

**RELIABILITY OF WAVELET ANALYSIS OF HEART RATE
VARIABILITY DURING REST AND EXERCISE**

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ABSTRACT

The reliability of wavelet analysis (DWT), of heart rate variability during rest and exercise was examined for this study.

All twenty-three participants visited the lab on two separate occasions no less than four weeks apart. All twenty-three participants were subjected to the spontaneous breathing (SB1), and HG60 exercise condition. Of those twenty-three participants, nine performed the HG20 exercise condition as well.

It was found that during the SB1 condition, the R-R intervals were fairly reliable between days. However, the reliability of all the HRV parameters (SDNN, spectral components and wavelet components) were quite poor. Interestingly, however, during HG20, the reliability of the HRV parameters was much more promising.

The ability of DWT to detect changes in sympathovagal balance with incremental handgrip exercise was seen, despite a very low number of participants.

INTRODUCTION

A significant relationship between cardiovascular mortality, including sudden cardiac death, and the autonomic nervous system has been seen over the last few decades (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). It has been shown that there is an increased incidence of total mortality and cardiac events in both apparently healthy middle-aged and elderly adults as well as post myocardial infarction patients who have reduced heart rate variability (HRV). A reduced HRV is a reflection of elevated sympathetic activity. This is a condition that may decrease the fibrillation threshold and thus predispose to ventricular fibrillation (Schuit et al., 1999). Decreased parasympathetic tone or sympathetic overstimulation reduces the magnitude of HRV and lowers the threshold for the origin of arrhythmias. Reduced levels of HRV are related to all-cause mortality, the incidence of new cardiac events (angina pectoris, myocardial infarction, coronary heart disease, death, or congestive heart failure, and risk of sudden cardiac death in asymptomatic individuals (Melanson, 2000).

Empirical evidence for an association between propensity for lethal arrhythmias and signs of either increased sympathetic or reduced vagal activity has spurred efforts for the development of quantitative markers of autonomic activity. HRV has the potential to provide additional valuable insight into pathological and physiological conditions and to enhance risk stratification (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Heart rate variability represents one of the most promising quantitative markers of autonomic activity. HRV has been described as the variations of both RR intervals and instantaneous heart rate.

The basis for heart rate variability as a measure of autonomic modulation is that the parasympathetic branch of the autonomic nervous system (ANS) has a very rapid tissue response and a rapid recovery from neural stimulation, which allows for a high frequency of neural activity. The rapid response to parasympathetic stimulation is acetylcholine released at the post-ganglionic synapse. This acts directly on the sinoatrial node and atrial conduction fibers to open potassium channels and hyperpolarize the cell. The sympathetic nervous system (SNS) has a low frequency of neural activity because it has a very slow onset of tissue response and recovery from the neural stimulation. The reason for this response is due to the sympathetic post-ganglionic nerves releasing norepinephrine at the synapse. This in-turn stimulates a beta-receptor to initiate the second messenger cascade for cyclic AMP. Once this occurs the result is the opening of the sodium and calcium channels in the tissues and results in excitability of the tissues. The lengthened recovery from sympathetic neural activation is due to the time it takes for the calcium and sodium pumps to clear ions from the target cells.

Mostly parasympathetic control of the heart results in high variation in the heart rate due to the rapid onset and recovery of the parasympathetic system. Most sympathetic stimulation of the heart results in a higher heart rate with less variability because of the extended tissue excitability brought on by sympathetic neural contribution. With this hypothesis, it is likely to suggest that the standard deviation of the normal sinus R-R intervals (SDNN) show a predominant sympathetic or parasympathetic modulation of the heart. There is evidence to support SDNN as way to measure autonomic modulation of the heart in that when the vagus nerve was cut in animals and humans, the SDNN decreases. Also, during exercise, SDNN decreases when there is an increase in sympathetic stimulation and there is parasympathetic withdrawal. In studies that used

paced breathing to cause an increase in parasympathetic modulation of the heart, SDNN increased. Many studies have also shown that lower SDNN over a 24-hour period predict an increased risk for sudden death. On the other hand, SDNN does not differ between the modulations by branches of the autonomic nervous system and is influenced by the heart rate at which the measurement was taken, hence reducing the value of comparison between people.

Heart rate variability can be measured by a different means and that is the frequency domain. This technique attempts to discriminate between R-R intervals occurring in a frequency band of sympathetic neural stimulation of the heart (low frequency) and in a frequency band of parasympathetic stimulation of the heart (high frequency), using chaos mathematics. The validity of classifying different frequencies to sympathetic and parasympathetic activity is based on numerous studies. In both animals and humans results showed a reduction in low frequency domain when a beta blockade was used. The power of a high frequency domain was reduced when the parasympathetic blocker atropine was used, however there was a decrease in the low frequency domain to some extent. Exercise has been shown to cause an increase in the low frequency domain, and a reduction in the high frequency domain, however, a reduction in total power of the frequency spectrum is seen. The results of these studies show that frequency domains are an artificial construct and that further work needs to be done as to whether the bands are changed or affected by certain conditions such as pathology, age and exercise.

It has been widely assumed that regular physical activity induces adaptations in the autonomic nervous system. Variations in mean HR and in catecholamine levels observed during changes in performance have suggested a strong interaction with the autonomic nervous system (Pichot, et al., 2000). A possible adaptation is that there is an increase in

parasympathetic activity and HRV. Until recently, most studies have been conducted in chronic heart failure or myocardial infarction patients. Most of these studies have shown a considerable and significant increase in HRV after a time of physical training. We will use time and frequency domain analysis of HRV because it has good reproducibility and is noninvasive.

Wavelet transform is based on recursive sums and differences of the vector components. The wavelets are the unit vectors; they correspond to the cosine and sine basis functions of Fourier transform. One of the advantages of wavelet is that an event can be described in the frequency domain as well as the time domain. This is unlike Fourier transform, in which an event is described as the time or frequency domain.

Wavelet analysis is devoted to the analysis of nonstationary signals. Therefore, there is no prerequisite regarding the stability frequency content along the signal analyzed; this allows for access at any time to the status of HRV. This analysis is devoted to the extraction of characteristic frequencies, contained along a signal, which was composed by consecutive intervals between RR interval series (Pichot et al., 2000).

Another characteristic of wavelet analysis is the shape of the wavelet-transform-analyzing equation. It can be designed to fit the shape of the analyzed shape. This allows for a better quantitative measurement.

This newer technique of analyzing heart rate variability seems to have advantages over using Fourier transform. Therefore, one of the purposes of this study is to examine the test-retest reliability of wavelet analysis of heart rate variability. Another purpose is to compare the change in wavelet under the conditions of spontaneous breathing and exercise.

LITERATURE REVIEW

INTRODUCTION

Cardiovascular control during exercise involves a complex system of redundant mechanisms that ensure adequate oxygen delivery to working muscles. These cardiovascular control mechanisms are both central and peripheral with each providing unique information to the brain.

During exercise, the autonomic nervous system responds to reduce blood flow to non-working tissues and maximize blood flow to working tissues, while controlling blood pressure. This occurs initially through a withdrawal of parasympathetic nervous activity and an increase in sympathetic nervous activity, which result in increased heart rate, stroke volume, and vascular resistance in the GI tract and non-working tissues (Fagraeus & Linnarsson, 1976; Peterson, Armstrong & Laughlin, 1988; Rowell, 1997). The signals to induce autonomic nervous stimulation to various parts of the body during exercise are from central command, skeletal muscle afferents, chemoreceptors, mechanoreceptors, metaboreceptors and baroreceptors.

AUTONOMIC CONTROL OF THE HEART

Anatomy

The parasympathetic division of the autonomic nervous system has long preganglionic neurons that originate in the brainstem and lateral horns of sacral levels of the spinal cord and short postganglionic neurons. The cranial portion supplies parasympathetic innervation to the muscles and glands of the head, neck, thorax and most of the abdominal viscera. The sacral portion supplies parasympathetic innervation to the stomach muscle and glands of the viscera in the lower abdomen and pelvis.

The sympathetic division of the autonomic nervous system arises from cell bodies in the lateral gray horn of the spinal cord. The myelinated nerve fibers emerge from the spinal cord in the ventral nerve roots of the 12 thoracic and first two or three lumbar spinal nerves. This emergence of fibers is known as the thoracolumbar outflow. These preganglionic fibers form small nerve bundles called white rami. They are white because the nerve fibers are myelinated. The fibers then pass to the paravertebral ganglia of the sympathetic chain.

Parasympathetic Stimulation

Acetylcholine is the neurotransmitter of the parasympathetic nervous system. Preganglionic and postganglionic neurons are classified as cholinergic neurons because they release acetylcholine.

Two types of cholinergic receptors are 1) muscarine receptors – receptors that respond to muscarine, an alkaloid produced by the poisonous mushroom *Amanita muscaria*, 2) nicotinic receptors – receptors that respond to nicotine, a derivative from the tobacco plant.

Nicotinic receptors are found on sympathetic and parasympathetic postganglionic neurons of both the parasympathetic and sympathetic divisions, and on the postsynaptic membranes of skeletal muscle cells in the somatic nervous system. Nicotinic receptors respond to acetylcholine released from both sympathetic and parasympathetic preganglionic fibers. Muscarine receptors are found on effectors innervated by the parasympathetic postganglionic neurons of the autonomic nervous system. These receptors bind with acetylcholine, which is released from parasympathetic postganglionic neurons and usually inhibits smooth muscle and cardiac pacemaker cells, but excites smooth muscle in other areas.

Usually, the parasympathetic system responds to a specific stimulus in a discrete region for a short time. This effect occurs because the postganglionic neurons have short axons that are distributed for short distances to specific areas. The rapid deactivation of acetylcholine by acetylcholinesterase results in a short-term effect by the neurotransmitter.

Sympathetic Stimulation

The neurotransmitter released by the preganglionic nerve terminals is acetylcholine. The neurotransmitter released by the postganglionic nerve terminal is norepinephrine. However, there are a few exceptions. Sympathetic postganglionic fibers to most sweat glands and some blood vessels release acetylcholine and not norepinephrine.

Sympathetic postganglionic neurons are classified as adrenergic because most postganglionic neurons of this division release norepinephrine. The preganglionic neurons of the sympathetic division release acetylcholine and these are classified as cholinergic neurons.

The sympathetic division of the autonomic nervous system is anatomically and physiologically organized to affect widespread regions of the body, or even the entire body, for sustained periods of time. Long-lasting sympathetic effects are directly related to the slow inactivation of norepinephrine and to the extensive distribution of norepinephrine and release of epinephrine from the adrenal medulla into the bloodstream.

Central Command

Central command are signals that initiate and maintain the close matching between blood flow and metabolism during exercise. It causes activation of both cardiovascular and somatomotor systems. Central command is a feed forward controller of circulation (Rowell, 1993). Central command of cardiovascular control originates in the subthalamic locomotor region of the brain and acts to induce parasympathetic withdrawal and increase

sympathetic activity by acting on the cardiovascular control center. The hypothalamus is active during exercise where there is an increase in body temperature. The hypothalamus then acts to vasodilate the skin blood vessels (Brooks, Fahey & White, 1996).

Autonomic Input

Sensory receptors may be classified according to their location, structure, type or stimulus, or type of sensation. Receptors play a role in autonomic input during rest and exercise.

Chemoreceptors (chemical receivers), respond to chemical stimuli that result in taste and smell; they also respond to changes in the concentrations of carbon dioxide, hydrogen and oxygen ions in the blood, as well as other chemical changes (VanWynsberghe, Noback & Carola, 1995).

Mechanoreceptors (mechanical receivers), respond to and monitor such physical stimuli as touch, pressure, muscle tension, metabolites in the muscle, joint position changes, air vibrations in the cochlear system of the ear and head movements detected by the vestibular system of the ear. Mechanoreceptors, such as golgi tendon organs and muscle spindles, detect movement and stretch within the tendons and muscle of active tissue. These receptors have no direct link to the cardiovascular control center, but do exert some influence through central command and possibly through interneuron links in the spinal cord with other skeletal muscle afferents (Thomas et al., 1998; Brooks et al., 1996; Appelberg, Hullinger, Johansson & Sojka, 1983; Pedersen, Ljubisavljevic, Bergenheim & Johansson, 1998). They are the most widespread of all the sensory receptors. They are also the most varied in structure and sensitivity.

Baroreceptors detect change in arterial pressure and induce changes in autonomic nervous stimulation in order to maintain blood pressure within a narrow range.

During exercise, baroreceptors are responsible for maintaining sympathetic activity and to prevent arterial hypotension (Rowell, 1997).

Proprioceptors (received from one's own self), respond to stimuli in such deep body structures as muscles tendons, joints and the vestibular apparatus of the ear. They are involved with sensing where parts of the body are in relation to each other and the position of the body in space.

MEASUREMENT OF AUTONOMIC CONTROL

Time Domain Methods

Time domain methods of measuring autonomic activity are the simplest to perform. In these methods, either the heart rate at any point in time or the intervals between successive normal complexes are determined. In a continuous ECG record, each QRS complex is detected, and the so-called normal-to-normal (NN) intervals (that is, all intervals between adjacent QRS complexes resulting from sinus node depolarizations) or the instantaneous heart rate is determined.

Simple time domain measures that can be calculated include the mean NN interval, the mean heart rate, or the difference between night and day heart rate (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). There are other time domain measures that can be used. They include variations in instantaneous heart rate secondary to respiration, tilt, Valsalva maneuver, or phenylephrine infusion.

More complex statistical time domain measures can be calculated using instantaneous heart rates or cycle intervals, particularly those recorded over longer periods of time, usually 24 hours. The simplest variable to calculate is the standard deviation of the NN intervals (SDNN), that is the square root of the variance. SDNN reflects all the cyclic

components responsible for variability in the period of recording. In many studies, SDNN is recorded over a 24-hour period and thus encompasses short-term HF variations as well as the lowest-frequency components seen in a 24-hour period.

Another commonly used statistical variable calculated from segments of the total monitoring period include SDANN, the standard deviation of the average NN intervals over short periods, usually 5 minutes, which is an estimate of the changes in heart rate due to cycles longer than 5 minutes, and the SDNN index (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The series of NN intervals can also be converted into a geometric pattern such as the sample density distribution of NN interval durations or a sample density distribution of differences between adjacent NN intervals.

Frequency Domain Methods

Power spectral analysis provides the basic information of how power (variance) distributes as a function of frequency. There are three main components that are used in spectral analysis. They are VLF (very low frequency), LF (low frequency) and HF (high frequency). The measurement of these is usually made in absolute values of power (milliseconds squared). LF and HF may also be measured in normalized units, which represent the relative value of each power component in proportion to the total power minus the VLF component (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

LF and HF components in normalized units emphasize the controlled and balanced behavior of the two branches of the autonomic nervous system. The normalized units tend to minimize the effect of changes in total power on the values of LF and HF

components (Pagani, Lombardi, Guzzetti, Rimoldi, Furlan, Pizzinelli, Sandrome, Mafatto, Dell, Piccaluga, Turiel, Baselli, Cerutti & Malliani, 1986).

Rhythm pattern analysis measures blocks of RR intervals determined by properties of the rhythm and investigating the relationship of such blocks without considering the internal variability.

HEART RATE VARIABILITY

Heart Rate Variability Used to Measure Autonomic Activity

Heart rate variability is a recognized parameter for assessing autonomous nervous system activity. The RR interval variations during resting conditions represent a fine tuning of the beat-to-beat control mechanisms (Saul, Rea, Eckberg, Berger & Cohen, 1990). Vagal afferent stimulation leads to reflex excitation of vagal efferent activity and inhibition of sympathetic efferent activity. The opposite reflex effects are mediated by the stimulation of sympathetic afferent activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Efferent vagal activity is seen by the high frequency component of spectral analysis and sympathetic efferent activity is seen by the low frequency component of spectral analysis.

Multivariate Risk Stratification

Evidence is given that multivariate models – which allow evaluation of the interactions between changes in blood pressure, heart rate, and other biological signals (such as respiratory activity) in the time of frequency domains – offer a more comprehensive approach to the assessment of cardiovascular regulation than that represented by the separate analysis of fluctuations in blood pressure or heart rate only. It was found that the potential importance of spectral and time domain techniques is particularly related to the possibility they offer information to be obtained on

cardiovascular regulation in real life conditions i.e. in conditions free from artificial laboratory stimulation (Parati, Saul, DiRienzo & Mancia, 1995).

Nakata, Takata, Yuasa, Shimakura, Maruyama, Nagai, Sakagami, and Kobayashi, 1998, found that the effect of LF component of MSNA on arterial pressure showed no change in response to propranolol, but was diminished by phentolamine. The effect of the LF component of MSNA on RR intervals was not altered by pharmacological sympathetic nerve blockade. The HF component of MSNA did not influence other variables, but was influenced by RR intervals, arterial pressure and respiration. These findings indicate that the LF component of MSNA reflects autonomic oscillations, whereas HF component is passive and influenced by other cardiovascular variables.

Bloomfield, Zweibel, Bigger Jr. and Steinman, 1998, found that phenylephrine infusions produced baroreflex-mediated increases in both time and frequency domain measures of RR variability known to reflect parasympathetic nervous system activity in normal healthy adults taking no medication.

Clinical Uses of Heart Rate Variability

Depressed HRV can be used as a predictor of risk after an acute MI and as an early warning sign of diabetic neuropathy.

Baroreflex sensitivity (BRS) and the HRV were studied in conscious rats after myocardial infarction (MI). It was concluded that BRS is transiently depressed in rats with left ventricular dysfunction after an MI probably due to a reduced reflex vagal activity. Even though basal HR and HRV are unchanged after and MI, a temporary attenuation of tonic vagal activity is unmasked after autonomic blockade (Kruger, Kalenko, Haunstetter, Schweizer, Maier, Ruhle, Ehmke, Kubler & Haass, 1997).

Bigger et al., 1991 found that spectral analysis of HRV in patients that survived and acute MI revealed a reduction in total and in individual power of spectral components. However, Lombardi et al., 1987, 1991, found that when the power of LF and HF was calculated in normalized units, an increased LF and a diminished HF were observed during both resting controlled conditions and 24-hour recordings analyzed over multiple 5-minute periods. The changes seen may be an indication in a shift of sympathovagal balance toward a sympathetic predominance and a reduced vagal tone.

It was found that in post-MI patients who had a very depressed HRV, were found to have most of the residual energy distributed in the VLF frequency range below 0.03 Hz, with only a small respiration-related HF (Bigger, Fleiss, Steinman, Rolnitzky, Kleiger & Rottman, 1992). These spectral profile characteristics are similar to those that were observed in an advanced cardiac failure or after a transplant, and are likely to either reflect a diminished responsiveness of the target organ to neural modulatory inputs (Mallini, Lombardi & Pagani, 1994). Another possible factor affecting HRV in post-MI patients is a saturating influence on the sinus node of a persistently high sympathetic tone (Malik & Camm, 1993).

Neuropathy associated with diabetes mellitus is characterized by alteration of small nerve fibers, a reduction in time domain parameters of HRV seems not only to carry negative prognostic value, but also to precede the clinical expression of autonomic neuropathy (Ewing, Neilson & Traus, 1984; Kitney, Byrne, Edmonds, Watkins & Roberts, 1982; Pagani, Malfatto, Pierini, Casati, Masu, Poli, Guzzetti, Lombardi, Cerutti & Mallini, 1988; Freeman, Saul, Roberts & Berger, 1991; Bernardi, Ricordi, Lazzari, Solda, Calciati, Ferrari, Vandea, Finardi & Frantino, 1992). It was shown that in diabetic patients without evidence of autonomic neuropathy, there was a reduction in the absolute

power of LF and HF during controlled conditions (Pagani et al., 1988). However, when the LF/HF ratio was considered or when LF and HF were analyzed in normalized units, no significant difference in comparison to normal subjects was present. This shows that in the initial manifestation of neuropathy there is more likely to be involvement in both efferent limbs of the autonomic nervous system (Pagani et al., 1988; Bernardi et al., 1992).

Other Clinical Potential

It has been shown that after 12 weeks of ACE inhibitor treatment, HF component of HRV increased. This could potentially show that significant augmentation of parasympathetic tone is associated with ACE inhibitor therapy (Binkley, Nunziata, Haas, Nelson & Cody, 1991).

Congestive heart failure patients have shown a decreased HF power and the LF/HF ratio increased in normal subjects when resting supine. This showed that there is a parasympathetic tone withdrawal in CHF. CHF patients have an imbalance of autonomic tone with a decreased parasympathetic tone and a predominance of sympathetic tone (Binkley, Haas, Starling, Nunziata, Hatton, Leier & Cody, 1993).

It was demonstrated that alterations of baroreceptor control of sympathetic nervous system function occurred in chronically overloaded rats. Because the baroreflex control of efferent sympathetic activity is dissociated from the control of heart rate, these alterations can only be detected if direct sympathetic nerve activity recordings are performed and if the cardiovascular system is stimulated by procedures such as baroreceptor unloading. Analysis of the arterial baroreflex control of heart rate alone might not be sensitive enough to detect early changes in autonomic nervous system function (Willenbrock, Stauss, Scheuermann, Osterziel, Unger & Dietz, 1997).

Hypertensive patients showed reduced parasympathetic when stressed. This shows support for the use of nonpathological therapy of hypertension that increases vagal tone, such as the use of exercise training (Langewitz, Ruddle & Schachinger, 1994).

PHYSICAL FITNESS, PHYSICAL ACTIVITY AND AUTONOMIC ACTIVITY

Physical Activity and Autonomic Modulation

Melanson, 2000 found that heart rate and HRV are highly reproducible, regardless of physical activity level. Additionally, although time and frequency domain measures of HRV may be greater in active than sedentary individuals, HRV does not appear to be increased in a dose-dependent manner with increasing levels of physical activity.

It was found that heavy training shifted the cardiac autonomic balance toward a predominance of the sympathetic over the parasympathetic drive. When recorded during the night, heart rate variability appeared to be a better tool than resting heart rate to evaluate cumulated physical fatigue, as it magnified the induced changes in autonomic nervous system activity. These results could be of interest for optimizing individual training profiles (Pichot et al., 2000).

Because HRV and blood pressure variability (BPV) response is different when induced by static or dynamic exercise, differences in the autonomic activity can be advised. Instead of the vagal withdrawal and sympathetic augmentation observed during dynamic exercise, the increase in the overall HRV and the middle frequency (MF) component during static exercise suggest an increased activity of both autonomic branches (Gonzalez-Camarena, Carrasco-Sosa, Roman-Ramos, Gaitan-Gonzalez, Medina-Banuelos & Azpiroz-Leehan, 2000).

It has been demonstrated that the sensitivity of baroreflex modulation of muscle sympathetic nerve activity, (MSNA), is elevated by muscle metaboreceptor stimulation,

whereas the sensitivity of baroreflex of modulate heart rate is unchanged during posthandgrip muscle ischemia (Cui, Wilson, Shibasaki, Hodges & Crandall, 2001).

HRV and MAP intensity dependent changes occurred during 20% MVC handgrip exercise. In contrast, changes in SDNN did not change in an intensity dependent manner. Rather, the changes are consistent with parasympathetic withdrawal at low intensity (20% MVC) and but no further change in parasympathetic modulation of the the heart at higher workloads (60% MVC). These findings support the use of HRV and as index of parasympathetic modulation of the heart during dynamic handgrip exercise. Furthermore, this study suggests that the coefficient of variation of RR intervals provides a unique method of characterizing cardiac chronotropic activity (Kluess, Wood & Welsch, 2000).

Age, Gender and Autonomic Regulation

The autonomic nervous system drives variability in heart rate, arterial pressure, vascular tone, and cardiac ejection. Gender differences in autonomic regulation of vascular tone, arterial pressure, and cardiac ejection are not well documented.

Spectral analysis was used to calculate variability in heart rate (RR interval, RRI), arterial pressure, stroke volume, and total peripheral resistance (TPR). Circulating levels of catecholamines and pancreatic polypeptide were measured as well in two groups of 25-year-old healthy men and healthy follicular women. Subjects were studied supine and were either administered muscarinic autonomic blockade or were drug free. The results suggest that there is a predominance of sympathetic vascular regulation in men compared with a dominant parasympathetic influence on heart regulation in women (Evans, Ziegler, Patwardhan, Ott, Kim, Leonelli & Knapp, 2001).

The correlates of baroreflex sensitivity were evaluated in healthy subjects. Participants were normal weight, non-smoking male and female adults between the ages of 23-77 years old. Baroreflex control of heart rate was measured by using the phenylephrine bolus-injection technique. It was concluded that physiological factors, particularly age and gender, have a significant impact of BRD in healthy subjects. In addition, it was demonstrated that BRS values that have been proposed to be useful in identifying postinfarction patients at high risk of sudden death are frequently found in healthy subjects (Laitinen, Hartikainen, Vanninen, Niskanen, Geelen & Lansimies, 1998).

Normal populations of middle-aged women and men ranging in the age from 40-79 years were studied in order to determine the influence of gender on sympathetic and parasympathetic control of heart rate and on the subsequent aging process, and heart rate variability. This was conducted through ECG monitoring in the daytime for five minutes with the subjects lying quietly and breathing normally. It was concluded that middle-aged women and men have a more dominant parasympathetic and sympathetic regulation, respectively. The gender-related difference in parasympathetic regulation diminishes after age 50, whereas a significant time delay for the disappearance of sympathetic dominance occurs in men (Kuo, Lin, Yang, Li, Chen & Chou, 1999).

RR interval variability and systolic blood pressure (SBP) variability is reduced in the elderly. However, little is known about the very elderly (over 70 years old). More important than that, is not knowing which frequency components of SBP and RR interval variability are affected significantly. This was addressed by broadband spectral analysis in very elderly adults and adult subjects who were monitored using noninvasive finger blood pressure and ECG for 30 minutes in the supine position and 15 minutes in the

upright position. It was found that in very elderly subjects that a reduction in overall RR interval variability is accounted for by a reduction in all of its frequency components.

The accompanying increase in overall BP variability however, results from a nonhomogeneous behavior of its frequency components, which consists of an increase in the very low frequency and concomitant reduction in the higher frequency powers. The mechanisms responsible for these changes may be complex, but at least they may in part reflect the baroreflex impairment and autonomic dysfunction that characterize aging (Parati, Frattola, DiRienzo, Castiglioni & Mancia, 1997).

NEW TECHNIQUE-WAVELET ANALYSIS

Analyzes Sequences of RR Intervals

Multi-resolution Wavelet Analysis is one of the most successful techniques to analyze non-stationary times series. Recently, this technique was utilized in order to analyze a sequence of RR intervals.

Peng, Havlin, Stanley, and Goldberger, 1995, were able to distinguish between healthy subjects and patients with heart failure by the use of the detrended fluctuation analysis. Later, Thrurner, Feuerstein, and Teich, 1998, used a similar procedure, but focused on the values of the variance than on the scaling exponent. For the scale windows of $m=4$ and $m=5$ heartbeats, the standard deviation of the wavelet coefficients for normal individuals and heart failure patients were divided into two disjointed sets. In this way they were able to succeed in classifying subjects from a test group as either being a part of the normal group or the heart failure group. They did this with 100% accuracy.

Wavelet analysis is based on recursive sums and differences of the vector components; the differences can be compared with the high frequency amplitudes in the Fourier transform and the sums can be compared with the low frequency amplitudes in the Fourier transform. The wavelets are unit vectors; they correspond to the cosine and sine basis functions of the Fourier transform. Wavelet analysis allows for a temporally localized sliding analysis of the signal. The condition of heart rate variability can be accessed anytime.

Wavelet analysis differs from Fourier transform and can be designed to better fit the shape of the analyzed signal - this allows for a better quantitative measure.

Ashkenazy, Lewkowicz, Levitan, Moelgaard, Thomsen and Saermark, 1998, applied the above-mentioned technique used by Thurner et al., 1998. They found that healthy subjects exhibit greater fluctuations than patients. This difference in fluctuations become most evident on the scale 4 to 5 (corresponding to windows of 16 and 32 heartbeats), but in their study it is apparent at all scales from 1 to 7 (windows of 2 to 128 heartbeats).

Advantages of Wavelet Analysis

In using wavelet analysis, an event can be simultaneously described in the time domain as well as in the frequency domain. Unlike Fourier transform, where an event is accurately described either in the frequency or in the time domain, wavelet analysis allows a multi-resolution analysis of data with different behavior on different scales. Because of this, large classes of biological data can be analyzed, such as RR intervals and ECG series, using this method.

The respective yields of Fourier and wavelet transforms were compared in analyzing heart rate variability during dynamic changes in autonomous nervous system balance induced by atropine and propranolol. Fourier and wavelet transforms were applied to

sequences of heart rate intervals in six subjects receiving increasing doses of atropine and propranolol. They found that the lowest doses of atropine administered, heart rate variability increased, followed by a progressive decrease with higher doses. With the first dose of propranolol, there was a significant increase in heart rate variability, which progressively disappeared after the last dose. The wavelet transform gave them significantly better quantitative analysis of heart rate variability than did Fourier transform during autonomous nervous system adaptations induced by both atropine and propranolol and provided temporally localized information (Pichot, Gaspoz, Molliex, Antoniadis, Busso, Roche, Costes, Quintin, Lacour & Barthelemy, 1999).

SUMMARY

Heart rate variability represents one of the most promising quantitative markers of autonomic activity. HRV has been described as the variations of both RR intervals and instantaneous heart rate.

There is an increased incidence of total mortality and cardiac events in both apparently healthy middle-aged and elderly adults as well as post myocardial infarction patients who have reduced heart rate variability (HRV). A reduced HRV is a reflection of elevated sympathetic activity. This is a condition that may decrease the fibrillation threshold and thus predispose to ventricular fibrillation (Schuit et al., 1999). Decreased parasympathetic tone or sympathetic overstimulation reduces the magnitude of HRV and lowers the threshold for the origin of arrhythmias. Reduced levels of HRV are related to all-cause mortality, the incidence of new cardiac events (angina pectoris, myocardial infarction, coronary heart disease, death, or congestive heart failure, and risk of sudden cardiac death in asymptomatic individuals (Melanson, 2000).

Time domain methods of measuring autonomic activity are the simplest to perform. In these methods, either the heart rate at any point in time or the intervals between successive normal complexes are determined. In a continuous ECG record, each QRS complex is detected, and the so-called normal-to-normal (NN) intervals (that is, all intervals between adjacent QRS complexes resulting from sinus node depolarizations) or the instantaneous heart rate is determined.

Power spectral analysis provides the basic information of how power (variance) distributes as a function of frequency. There are three main components that are used in spectral analysis. They are VLF (very low frequency), LF (low frequency) and HF (high frequency). The measurement of these is usually made in absolute values of power (milliseconds squared). LF and HF may also be measured in normalized units, which represent the relative value of each power component in proportion to the total power minus the VLF component (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Wavelet transform is based on recursive sums and differences of the vector components. The wavelets are the unit vectors; they correspond to the cosine and sine basis functions of Fourier transform. The differences can be compared with the high frequency amplitudes in the Fourier transform and the sums can be compared with the low frequency amplitudes in the Fourier transform. Wavelet analysis allows for a temporally localized sliding analysis of the signal. The condition of heart rate variability can be accessed anytime.

One of the purposes of this study is to examine the test-retest reliability of wavelet analysis of heart rate variability. Another purpose is to compare the change in wavelet under the conditions of spontaneous breathing and exercise.

It is hypothesized that wavelet transform will perform differently from Fourier transform in analyzing heart rate variability. With exercise, wavelet analysis will perform better than Fourier transform from a quantitative viewpoint in separating different levels of heart rate variability. We expect to see better reliability within the exercise conditions with wavelet analysis when compared with Fourier transform, but are not sure of what will occur within the spontaneous breathing condition.

MATERIALS AND METHODS

PARTICIPANTS

Volunteers (age 18 to 30 years) were asked to participate in this study. All volunteers were healthy adults, both male and female, without any over signs such as heart disease, hypertension, diabetes, emphysema, gastrointestinal disturbances or bleeding or gynecological problems. Individuals with current medical problems (including those listed above), current infection, or on medication known to affect cardiovascular function were excluded from the study. In addition, anyone that had taken aspirin within two weeks prior to testing was excluded. Each participant came to the laboratory on two occasions and was asked to perform one of two experimental protocols. Protocol one (n=9) involved 2 sessions of incremental dynamic handgrip exercise at intensities of both 20% and 60% of maximal voluntary contraction (MVC). Protocol 2 (n = 24) involved 2 session of dynamic handgrip exercise at 60% of MVC. These protocols are discussed in greater detail below. All visits occurred prior to 11 AM and participants were asked to abstain from eating, drinking, and exercising at least 12 hours prior to the test. Upon arrival at the laboratory, participants were be asked a few general health questions about their health history in order to evaluate for the presence of any disease, condition, or medical therapy that might affect their cardiovascular function.

PROTOCOL 1

After lying in a supine position for 20 minutes, the participant was asked to squeeze the handgrip dynamometer attachment (Biodex, New York) “as hard as [they] can for 5 seconds”. They were permitted three trials with 30 seconds in between each trial. The three MVC's were then be averaged and multiplied by 0.20 and 0.60 to get the peak

torque that will be used during the exercise conditions. The participant was then instrumented with equipment to record ECG (see Figure 1 for a diagram of instrumentation for testing the arm).

Heart rhythm was continuously collected using a 3-lead ECG interfaced with a Biopac MP100 and its companion software Acqknowledge (model MP100A, Biopac Inc., Santa Barbara, CA). All data were collected at 200hz. The participant was then asked to perform a handgrip exercise task preceded by 5 minutes of quiet rest (spontaneous breathing). The handgrip exercise task consisted of squeezing a handgrip-measuring device once every two seconds at 20% MVC for 5 minutes. They then rested for another 5 minutes and then exercised again at 60% MVC for 5 minutes. The participant had visual feedback regarding the amount of force they are exerting and an auditory cue for the cadence. In addition, they received verbal encouragement to maintain the force and cadence. Throughout the testing procedures, ECG data were acquired. The procedure was repeated 4 weeks later.

PROTOCOL 2

While lying in a supine position, the participant was asked to squeeze a handgrip dynamometer (Biodex, New York) “as hard as [they] can for 5-seconds”. They were permitted three trials with 30 seconds in between each trial. The three MVC's were then averaged and multiplied by 0.60 to get the peak torque that was used during the exercise condition. The participant was then instrumented with equipment to record ECG data (see Figure 5 in appendix for a diagram of instrumentation for testing the arm).

The ECG data were continuously collected using Biopac MP100 and its companion software Acqknowledge (model MP100A, Biopac Inc., Santa Barbara, CA). All data were collected at 200hz.

The participant was then asked to perform a handgrip exercise task preceded by 5 minutes of quiet rest (spontaneous breathing). The handgrip exercise task consisted of squeezing a handgrip-measuring device once every two seconds at 60% MVC. The participant was given visual feedback regarding the amount of force they are exerting and an auditory cue for the cadence. In addition, they received verbal encouragement to maintain the force and cadence. The participant returned to the laboratory 4 weeks later to repeat the experimental protocol.

DATA TREATMENT

ECG Analysis

The ECG data were analyzed using Acqknowledge 3.0 software. The ECG data were visually inspected for non-sinus beats and converted to a tachogram of R-R period. Matlab 6.5 was used to analyze 2.2-minute segments of tachogram data for frequency and time domain parameters. Tachogram segments were taken from a representative section of data at least 90 seconds into the data collection of spontaneous breathing and exercise. Raw data were reported as mean heart period (mean R-R interval), standard deviation of the normal sinus R-R intervals (SDNN), low frequency power (LF: 0.04 hz to 0.15 hz) and high frequency power (HF: 0.15 hz to 0.40 hz). The frequency domain parameters were calculated using the squared modulus of the fast Fourier transformation and reported in normalized frequency units (LFnu or HFnu: $\ln[\text{power}/\text{sum of } \ln(\text{LF}) \text{ and } \ln(\text{HF})]$).

Wavelet Analysis

256 consecutive normal RR intervals are subjected to Daubuchies-4 Tap Wavelet Analysis (Matlab 5.3). This complex transformation yields 7 levels of power that area related to the length of the heart rate tachogram. Each level is associated with time

periods equivalent to 2^n intervals. That is level 1 is associated with changes that occur over each 2 intervals (i.e., very quickly or highest frequency), whereas level 7 corresponds to events that cycle over every 128 consecutive intervals (i.e., rather slowly or low-frequency). The specific frequency for each level is thus dependent upon not only the number of intervals, but the length of each the set of intervals. Typically, however, levels 2 and 3 are approximately reflecting the power in the “low-frequency band.” Likewise, levels 4,5 and possibly 6 are often reflecting the power in the “high-frequency” band. The ratio of low-to-high frequency power derived from the DWT is calculated as the sum of the coefficients for the levels corresponding to representing LF powers, divided by the sum of the coefficients corresponding to HF powers. Thus the wavelet transformation provided us with power at seven levels of signal reconstruction.

STATISTICAL ANALYSIS

A repeated measures ANOVA was used to evaluate differences among the conditions (SB1, HG20 and HG60) in mean R-R, SDNN, LFnu, Hfnu and wavelet powers at all levels, including those that correspond to low- and high- frequency variations in heart rate. Repeated measures were also used to calculate ICC's for Day 1 and Day 2 comparisons.

Alpha was set at $p < 0.05$, and Tukey post-hoc comparisons were made where indicated.

RESULTS

Eleven men and twelve women participated in this study. All twenty-three participants were subjected to the SB1 and HG60 conditions. Of those twenty-three, nine performed the HG20 condition. Table 4.1 summarizes the participant characteristics for this study.

Table 1: Participant Characteristics

Men	11
Women	12
Age (years)	21.53 \pm 3.47
Weight (pounds)	151.07 \pm 58.93
Height (inches)	66.21 \pm 4.79

The results of the ANOVAs revealed that exercise resulted in a decrease in RR interval and SDNN ($p < 0.05$) (Figures 4.1 and 4.2). However in the case of the nine participants who performed SB vs. HG20 and HG 60 conditions (figure 4.2), while RR decreased incrementally, SDNN decreased during HG20 ($p < 0.05$), but appeared to subsequently increase during HG60. With respect to spectral parameters, ANOVA did not detect any effect of test condition in either the nine participants who performed all three test conditions, or among the 23 who performed SB and HG60 (Figures 4.3 and 4.4). However, there was a main effect of test condition on the discrete wavelet

transformation derived LF/HF ratio such that the ratio increased during exercise (Figure 4.4). Furthermore, post-hoc testing using the data from the nine participants who performed all three conditions indicate an incremental increase with increasing workload.

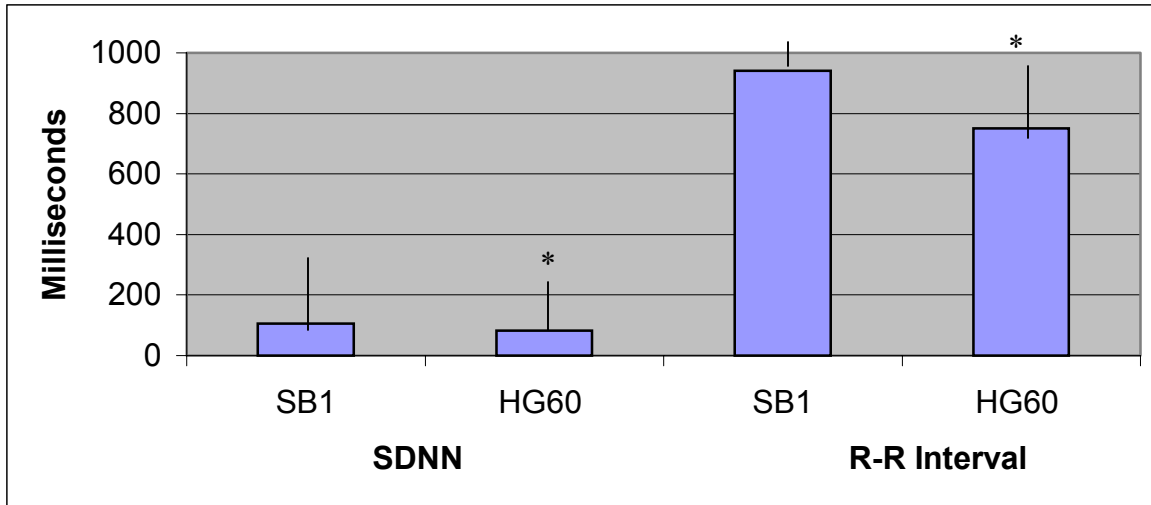


Figure 1: Effect of 60% MVC handgrip exercise on SDNN and R-R Interval. Values are msec. SDNN =standard deviation of normal RR intervals collected over 2 minutes. * = different from SB ($p < 0.05$).

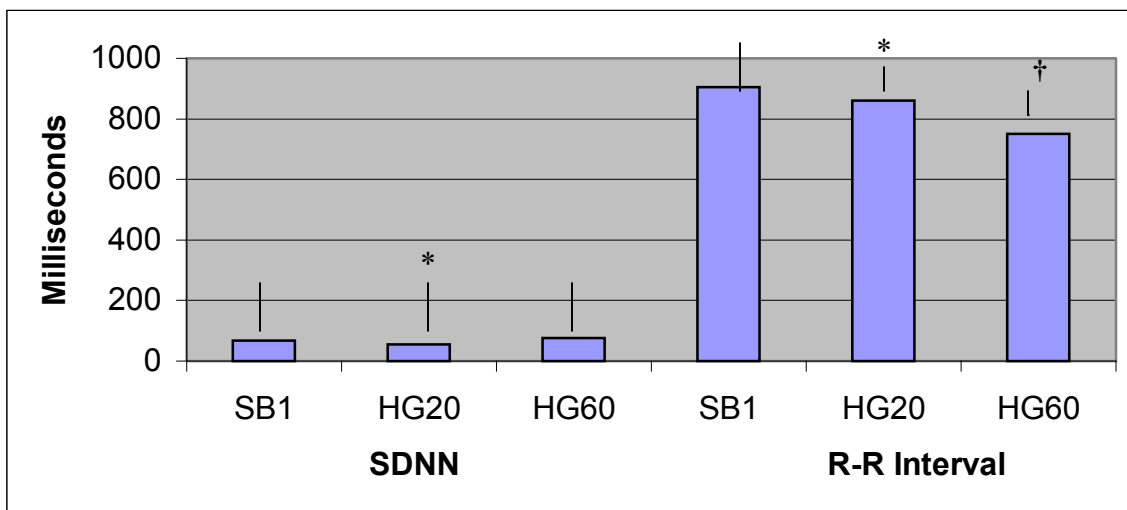


Figure 2: Effect of 20% and 60% Handgrip Exercise on SDNN and R-R Interval. Values are milliseconds SB= spontaneous breathing HG20= dynamic handgrip exercise at 20% MVC HG60= dynamic handgrip exercise at 60% MVC SDNN =standard deviation of normal RR intervals collected over 2 minutes. * = different from SB ($p < 0.05$). † = different from SB and HG20 ($p < 0.05$).

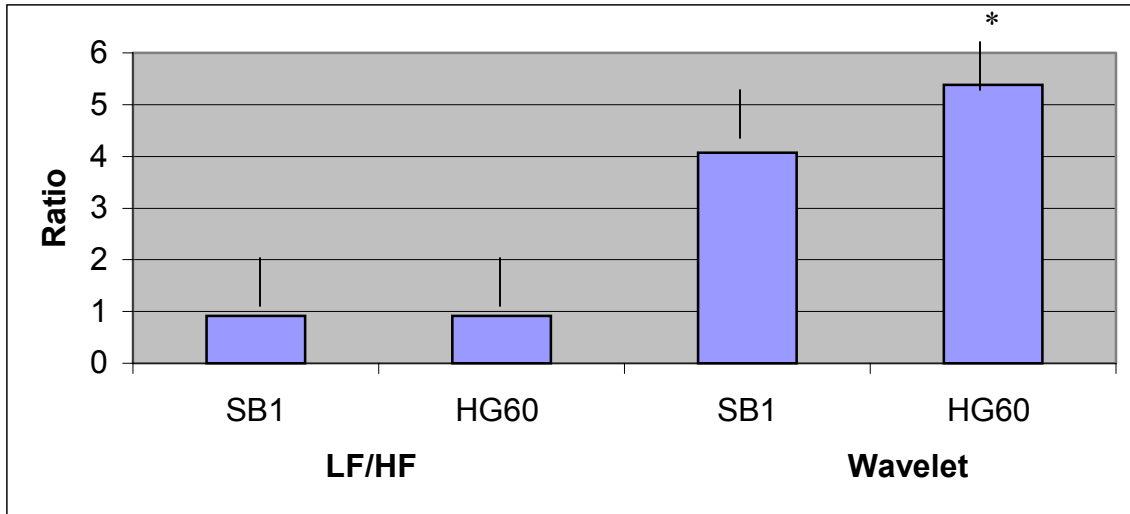


Figure 3: Effect of 60% handgrip exercise on sympathovagal balance
 LF/HF = ratio of low- to high-frequency normalized units derived by FFT.
 Wavelet = ratio of coefficients for the DWT that correspond to low- to high-frequency parameters
 * = different from SB ($p < 0.05$).

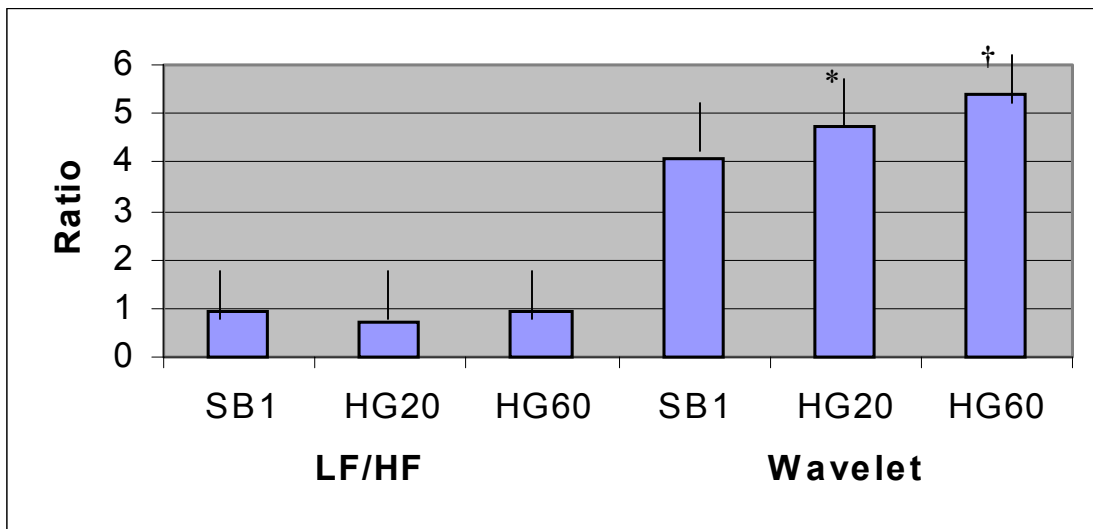


Figure 4: Effect of 20% and 60% handgrip exercise on sympathovagal balance
 LF/HF = ratio of low- to high-frequency normalized units derived by FFT.
 Wavelet = ratio of coefficients for the DWT that correspond to low- to high-frequency parameters
 * = different from SB ($p < 0.05$).
 † = different from SB and HG20 ($p < 0.05$).

Table 2: Mean Values of Wavelet Level Coefficients (Day 2 only)

Condition	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Ratio
SB1	0.60±0.29	0.51±0.18	1.07±0.46	.034±0.25	1.25±0.40	5.36±1.54	4.07±1.90	3.10±0.85
HG20	0.29±0.09	0.10±0.04	0.78±0.21	0.23±0.05	0.95±0.17	2.95±0.57	4.88±1.18	3.84±0.18
HG60	0.33±0.71	0.09±0.38	0.66±0.28	0.15±0.21	0.62±0.98	1.24±0.96	2.44±2.22	5.39±0.77

SB= spontaneous breathing

HG20= dynamic handgrip exercise at 20% MVC

HG60= dynamic hamdgrasp exercise at 60% MVC

Table 3: Time and Frequency Domain Correlation Coefficients

Condition	Number of Participants	R-R Interval (milliseconds)	SDNN (milliseconds)	Spectral Components (normalized units)
SB1	22	0.75	0.29	0.16
HG20	9	0.95	0.46	0.82
HG60	23	0.54	0.17	0.09

Values are intraclass correlation coefficients for Day1 vs. Day 2 comparisons of the parameters indicated.

SB= spontaneous breathing

HG20= dynamic handgrip exercise at 20% MVC

HG60= dynamic handgrip exercise at 60% MVC

Table 4: Wavelet Autonomy Level Plus Ratio Correlation Coefficients

Wavelet Autonomy Level Plus Ratio	Number of Participants	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Ratio
SB1	23	0.25	0.16	0.57	0.11	0.15	0.54	0.74	0.24
HG20	9	0.92	0.94	0.81	0.23	0.47	0.40	0.92	0.34
HG60	23	0.20	0.02	0.64	0.12	0.21	0.74	0.38	0.11

Values are intraclass correlation coefficients for Day1 vs. Day 2 comparisons of the parameters indicated.

SB= spontaneous breathing

HG20= dynamic handgrip exercise at 20% MVC

HG60= dynamic hamdgrip exercise at 60% MVC

DISCUSSION

The main purpose of this study was to report the test-retest reliability of heart rate variability derived using the traditional FFT, and the newly proposed discrete wavelet transformation (DWT). Further, the construct validity of these approaches is considered by including an analysis of these measures of HRV under conditions of spontaneous breathing and during dynamic handgrip exercise.

The population used for this study was a relatively homogenous group in that all participants were apparently healthy individuals between the ages of 18 and 25. In consideration of the age and relatively good health of the participants the spectral powers of heart rate variability data appear to be within the expected range of values. For example, Parati et al, 1995 examined HRV under a number of laboratory conditions and reported time domain and HRV spectral powers within the same range as those of the present study. While only a small number of studies have employed the wavelet approach, our wavelet-derived values for the sympathovagal ratio are similar to those reported by Pichot et al., 2000.

The response of HRV to dynamic handgrip exercise was employed to examine the construct validity of HRV as measured using the time, frequency, and wavelet approaches. The results indicate that R-R- interval and SDNN both decreased during exercise. This decrease in the R-R is consistent with countless studies examining the chronotropic activity of the heart, and is likely due to both vagal withdrawal and heightened sympathetic activation. The unusual behavior of the SDNN, however, is not inconsistent with previous findings from our laboratory (Kluess et al., 2000) who suggested that SDNN dropped during HG20 due to vagal withdrawal, but experienced no further decline with increasing intensity because SDNN is by an large a measure of vagal

modulation and not sympathetic. The additional variability that appeared in this study at HG60 may have been due to large changes in the mean heart rate during HG60 condition.

The greater emphasis for this study was on the examination of the autonomic contribution to the chronotropic activity of the heart by utilizing power frequency and wavelet analysis techniques. While the ANOVA failed to detect an exercise induced change in the LF/HF ratio, these data are not inconsistent with the findings of Kluess et al., 2000, who reported an increase in sympathovagal balance (LF/HF ratio) during dynamic handgrip exercise. With respect to the DWT-derived ratio, however, we did observe an incremental increase in sympathovagal balance with intensity of dynamic handgrip exercise. The only data similar are from Pichot et al. 2000, who reported heightened wavelet ratio (i.e., sympathovagal balance) for several hours following exhaustive exercise.

The primary purpose of this paper was to examine the reliability of FFT and DWT of HRV during rest and exercise. We found that during SB1, the R-R intervals were fairly reliable between days. However, the reliability of all of the HRV parameters (SDNN, spectral components and wavelet components) were quite poor. Interestingly, however, during HG20, the reliability of the HRV parameters was much more promising. We found that that the intraclass correlation coefficients for the spectral and wavelet parameters were roughly 0.70 or higher during this condition. At HG60, however, the intraclass correlation coefficients for the HRV parameters were very weak.

When the wavelet levels and ratios were analyzed for reliability, only level 7 of SB1 was found to have an acceptable strength of reliability with an ICC of 0.74. However, Levels 1, 2, 3, and 7 of the exercise conditions HG20 were all found to have a correlation coefficient of .81 or greater. Wavelet level 6 of the exercise condition HG60 was found

to have a correlation of .74. The indication of generally poor reliability of these measures appears to be inconsistent with previous studies. For example, Melanson, 2000 found that heart rate and HRV and highly reproducible, regardless of physical activity level. Melanson had correlation coefficients of .97 and .98. One possible explanation is that Melanson analyzed 5-minute segments, whereas the present investigation utilized 2-minute segments. While the standards for examining HRV indicate that 2-minute segments are acceptable (Task Force of the European Society for Pacing and Electrophysiology, 1996), the present findings may suggest that a greater length than two minutes of data collection is required in order to see the sympathovagal changes that occur with exercise. Melanson also had his subjects report for five visits to the lab. While two visits to the lab are acceptable, the present findings may suggest that more days of testing may be needed in order for an subject to be acclimated to the conditions being tested.

Previous data from our laboratory do agree with Melanson. However, these data indicate that HRV is very reproducible if a prior exposure to the testing environment is included. Lee et al. (2000) found that Days 2 and 3 of a repeated data collection were very reproducible, however, the intraclass correlation coefficients for HRV parameters between Days 1 and 2, and Days 1 and 3 were considerably lower than those observed between days 2 and 3. As the present investigation only examined two days, the correlation coefficients are not entirely surprising.

Other possible threats to the reliability of this data could have been seasonal changes that occurred over the course of the data collection time period. Changes in the environment can cause sickness and allergies which in turn affects an individual's response to exercise.

Another possible threat was that our group of participants was an homogenous group of individuals. This possibility is supported by a relatively narrow range of scores for the parameters investigated.

Of interest, however, is the indication that the reliability coefficients for the HRV parameters appear to be consistently stronger during HG20 than during SB and HG60. This may be a consistent phenomenon that is related to the vagal withdrawal that occurs during HG20 in the absence of changes in sympathetic activation. With HG60, however, perhaps the picture becomes more difficult to interpret as sympathetic activation is heightened significantly. Camarena et al., 2000, found that when an individual exercised at 60% of their MVC, the interpretation of the HRV spectral analysis was skewed due to respiratory non-neural and/ or other humoral mechanisms.

The most interesting finding of this investigation was the ability of the DWT to detect changes in sympathovagal balance with incremental handgrip exercise, despite a very low number of participants (n=9). However, the application of this approach to exercise training studies is somewhat challenged by the apparently low reproducibility of HRV measures, whether DWT or FFT are applied. Nonetheless, the results are somewhat encouraging as there may be some evidence of an advantage to the DWT over the FFT. Moreover, the low reproducibility if the wavelet ratio is made less troublesome by the large change in the ratio during exercise. While the standard deviation of wavelet ratios under any condition hovered under 1.0 units, the average change during exercise was roughly 2.5 units. Therefore, the low reliability of this parameter may be less troublesome in the face of a large effect size.

More study is needed. Perhaps the optimum length of data collection still needs to be investigated, and greater acclimatization to the testing environment may also enhance

the opportunities to use the DWT as a surrogate for sympathovagal balance in longitudinal studies. The ability to present a local analysis of heart rate variability and to obtain a better quantification of it represents the major advantages of wavelet transform compared with Fourier transform. Fast autonomic nervous system adaptations could be precisely monitored by using the feature of temporally localized analysis. The relationship between parasympathetic tone and cardiac function could probably also be further explored, benefiting both from the quantitative and temporal feature of this mathematical analysis. This additional novel and more precise temporal localization, brought by the ability of wavelet transform to analyze nonstationary signals, makes wavelet transform a promising tool for analyzing other temporary situations, such as progressive adaptations to exercise or progressive effects of pharmacological test, in which it could provide information not easily shown by more traditional methods (Pichot et al., 1999).

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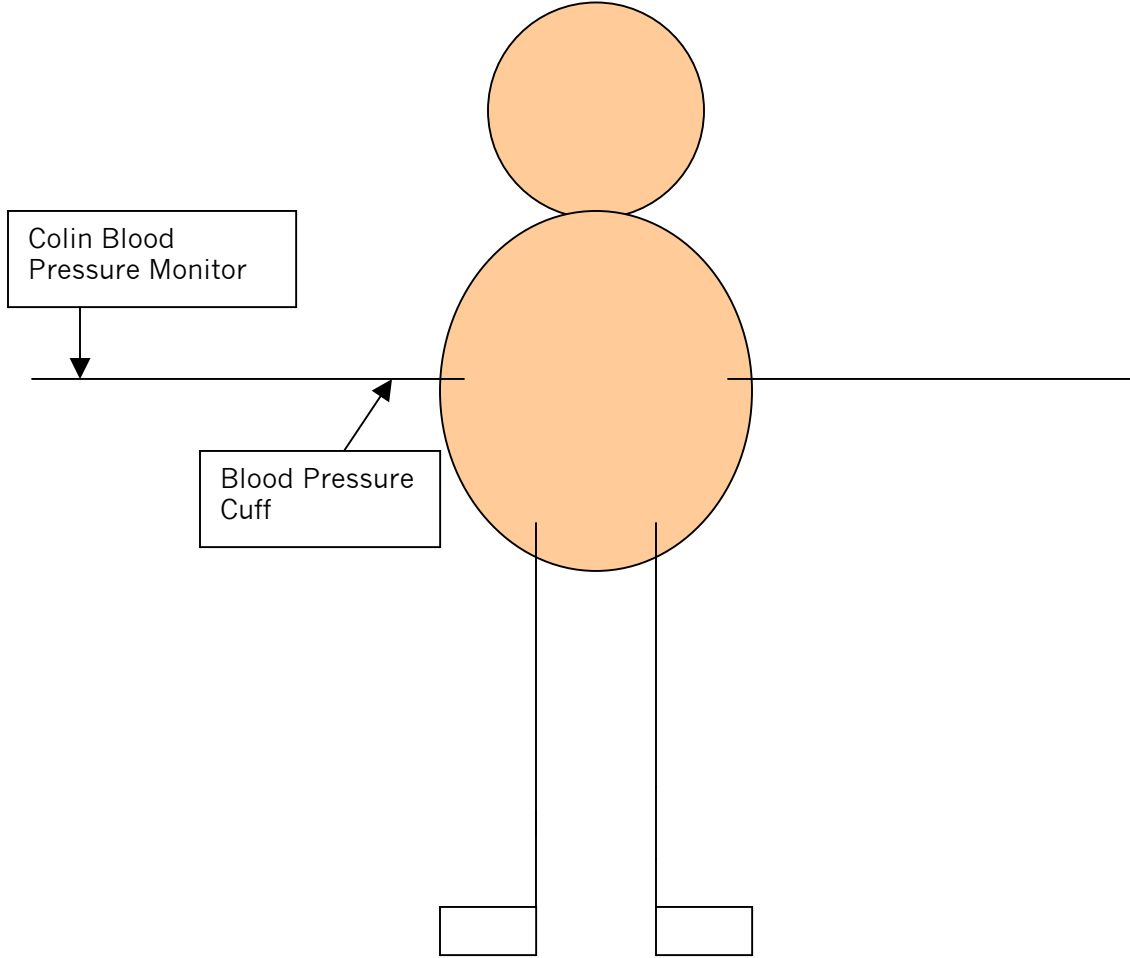
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APPENDIX: RELEVANT EXTRA MATERIAL

Figure 5:



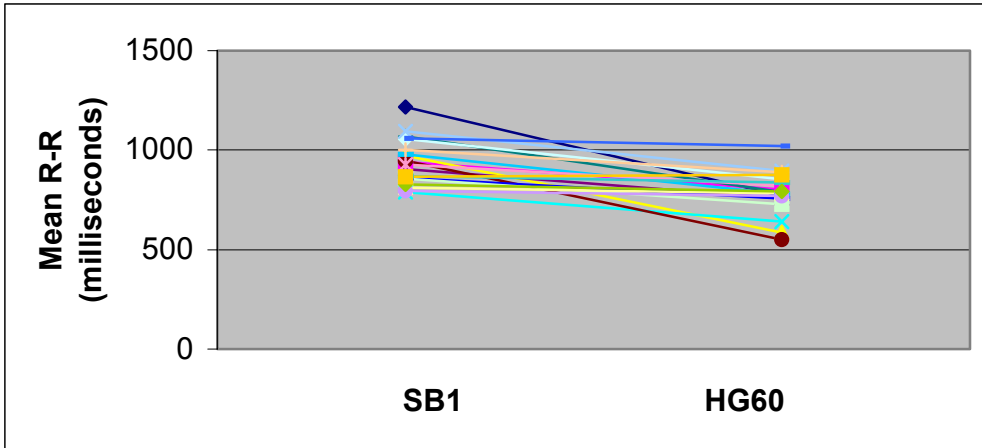


Figure 6: Individual responses for mean R-R to the conditions, n=21.

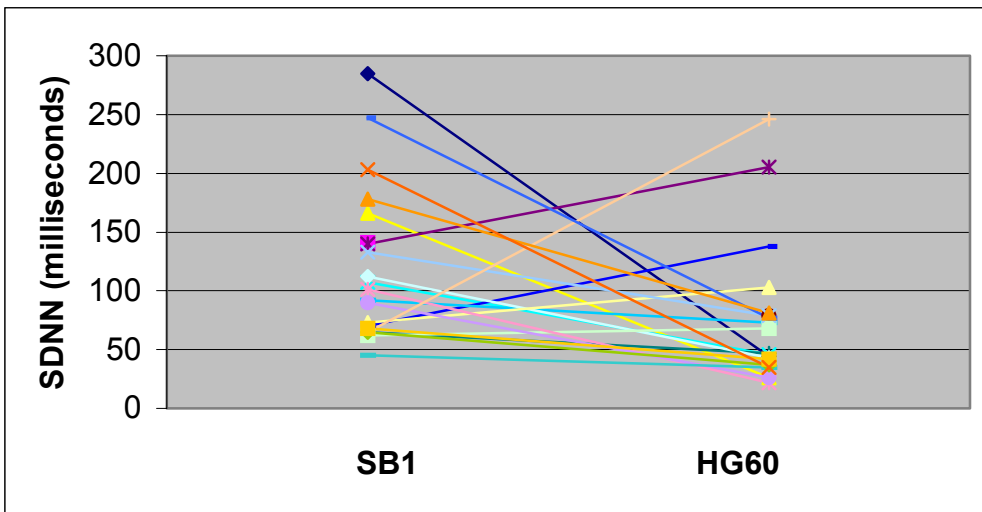


Figure 7: Individual responses for SDNN to the conditions; n=21.

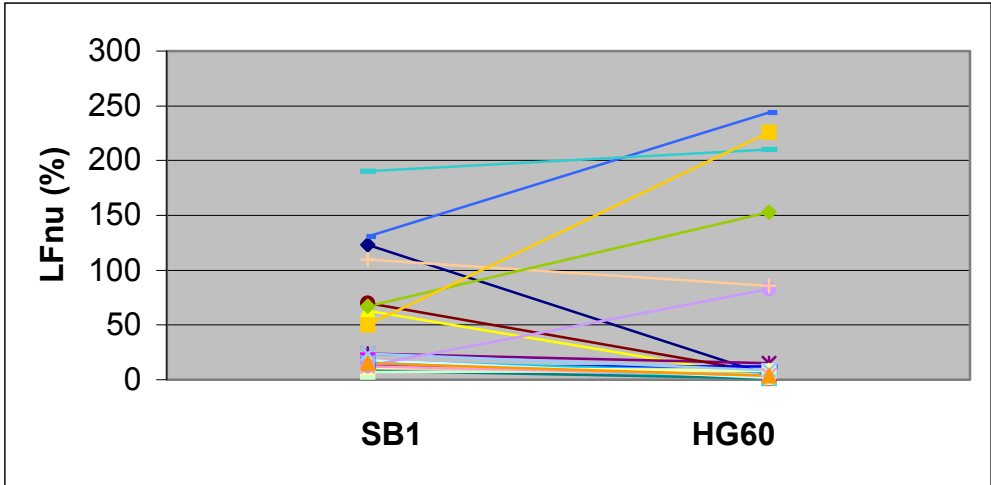


Figure 8: Individual responses for LFnu to the conditions; n=21.

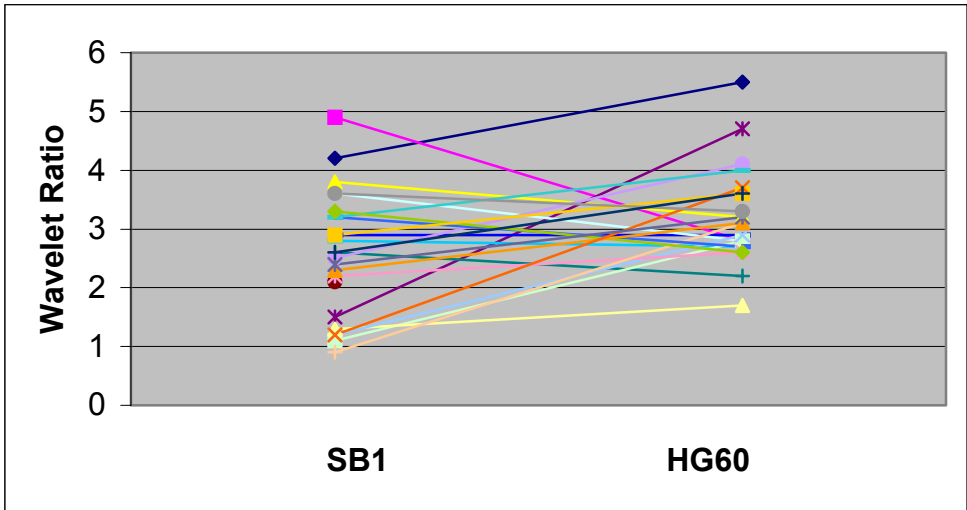


Figure 9: Individual wavelet ratios to the conditions; n=21.

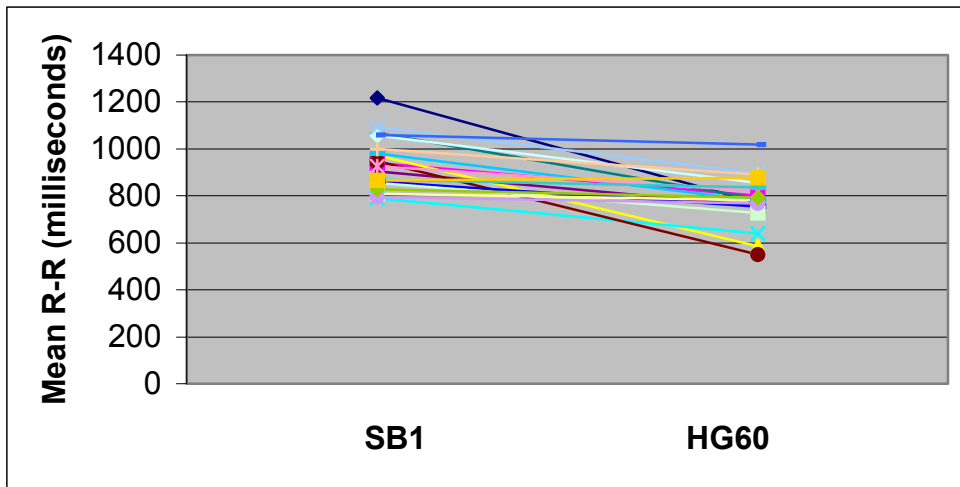


Figure 10: Individual responses for mean R-R to the conditions; n=21.

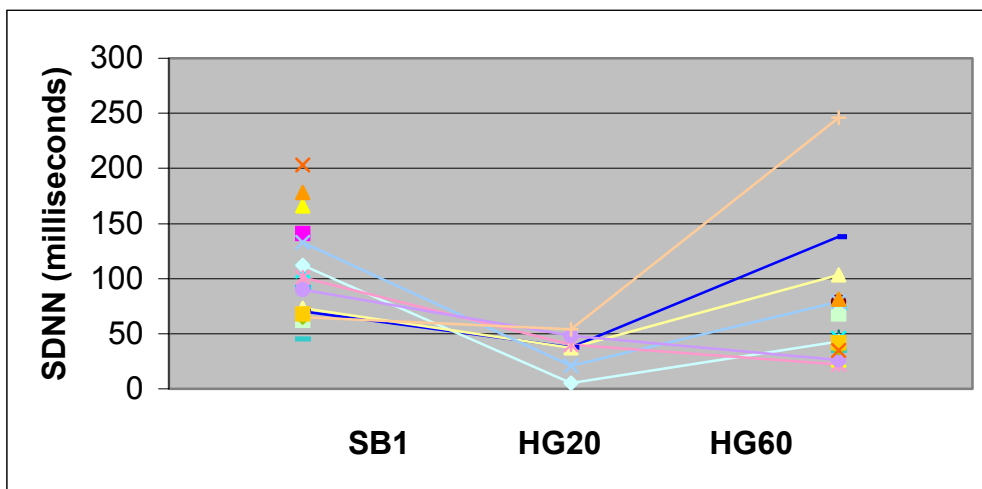


Figure 11: Individual responses for SDNN to the conditions; n=21.

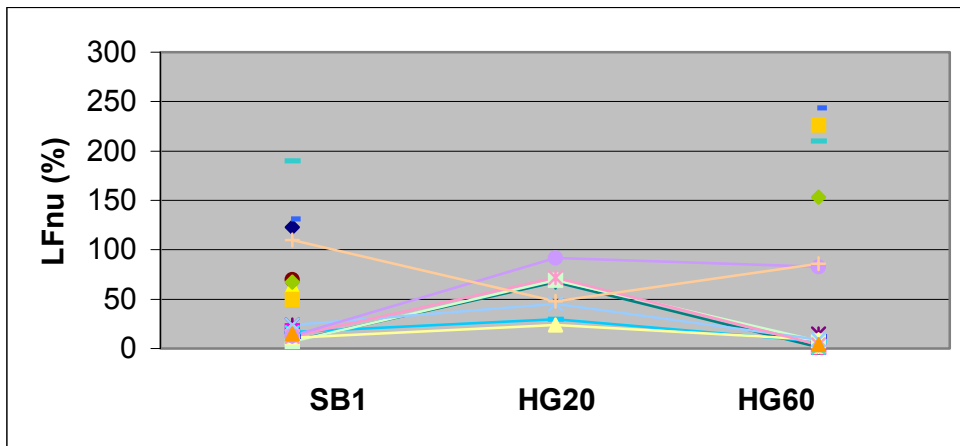


Figure 12: Individual responses for Lfnu to the conditions; n=21.

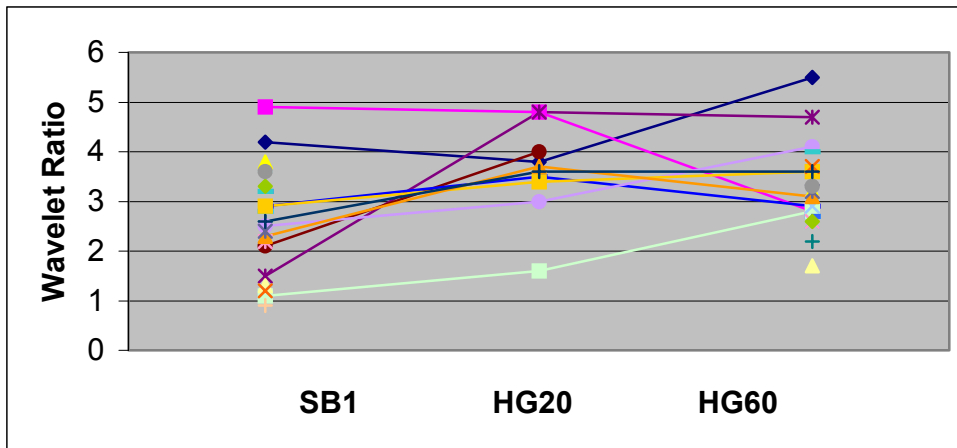


Figure 13: Individual wavelet ratios to the conditions; $p < 0.60$; n=21

VITA

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