1, Trial 2; 3, Schiller Day2, Trial 1; 4, Schiller Day 2, Trial 2; 5, Polar Day 1; 6, Polar Day 2.

Figure 5. High Frequency Power (ms^2) with the Schiller HRV method and Polar HRV method.



Legend: 1, Schiller Day 1, Trial1; 2, Schiller Day1, Trial 2; 3, Schiller Day2, Trial 1; 4, Schiller Day 2, Trial 2; 5, Polar Day 1; 6, Polar Day 2.

Figure 6. Low Frequency/High Frequency Power ratio (ms^2) with the Schiller HRV method and Polar HRV method.



Legend: 1, Schiller Day 1, Trial1; 2, Schiller Day1, Trial 2; 3, Schiller Day2, Trial 1; 4, Schiller Day 2, Trial 2; 5, Polar Day 1; 6, Polar Day 2.

Figure 7. Bias plot for HRV variable, mean R-R interval, for the Schiller and Polar HRV method where the line of identity is the Polar method.

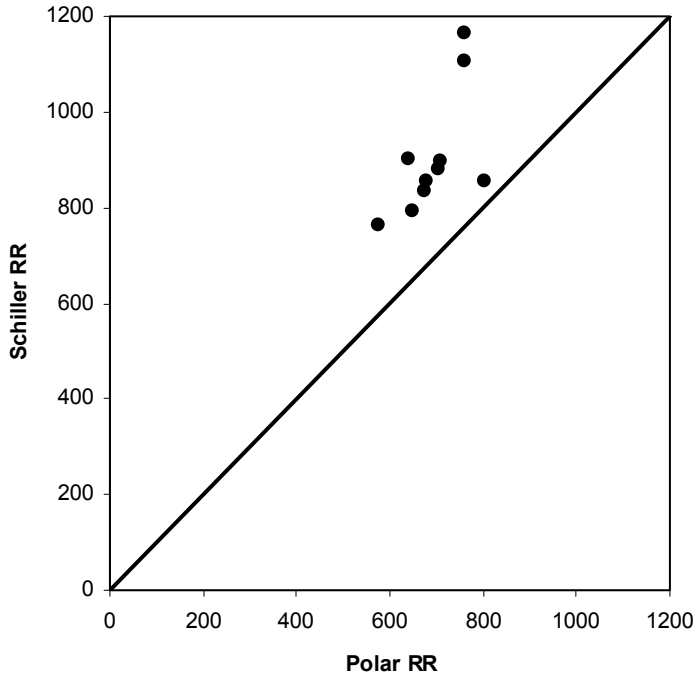


Figure 8. Bias plot for HRV variable, standard deviation of R-R intervals, for the Schiller and Polar HRV method where the line of identity is the Polar method.

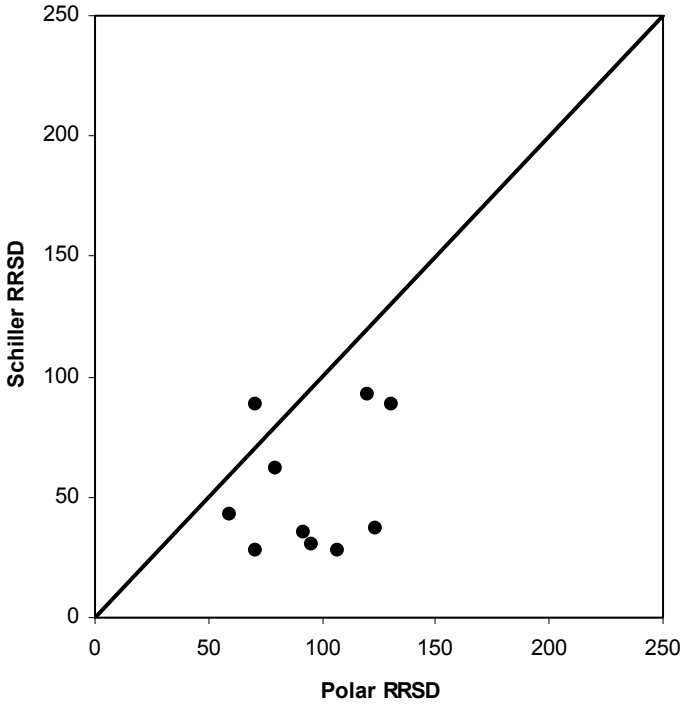


Figure 9. Bias plot for HRV variable, percent of R-R intervals that differ by more than 50ms to the adjacent interval (pNN50), for the Schiller and Polar HRV method where the line of identity is the Polar method.

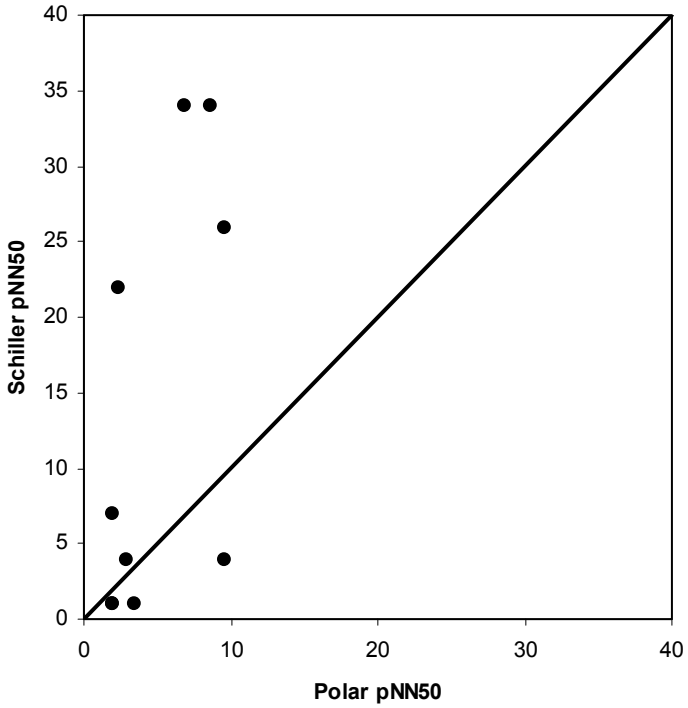


Figure 10. Bias plot for HRV variable, low frequency power (%ms^2 of the total power minus very low frequency) for the Schiller and Polar HRV method where the line of identity is the Polar method.

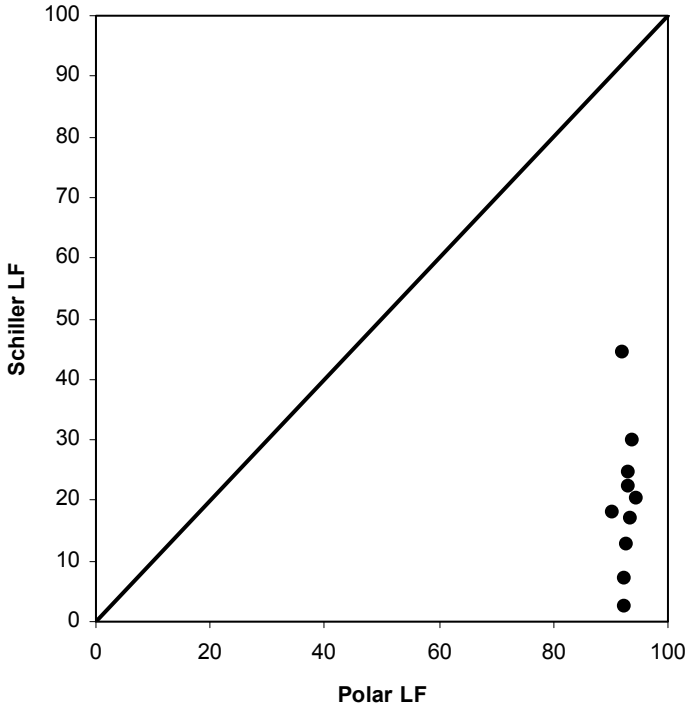


Figure 11. Bias plot for HRV variable, high frequency power (%ms^2 of the total power minus very low frequency) for the Schiller and Polar HRV method where the line of identity is the Polar method.

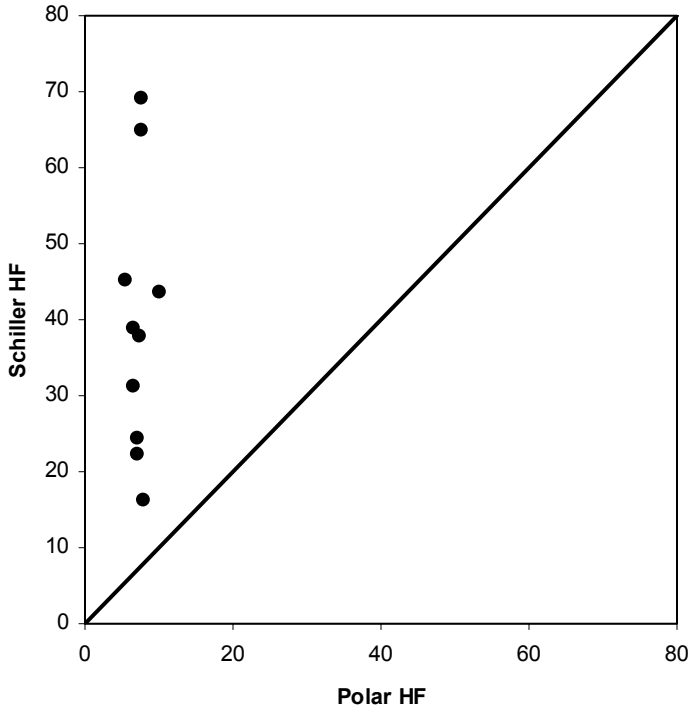
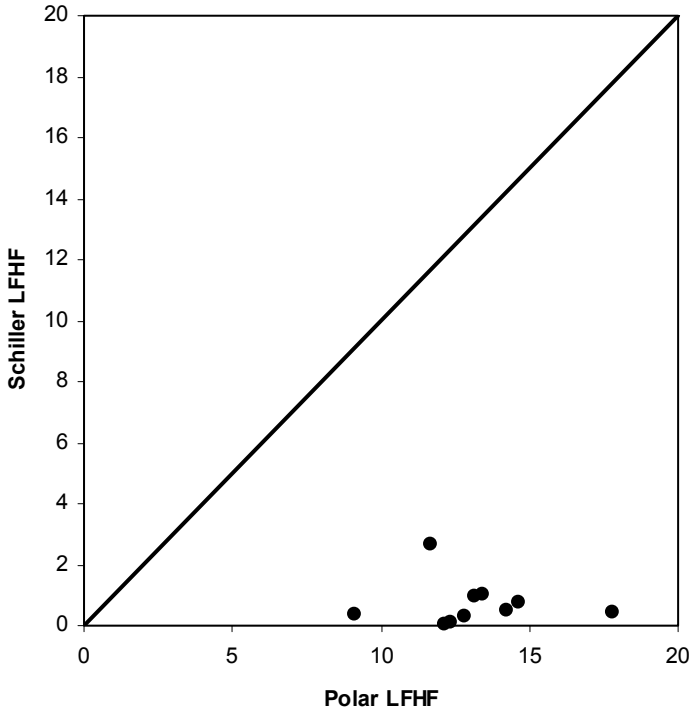


Figure 12. Bias plot for HRV variable, the ratio of low frequency/ high frequency power (%ms^2) for the Schiller and Polar HRV method where the line of identity is the Polar method.



APPENDIX H

INSTRUCTIONS FOR OPERATING

POLAR R-R RECORDER

R-R Recorder Method:

1. **Charging prior to use:** Polar device must be charged for about 3-4 hours prior to use. (Red when charging and will go out when full). Green blinks when parameters are set and ready to go.
2. **Cleaning prior to use:** Make sure to clean off the black part of the chest strap prior to hooking up the next person.
3. **Setting the parameters:**
    a. The electrode chest belt should be secured on the subject right below the chest muscles. The strap can adjust to the length needed to fit snug and secure to the subject. May need to wet the backside of the electrode belt (the part that is grooved and against the skin of the subject) with the ECG spray. A second option is to use the electrodes provided by the lab.
    b. Connect the RR Recorder to the to serial port on the computer with the optical serial interface cable.
    c. Start the software. (Select Options to set the path and port, only if needed).
    d. Download the data that was currently on the recorder from the person before by selecting RECORDER----READ RECORDER---transfer ECG and RR data, type in name, subject number, and time point (baseline, 3-week, 6-week, or 12-week) into boxes. Then select START. If an error should occur just press start again for it to transfer again.
    e. Connect the person to the computer by connecting the red lead wire to the left electrode and black to the right electrode. And the opposite ends should fit into the recorder to the appropriate color-coded slot. Make sure the connections are in properly.
    f. Next set parameters. Select RECORDER----RECORDER SET UP---
        i. PARAMETERS: TIME & DAY-PUSH GET REALTIME
        ii. PARAMETERS: RECORDING TIME---ENTER TIME FOR THE SUBJECTS TRIAL PERIOD---REMEMBER TO SELECT START BY BUTTON
        iii. ECG RECORDING---UPPER AND LOWER LIMIT SHOULD BE ACTIVATED (at 300-1500), AND % LIMIT SHOULD BE SET AT 20.
        iv. PRESS SET RECORDER AFTER EACH OF THESE STEPS
    g. Next select RECORDER----REALTIME ECG. When the EGC appears, select SPECIAL -- STOP (to stop displaying new data). May zoom in and out by using compress or expand. Then use the markers to frame a QRS complex that you would like to use (there are restrictions on how many ms you may include in your selection-between 20-128ms) next you must select SPECIAL---FILTER OUTPUT-select COMBED OR MATCHED then push RESTART.
4. Select OPTION---PREFERENCES---12-HOUR

5. Disconnect subject from the computer. The device should blink green to signal that the Recorder is ready to go. Give the subject the Recorder, wires, electrodes or chest strap then keep the optical serial interface cable with you. You can give them the battery charger if needed.
6. When the recording should begin, press the start button on the Recorder, hold for 5 seconds UNTIL THEY HEAR A BEEP. Tell them to check periodically to see that the red light is flashing meaning it is recording data.
7. The subject should wear the Recorder for all wakeful hours of the day.
8. When taking it off, have them press the same button (as the start) to stop the device.
9. The information will have to be downloaded to the computer between trials.

Filtering Data

1. Download all the RR and ECG data to computer labeling it with name, subject number, and time point of study. It may take several times for it to download without stumbling over some error messages. Just restart it each time.
2. After downloaded, open the recording and begin filtering. CHECK TO MAKE SURE THE DATA IS USEABLE.
3. To filter, select a small portion by dragging the mouse over the data area while left clicking and holding.
4. Select EDIT----ERROR CORRECTION---CHECK THE FILTER BOX THEN OK (Use the default HR criterion for errors, standard deviation criterion for errors, and the protection zone to get all the errors to show up. Use preview to show the errors highlighted).
5. Scan through the entire recording doing this with the error correction. Save occasionally so you do not lose any data if the program shuts down on you.
6. After filtering the entire recording, compress the entire recording to zoom all.
7. Hold the curser down on the bottom of the x-axis under the rr data and drag through the recording section you want. Press selection info and wait for recording information to calculate (there is a delay so wait patiently).
8. Use the frequency measures from the AR spectrum. For total power subtract out very low frequency. Then use the HF divided by the total power minus VLF. Do the same for LF. Then the ratio is LF/HF.
9. Export all text files into excel file. This excel file will take the data through 6 steps. Be patient, it takes a while for it to perform all the calculations. This excel file that has been developed will calculate the time domain measures: mean RR, SD of RR, and pNN50 after it manually filters data (excluding values that are not physiological). Steps:
    a. Calculates the difference between consecutive RR intervals
    b. Excludes any intervals that are $\geq$100ms from the adjacent interval.

c. Identifies any intervals that differ by 50ms from the adjacent interval.
d. Calculates pNN50
e. Excludes any intervals that are less than 300ms.
f. Excludes any intervals that exceed 1500ms.
g. Calculates mean and standard deviation of all RR intervals.

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PUBLICATIONS

Chittenden TW, Kaleth AS, Blevins JS, Ballentine HM, Arner AE, Gregg JM, Zedalis D, Herbert WG. Increasing physical activity in the obstructive sleep apnea patient: part 1 – clinical presentation, pathogenesis, and medical management. Am J Med Sport. 2002;4:31-40

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