Spring 2018

Repeatability of the heart rate variability threshold during treadmill exercise

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Repeatability of the Heart Rate Variability Threshold During Treadmill Exercise

An Honors College Project Presented to

the Faculty of the Undergraduate

College of Health and Behavioral Studies

James Madison University

by Shane Alexander Chambers

April 2018

Accepted by the faculty of the Department of Kinesiology, James Madison University, in partial fulfillment of the requirements for the Honors College.

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PUBLIC PRESENTATION

This work is accepted for presentation, in part or in full, at the Kinesiology Honors Dinner on 4/20/18.

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Acknowledgements

I would like to express gratitude to my advisor Dr. Trent Hargens for his patience and guidance through the process of the completion of this project. The assistance you provided is something that has allowed me to grow both academically and personally, and this project would not have been possible without your assistance.

I would like to thank my readers Dr. Nicholas Luden and Dr. Chris Womack for dedicating their time to assuring that this project was the highest quality possible.

I would also like to extend my thanks to Mr. Patrick Kelley for assisting with much of the data collection for this endeavor. This project was made much easier with your assistance.

I would like to finally thank the James Madison Kinesiology department for giving me the opportunity to conduct this research. The experience I've gained in the process of this project will surely benefit me in my future endeavors.

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Abstract

Purpose: Heart rate variability threshold (HRVT) is a clinical parameter used to gain insight into autonomic balance. Various studies have validated the use of cycle ergometers in determining HRVT, although the literature lacks evidence that treadmill use is a viable means of determining this threshold. We examined the repeatability of HRVT occurrence using standard deviation of normal R-R intervals (SDNN), root mean square of successive differences of continuous R-R intervals (RMSSD), and standard deviation of instantaneous beat intervals (SD1) in college aged males using treadmill exercise to see if this is a reliable method of threshold determination.

Methods: Ten healthy, college-aged males underwent two treadmill max tests while heart rate and ventilation data was obtained using a polar V-800 watch and a PARVO metabolic cart, respectively. Paired t-tests were used to compare consistency of average HRVT and VT occurrence between trials, and to find agreement of HRVT and VT occurrence together within trials.

Results: There was no difference between SDNN, RMSSD, SD1, or VT occurrence between trials one and two. There a significant difference between RMSSD and VT and SD1 and VT ($P = 0.003$ and 0.002, respectively), however there was agreement between SDNN and VT ($P = 0.06$).

Conclusion: Treadmill modality is a reliable method of finding HRVT in a population of healthy young males. HRVT and VT does not correspond on a treadmill as it has been reported to with cycle ergometry.

Keywords: Heart rate variability threshold, treadmill, reliability

Chapter I

Introduction

The heart is a vital organ which is involved in the delivery of blood to the various organs and tissues of the body. The heart has two groups of muscle cells: autorhythmic and contractile. The contractile cells are those that undergo mechanical contraction that result in atrial and ventricular contraction, which physically pumps the blood. The autorhythmic or pacemaker cells of the heart are responsible for exciting the contractile cells at a certain rate, which leads the observable phenomenon of heart rate. These autorhythmic cells are grouped into various nodes, allowing for signal transmission to be delivered to each part of the heart. The atrioventricular (AV) node is found in the interatrial septum, and the sinoatrial (SA) node is found in the right atrium. Heart rate can vary drastically dependent on various environmental or physiological conditions; this large range is the result of sympathetic and parasympathetic input from the nervous system. Sympathetic nerve fibers synapse with the SA and AV nodes to increase heart rate, and with the contractile cells of the ventricles to increase force of ventricular contraction. Parasympathetic nerve fibers synapse with the SA and AV node to slow the heart rate (1).

The autorhythmic cells send electrical impulses to the contractile cells to initiate contraction in different regions of the heart. These electrical impulses can be represented graphically with an electrocardiogram (EKG), and can be indicative of the mechanical work that is being done in the heart at a given time. The P-wave in an EKG graph represents atrial depolarization, which translates to atrial contraction. The QRS complex is an easily identifiable spike that represents ventricular depolarization, indicative of ventricular contraction. The last major event, the T-wave, represents ventricular repolarization (2). The largest, most identifiable

part of the QRS complex is the R-wave. The distance between two R-waves (two ventricular contractions) is the R-R interval. The R-R interval can be used to calculate heart rate, but can also be used for other biomarkers that can have clinical and sports-related significance. One of these biomarkers is known as heart rate variability. Heart rate variability if the deviation of time between R-R intervals, or heart beats. These deviations are the result of regulatory mechanisms of the body that control heart rate, and the frequency that these stimuli are applied to the heart. The heart is extensively regulated by both the sympathetic and parasympathetic components of the autonomic nervous system. The sympathetic nervous system releases catecholamines, which increase the rate of SA node depolarization, while the parasympathetic nervous system releases acetylcholine which slows the rate of SA node depolarization (1). The frequency that these inputs regulate heart rate create the phenomena of heart rate variability.

Sympathetic and parasympathetic input innervate the heart at different frequencies. These bands of frequency can be divided into ultra-low frequencies (ULF), thought to be the product of the circadian rhythm; very low frequencies (VLF), thought to be the product of temperature and endocrine regulation; low frequencies (LF), believed to be the product of both sympathetic and parasympathetic input; and high frequencies (HF), thought to be due to parasympathetic input and respiratory sinus arrhythmia (3, 4). For the purposes of this study, high and low frequency bands were emphasized. The HF band is found between .15 and .4 Hz, while the LF band is found between .04 and .15 Hz. Examining the presence or absence of these frequency bands via power spectral analysis is known as a frequency-domain analysis of HRV. Alternative ways to measure HRV are time-domain analysis. SDNN (the standard deviation of normal R-R intervals) is one method of time-domain analysis, which focuses on the standard deviation of R-R intervals.

RMSSD (root mean square of successive differences) is another time domain method of HRV analysis which is primarily used for short term HRV recordings (5).

HRV is generally high at rest in healthy individuals, and decreases with increasing exercise intensity because of increased sympathetic stimulation and parasympathetic withdrawal. HRV can also decrease due to chronic disease, increasing age, psychological stress, and other variables that will be discussed further. Heart rate variability is known to be the product of the balance between sympathetic and parasympathetic inputs, with parasympathetic input producing greater beat to beat variations than its counterpart due to the immediate effect of parasympathetic neurotransmitters versus the relatively tonic effect of sympathetic neurotransmitters (6, 2). Bonnemeier et al. found that HRV is associated with an individual's circadian rhythm, with peak vagal activity and higher levels of HRV occurring at night (7). Bonnemeier et al. also found that increasing age is associated with decreased HRV peaks during the circadian rhythm, presumably due to a decrease in parasympathetic activity at night. However, the circadian-dependent HRV cycles where HRV increases at night are based on reduced levels of sympathetic input during the night, not due to an increase in vagal tone (8). Not only do demographics and health status influence HRV, but emotional status does as well. Thayer et al. found that amygdala activity, a regulatory center of the brain associated with fear and threat perception also plays a role in controlling HRV, suggesting a linkage between stress and HRV presumably due to autonomic influence (9). The emotional stress-HRV relationship is further supported by Dikecligil et al, who found that emotional stress was directly related to a decrease in HRV (10). These principles are important to consider when assessing HRV to prevent transient stress from being misinterpreted as poor health.

Heart rate variability is an important biomarker which gives insight into an individual's autonomic functioning. This variable can be used clinically, as well as give insight into one's stress and mental health. HRV is a minimally invasive, low cost procedure that can assist in detecting and predicting cardiovascular disease, the leading killer in the United States. Tsuji found that in 2501 otherwise healthy men and women, HRV was a strong predictor of future coronary disease or congestive heart failure (11). Tsuji et al. also found in a previous study that in an elderly population, low levels of HRV is indicative of high levels of all-cause mortality, further bolstering the clinical significance of HRV (12). Seeing as the relationship between HRV and age is known, Umetai et al. supplemented this knowledge with a study that showed that not only does HRV decrease with age, but falls into a range that may classify otherwise healthy individuals greater than 65 years old to be at a heightened risk of all-cause mortality (13). However, HRV is susceptible to change with an exercise intervention. Previous literature supports the notion that resting HRV can increase with physical activity, presumably due to an increase in parasympathetic activity which increases the magnitude of respiratory-sinus induced HRV (14). Bär et al. demonstrated that an exercise intervention not only increased resting HRV levels, but also increases vagal activity at a given intensity during exercise (15). Stein et al. also showed that in a group of older individuals, an exercise intervention lead to higher levels of resting HRV than a non-intervention group (16).

Heart rate variability is a tool that can not only be used clinically, but may also give insight into other physiological processes associated with athletic performance. HRV decreases as exercise intensity increases due to sympathetic increase and vagal withdraw, but there is a

threshold that occurs when vagal activity is totally withdrawn, and the R-R interval variability remains constant. This occurrence is termed the heart rate variability threshold (HRVT). Dourado et al. stated that at roughly 50-60% of VO_{2max}, vagal activity is totally withdrawn, and all resulting changes in HRV is the result of varying levels of sympathetic stimulation (17). HRVT is also commonly found around the same intensity as lactate threshold (LT) and ventilatory threshold (VT) (18). VT is marked by a disproportionate increase in ventilation (VE) compared to VO_2 (19). Beaver et al. determined that anaerobic threshold is indicative of the bicarbonate buffering system maintaining a steady state blood pH, which generally occurs during the transition to primarily anaerobic metabolism. The bicarbonate buffering system causes a disproportionate increase in VCO_2 relative to VO_2, which is an observable phenomenon known as the ventilatory threshold or gas exchange threshold (GET) (20). LT occurs at roughly 60% of an untrained individual's VO_{2max} when lactate production exceeds lactate clearance (21). One contributing factor to LT is the increase of plasma catecholamine concentration as both exercise duration and intensity increases (22). These neurotransmitters serve to decrease HRV as well as increase the production of lactate by stimulating glycolysis, as well as decreasing the blood flow to the liver, limiting lactate clearance via gluconeogenesis (23). High levels of lactate are registered by chemoreceptors in the carotid artery, stimulating an increase in V_E which may be a contributing factor as to why VT and LT often happen around the same intensity (24, 25). Several studies have shown the relationship for HRVT and these two thresholds. Anosov et al. found that the HRVT also occurred at the same intensity as the anaerobic threshold, or the shift to primarily anaerobic metabolism indicated by LT and VT (26). Karapetian et al. found that HRVT occurred at roughly the same intensity as LT (18). Additionally, Cunha et al. studied the relationship between HRVT and VT, and found that there was a strong relationship between

HRVT and the VT in both running and cycling modalities (27). The use of HRVT to detect LT and VT adds convenience for both the practitioner and client in detecting these important physiological occurrences. LT and VT are two variables that are often tested for, and give insight into the conditioning status of the athlete. LT is a widely-accepted biomarker which is indicative of athletic performance (28), and has shown to be the determining factor in the intensity which marathon runners perform at (29). Powers et al. found that endurance performance and VT have a strong correlation, indicating the use of VT in athletic training contexts (30). In a cycling context, Amann et al. also found the VT to be a good indicator of performance, further supporting the value of determining VT (19). Seeing as closed circuit indirect calorimetry is needed to directly measure VT and measurement of LT is a generally invasive procedure, the traditional methods of detecting the anaerobic threshold are uncomfortable procedures for the subject and can be costly for the practitioner. The ability to use HRVT rather than blood draws or metabolic carts to assess VT poses benefits for all parties.

Heart rate variability threshold testing is a relatively novel procedure, and has not been extensively conducted in the literature in all exercise modalities. Generally, HRVT experiments are conducted via means of cycle ergometer (31, 32, 33). Several studies have examined HRVT using other alternative exercise protocols, but very few have been strictly running protocols. Dourado et al. collected HRVT data on a group of older individuals (greater than 40 years old) during an incremental shuttle test, but the trials were not repeated to ensure reliability (17). Mendia-Iztueta et al. conducted a HRVT study designed to mimic the mechanics of cross country skiing on a motorized treadmill, but this method was not a running-based protocol (34). Paschoal et al. found a correspondence between VT and HRVT in both obese and non-obese pre-

adolescents, but this study did not assess the repeatability of the HRVT-VT correspondence nor do their findings represent healthy young adults (35). Cunha et al. also found high correlation between VT and HRVT on a motorized treadmill, but their findings were not repeated to assess inter-test reliability (27). This experiment attempted to fill these gaps by assessing the repeatability of HRVT tests on a treadmill. The use of HRVT to assess LT and VT on a motorized treadmill is a noninvasive procedure that minimizes subject discomfort while also giving additional insight into their sympathetic/parasympathetic regulation. A treadmill procedure is the ideal modality to determine this with, seeing as most individuals are more familiarized with walking mechanics than they are with cycling mechanics, providing more accurate and applicable results. The purpose of this study was to determine whether HRVT occurs at the same workload, VO2, VE, and HR in the same subject on different days using a motorized treadmill based maximal exercise test. This study also aimed to determine whether there is a relationship between the workloads, VO2 values, HR, and V_E at which HRVT and VT occur using a treadmill modality, as their relationship has been demonstrated in cycling contexts.

Chapter II

Methodology

Subjects

10 healthy males age 19-21 from the James Madison University and Rockingham county area were recruited for this study. Only males were recruited to exclude possible bias induced from skewed HRV data because of the female menstrual cycle (24). Exclusion criteria for participation included musculoskeletal, metabolic, and cardiovascular disorders. Additional exclusion criteria included regular use of any medication, due to the potential impact on heart rate or HRV. Subjects were recruited by word of mouth. Subjects were instructed to abstain from caffeine and food for 12 and 4 hours prior to the trial, respectively.

Testing Procedure

The height and weight of each subject was recorded prior to the exercise testing procedures. Each individual participated in two maximal graded exercise tests with a minimum of 48 hours between each trial. Each individual completed both of their respective trials at the same time of day as to avoid diurnal variations of HRV. A standardized treadmill protocol with 3 minute stages was used for each graded exercise test. Heart rate was measured continuously for the entirety of each exercise trial. Each subject progressed through the stages of the protocol until volitional fatigue was reached and the subject willfully terminated the trial (36). A metabolic measurement system utilizing a high efficiency mixing chamber (TrueOne 2400, Parvo Medics, Sandy UT) was used to measure expired CO_2 per minute (VCO_2) and consumed O_2 per minute (VO2). Expired gases were also used to determine VT, which was evaluated as the graphical point where VCO_2 increases disproportionately to VO_2, as assessed automatically by the PARVO metabolic cart. Heart rate and R-R data were measured continuously throughout each test with a

polar v800 watch (Polar Electro Inc., Lake Success, NY). After each test, R-R data was downloaded to a computer, and analyzed for HRV variables via Kubios HRV software (Version 3.0, Kuopio, Finland).

HRVT measurement

For each trial, the RMSSD of each respective intensity was plotted for the last two minutes of HRV data of each stage of the protocol, and visual inspection was used to determine HRVT. The intensity at which there was no significant decline in HRV in subsequent intensities was assumed to be the HRVT. This inflection point was identified by two separate evaluators to avoid bias in the case of ambiguity. If the two evaluators disagreed on the inflection point, a third investigator decided which of the two proposed points was to be considered HRVT.

VT Measurement

VT was determined automatically using the proprietary software of the Parvo metabolic system using a V-slope method.

Statistical Analysis

To determine the repeatability of the HRVT across the treadmill trials, as well as to assess the relationship between the HRVT and the VT, paired sample t-tests were used to test mean difference in the VO2 at which the HRVT and the VT occurs.

Chapter 3

Manuscript

Repeatability of Heart Rate Variability Threshold During Treadmill Exercise

Purpose: Heart rate variability threshold (HRVT) is a clinical parameter used to gain insight into autonomic balance. Various studies have validated the use of cycle ergometers in determining HRVT, although the literature lacks evidence that treadmill use is a viable means of determining this threshold. We examined the repeatability of HRVT occurrence using standard deviation of normal R-R intervals (SDNN), root mean square of successive differences of continuous R-R intervals (RMSSD), and standard deviation of instantaneous beat intervals (SD1) in college aged males using treadmill exercise to see if this is a reliable method of threshold determination.

Methods: Ten healthy, college-aged males underwent two treadmill max tests while heart rate and ventilation data was obtained using a polar V-800 watch and a PARVO metabolic cart, respectively. Paired t-tests were used to compare consistency of average HRVT and VT occurrence between trials, and to find agreement of HRVT and VT occurrence together within trials.

Results: There was no difference between SDNN, RMSSD, SD1, or VT occurrence between trials one and two. There a significant difference between RMSSD and VT and SD1 and VT (P = 0.003 and 0.002, respectively), however there was agreement between SDNN and VT (P = 0.06).

Conclusion: Treadmill modality is a reliable method of finding HRVT in a population of healthy young males. HRVT and VT does not correspond on a treadmill as it has been reported to with cycle ergometry.

Keywords: Heart rate variability threshold, treadmill, reliability

Introduction

Heart rate variability (HRV) is a biomarker that can be used in athletic and clinical settings. HRV involves analysis of the inter-beat variability to give insight into an individual's autonomic input, with sympathetic tone leading to decreased HRV, and vagal tone leading to the opposite (1). Accordingly, HRV is generally high at rest with healthy individuals, and decreases with exercise intensity due to vagal withdraw and increased sympathetic input (1). As exercise intensity increases and HRV decreases, a threshold is eventually met where inter-beat variability no longer decreases with increasing exercise intensity. This is known as the heart rate variability threshold (HRVT) and has been shown to occur at approximately the same intensity as lactate threshold (LT) and ventilatory threshold (VT) (2). Ventilatory threshold is the intensity at which ventilation increases disproportionately to oxygen consumption (VO_2) (3). At this intensity, the bicarbonate buffering system is generating more CO_2 by neutralizing exercise-provoked H+. This induces hypercapnia, which increases V_E but not VO_2, an intensity that is reached at around 60% of VO_2 for untrained individuals (4, 5). VT is a threshold commonly used in clinical and research settings to give insight into conditioning status. This is supported by the work of Powers et. al showing that ventilatory threshold is associated with better running performance in trained men (21). Similar work by Amann et. al further validate the value of VT, showing that it is a better predictive tool than LT when considering average wattage in a cycling time trial (3). HRVT is a non-invasive procedure which, being linked to the other thresholds previously mentioned, may be a proxy for VT and LT, which require more resources and subject discomfort to collect.

The process of acquiring HRVT generally requires the subject to undergo a graded maximal exercise test. HRVT has primarily been tested during cycle ergometry (6, 7, 8). However various other modalities such as incremental shuttle tests, and mock-cross country skiing on a treadmill have been employed (1, 9). To our knowledge, the only prior study that used treadmill exercise to assess HRVT was used to detect HRVT in obese and non-obese adolescents. This study found that the HRVT occurred at a higher relative VO_2 in non-obese adolescents, although these results were not assessed for repeatability and are not representative of the general population (10). There is a lack of information regarding the reliability of treadmill use for HRVT detection, a gap that this study aims to fill. Although ergometer use is verified and frequently used for HRVT detection, this modality may introduce bias. Cycle ergometry generally leads to lower VO_{2max} values due to local fatigue induced by decreased muscle mass, and most subjects are generally more comfortable walking or running than on a stationary bike (27, 12).

HRVT is known to be a valuable tool due to its previously mentioned relationship with VT and LT. As a result of this relationship, HRVT can be used to give indirect insight to these thresholds; a much more cost efficient method if metabolic carts and lactate analyzers are not accessible. Ideally, the means of detecting the threshold should be a motion that is not new to the individual, and most subjects will be comfortable walking or running. Therefore, the primary purpose of this study was to examine the within-subject repeatability of HRVT detection on a treadmill. A secondary purpose was to examine the relationship of the HRVT to the VT, to determine if they occur at the same exercise intensity.

Materials and Methods

Subjects

10 healthy males from the James Madison University community were recruited to undergo 2 maximal exercise tests (table 1). Females were excluded to avoid bias due to the menstrual cycle's effect on HRV (11). Exclusion criteria included metabolic and cardiovascular disorders and regular use of medication due to their effect on HRV (13, 14, 15). Individuals with musculoskeletal disorders were also excluded because these individuals may be physically limited and unable to complete a true max test via treadmill modality.

Testing Procedure

Subject demographics were recorded for each individual (height, weight, age). Subject height was measured from standing using a stadiometer, and weight was recorded in kg using a Pelouze model 4040 scale. Each subject underwent two maximal graded exercise tests, separated by a minimum of 48 hours. To avoid diurnal variation of HRV, the time of day each subject completed their test was also kept constant. A standardized treadmill protocol with 3 minute stages was used for each graded exercise test. Ventilation data (expired O_2 and CO_2) was collected through the entirety of the test, and was later used to derive VT. Heart rate and R-R data was also monitored through the entirety of each test using a polar RS800CX watch (Polar Electro Inc., Lake Success, NY). Kubios HRV software was used to analyze the HRV data (Version 3.0, Kuopio, Finland).

HRVT determination

For each trial, three HRV parameters were used: standard deviation of normal R-R intervals (SDNN), root mean square of successive differences of continuous R-R intervals (RMSSD), and standard deviation of instantaneous beat intervals (SD1).

Three graphs were generated for each test (6 total per subject), each of which showed the respective parameter in the last minute of each stage of the max protocol, which was presumed to be steady state. These graphs were interpreted by two separate individuals, each of which selected which point they believed to represent HRVT. If the interpreters did not select the same point, a third individual determined which of the two points was to be identified as the HRVT. If the three individuals all selected different points for VT or on of the HRVT indices, the subject's data was not used for the analysis in question.

VT Measurement

The VT of each trial was detected automatically by a PARVO metabolic cart using the V-slope method (23).

Statistical Analysis

Paired t-tests were used to test mean difference in the intensity at which HRVT (as indicated by RMSSD, SD1, and SDNN) and VT occurred. When comparing the incidence of HRVT parameters, averaged values from trial one were compared to averaged values of trial two. When comparing the incidence of HRVT parameters to the incidence of VT, HRVT values of trial one and two were averaged for each respective variable, and then compared. Bland-Altman plots were utilized to evaluate agreement between HRVT values (RMSSD, SDNN, and SD1 derived) between each TM trial, and to evaluate agreement between HRVT and VT.

Results

Trial 1 to Trial 2 Comparison

Maximal testing information is presented in Table 2. There were no differences in VO_{2max}, V_E/VCO_2 slope, or respiratory exchange ratio between trial 1 and trial 2 (P = 0.24, 0.56, and 0.32, respectively). Maximal heart rate was significantly lower in trial 2 compared to trial 1

(P = 0.03). Several HRVT graphs were omitted from analysis due to investigator disagreement of inflection points. Data from two individuals was omitted for RMSSD graphs (N=8), data for three individuals were omitted for SD1 analysis (N=7), while no data was omitted for SDNN analysis (table 3). No significant differences were found between the VO_2 at which the HRVT occurred for RMSSD (P = 0.37), SDNN (P = 0.55), and SD1 (P = 0.83) between trials 1 and 2 (table 3). Additionally, there was no significant difference in the VO_2 at which VT occurred between trials 1 and 2 (P = 0.33). Bland-Altman analysis revealed good agreement between trials for the HRVT derived from SD1 (Figure 1), SDNN (Figure 2), and RMSSD (Figure 3). Bland-Altman analysis also showed good agreement between trials for the VO_2 at which VT occurred (Figure 4).

HRVT to VT comparison

Paired t-tests revealed that the VO_2 at which the HRVT occurred for RMSSD and SD1 was lower than the VO_2 at which the VT occurred, while the VO_2 at which the HRVT occurred for SDNN did not differ from the VT (P = 0.06) (Table 4). Bland-Altman analysis revealed similar findings in that the RMSSD (Figure 5) and SD1 (Figure 6) comparisons to VT did not show agreement (P = 0.003 and 0.002 for RMSSD and SD1, respectively), while the SDNN to VT comparison (Figure 7) did show agreement (P = 0.06).

Discussion

The results obtained from this study demonstrate there was no difference in HRVT occurrence between trials one and two. Previously, HRVT detection has been determined primarily by means of cycle ergometry (6, 7, 8). Demonstrating that this threshold can be consistently detected by treadmill testing is a finding that may allow HRVT detection to be a more accessible parameter to those without the resources to conduct a cycle ergometer max test.

The disparities between RMSSD vs. VT and SD1 vs. VT disagrees with previous literature where HRVT and VT were demonstrated to occur at same intensity (2,19,20, 2). A number of factors may explain the contradictory findings. For instance, Karapetian et al. recruited a subject base that was mostly female (15 females, 9 males), while our study examined only males (2). It is possible that the influence of the female menstrual cycle on HRV may have introduced some bias in their work, since it has been shown that the autonomic balance which dictates HRV can be shifted dependent on the phase of the menstrual cycle, which Karapetian's study did not control for (2, 11). Additionally, they used a visually determined V-slope method of identifying VT occurrence, while our study used a PARVO metabolic cart to automatically detect VT, possibly altering the identification of the threshold (2).

Finally, the group of subjects recruited for our study was relatively trained, having a mean VO_{2max} of 53.6 mL/kg/min, which is classified as being between the 85^{th} and 90^{th} percentile of age matched VO_2 values (15). The males used in Karapetian's study had an average VO_{2max} of 38.8 mL/kg/min, which falls between the 20^{th} and 25^{th} percentile (2,15). This is a similar case for the results obtained in Anosov's work, which found that the HRVT and VT occurred at the same point in a group of 13 females and 9 males. These individuals were all untrained, and were reported to exercise less than four hours per week (19). The detraining of these subjects and the mix of males and females in the subject pool may play a role in the difference in of results seen in their study and ours in terms of the occurrence of HRVT and VT. Finally, Cunha et al. also demonstrated that VT and HRVT occurred at the same intensity in 16 apparently healthy college aged males (20). In this study, similar methods as the other two

previously mentioned studies were employed: a manual V-slope detection method and a group of individuals less trained than those used in the current study. The average VO_2max of the males in Cunha's study was found to be 48.3 mL/kg/min, which the ACSM guidelines categorizes to be between the 70^{th}-75^{th} percentile (20,15).

We suspect that the most likely explanation for the uncoupling of HRVT and VT is the training status/fitness of the current sample. While there no established mechanism for this, HRV is used to give insight into autonomic input at rest, and is widely accepted as a trainable phenomenon (24, 25, 26). While no previous literature has examined the trainability of HRVT, which is the point at which HRV ceases with increasing exercise intensity, vagal input is totally withdrawn at roughly 60% exercise intensity, and increasing heart rate after this point is the result of increased sympathetic input which would also induce a change in HRV (1). Our results suggest that although HRV is a trainable phenomenon, HRVT may not be. Given that HRV adaptations to training indicates a shift in autonomic balance, it would be reasonable to assume that this training induced shift in autonomic balance would in turn influence the occurrence of HRVT. This may not be the case however, seeing as we demonstrate HRVT occurred at a lower intensity than VT, which was most likely trained to a higher intensity. The average HRVT and VT of Cunha's work was 56±11% and 53±7% of VO_{2max}, respectively, using SD1 to assess HRVT (20). Their HRVT results are consistent with the results of this study, where SD1 was detected at roughly 54±9% of VO_{2max} (table 3). However, our findings differ in terms of VT occurrence, where VT was detected at roughly 68±16% of VO_{2max} (table 3). This further supports that HRVT may not be a trainable parameter, and our cohort may have had higher VT occurrences compared to groups in similar studies. Seeing as HRVT is the point at which no

HRV is observed with increased intensity, and it is known that sympathetic input is still increasing past the HRVT to increase heart rate, it may be plausible that HRVT is the result of the structure of the pacemaker cells themselves, and that training does not alter their makeup in a way that could be reflected via HRVT. To date, no mechanism has been proposed to explain the occurrence of HRVT, so further studies are necessary to test this hypothesis. We propose that a training intervention be administered to a subject demographic similar to that used in this study to determine the trainability of HRVT vs. the trainability of VT.

No difference was found between the VO_{2max} achieved between trial one and two, as expected based on previous studies (12). Maximal heart rate was found to be significantly different between trials one and two ($P < .05$). However, it has been predetermined that max heart rate can vary by 10 beats per minute, and our average max heart rate for trial 1 and 2 falls within this range (16). No significant difference was found between the VE/VCO_2 slope nor the VT between trials one and two, further indicating that there was no change in the training status of these individuals between trials (17, 18). Possible limitations to this study include the method of VT detection previously mentioned and the low number of subjects recruited. Some analysis had to be completed on as little as seven subjects, which is not optimal when attempting to represent a population.

To summarize, treadmill modality is a reliable measure that can detect HRVT in a group of college aged males. However, it appears as though HRVT and VT do not occur at the same intensity, an observation that cannot be attributed to any one variable. The exercise modality used to detect HRVT may be the cause of observation, or this may be the effect of having a

trained population. Further work is needed to differentiate between these two explanations. Future work should be directed towards investigating the degree of trainability of HRVT, and the effect of exercise modality on the occurrence of HRVT and VT.

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Table 1. Subject Characteristics.

Characteristic	Mean ± Standard Deviation
Age (year)	20.5±.7
Height (cm)	174.1±7.1
Weight (kg)	78.4±6.5
BMI (kg/m^2)	25.9±2.2
VO_2 max (mL/kg/min) (n=10)	53.6±6.1

BMI = body mass index; VO_2 = oxygen consumption rate

Table 2. Maximal Exercise Test Data

	Trial 1 (Average ± Standard Deviation)	Trial 2 (Average ± Standard Deviation)	P Value
HRmax (bpm)	191.1±8.7	187.8±9.3	.03*
VO_2max (mL/kg/min)	53.3±6.2	54.1±6.4	0.24
VE/VCO_2 slope	29.3±4.6	29.0±3.9	0.56
RERmax (L/min)	1.1±.04	1.1±.05	0.32

*Correlation is significant ($p < 0.05$)
HR = heart rate; VO_2 = oxygen consumption rate; V_E = ventilitory equivalent; VCO_2 = carbon dioxide production; RER = respiratory exchange ratio

Table 3. HRVT and VT data for Trials 1 and 2

	Trial 1	Trial 2	P value
RMSSD HRVT (L/min)	2.4 ± .6	2.2 ± 0.3	0.37
RMSSD HRVT (% max) (n=8)	58.5 ± 10.3	53.5 ± 8.6	0.3
SDNN HRVT (L/min)	2.8 ± 0.5	2.7 ± 0.5	0.55
SDNN HRVT (% max)	69.0 ± 9.8	65.9 ± 7.4	0.48
SD1 HRVT (L/min)	2.4 ± 0.6	2.3 ± 0.6	0.83
SD1 HRVT (% max)	55.5 ± 9.8	53 ± 9.1	0.72
VT (L/min)	3.0 ± .3	3.1 ± .3	0.33
VT (% max)	68.0 ± 18	69.3 ± 14.4	0.86

RMSSD = root mean square of successive differences of continuous R-R intervals; HRVT = heart rate variability threshold; SDNN = standard deviation of normal R-R intervals; SD1 = standard deviation of instantaneous beat intervals; VT = ventilatory threshold

Table 4. HRVT and VT Comparison Data

Pair	Parameter	Mean	P-Value
RMSSD vs. VT	Mean RMSSD	2.3 ±.3	0.003*
	Mean VT	3.0±.3	
SDNN vs. VT	Mean SDNN	2.8±.5	0.06
	Mean VT	3.0±.3	
SD1 vs. VT	Mean SD1	2.3±.5	0.002*
	Mean VT	3.1±.3	

RMSSD = root mean square of successive differences of continuous R-R intervals; SDNN = standard deviation of normal R-R intervals; SD1 = standard deviation of instantaneous beat intervals; VT = ventilatory threshold

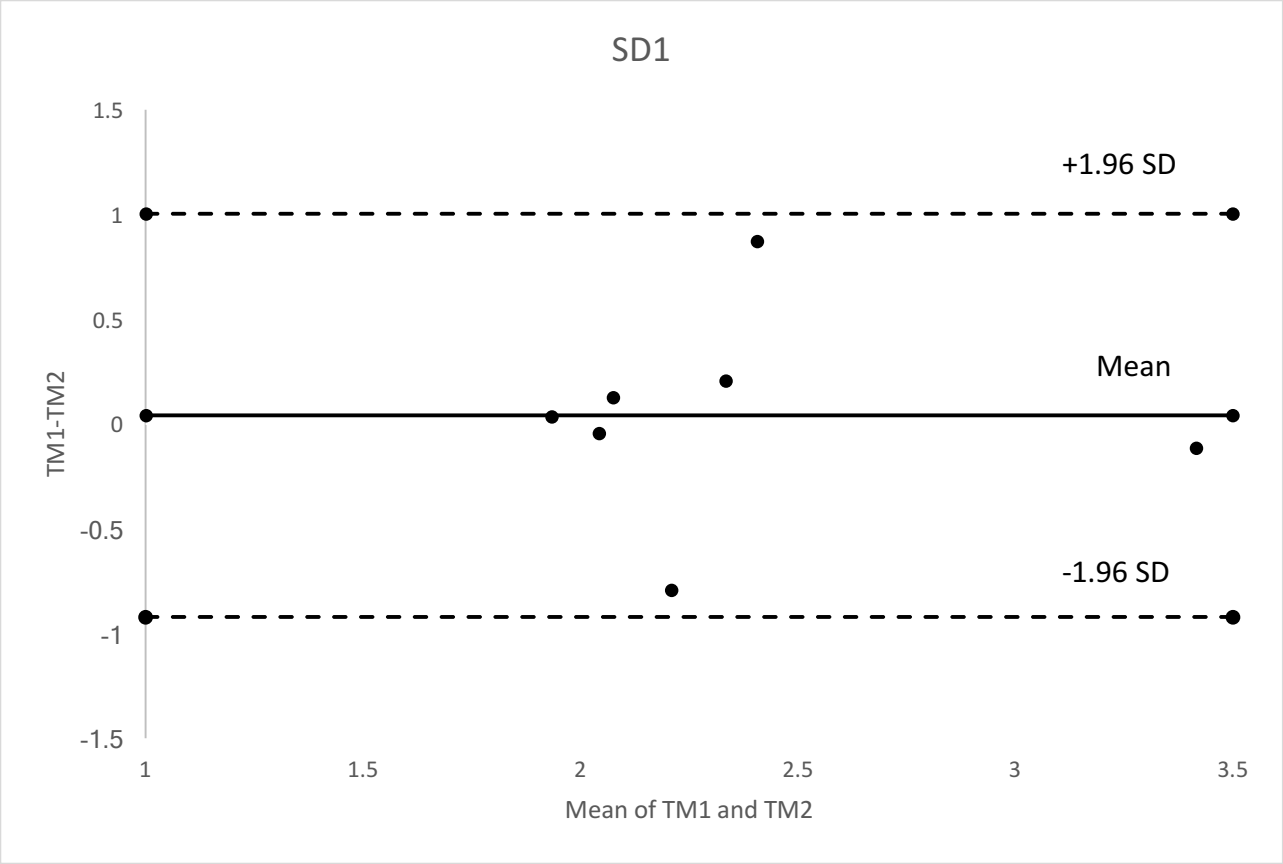

Figure 1. Bland-Altman displaying the average SD1 occurrence between trials one and two versus the difference between the two trials for each subject. Dashed lines represent 1.96 standard deviations from the mean indicated by the solid black line.

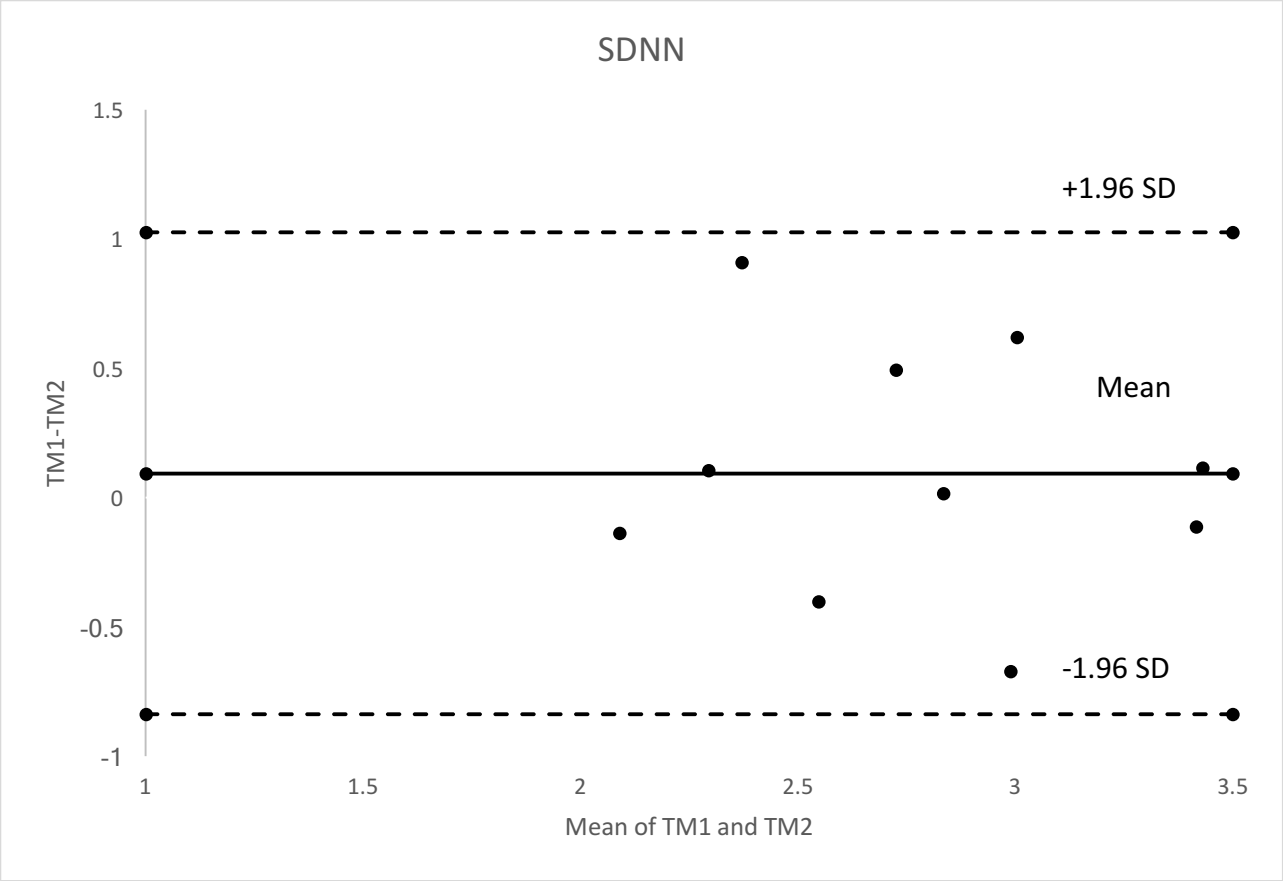

Figure 2. Bland-Altman displaying the average SDNN occurrence between trials one and two versus the difference between the two trials for each subject. Dashed lines represent 1.96 standard deviations from the mean indicated by the solid black line.

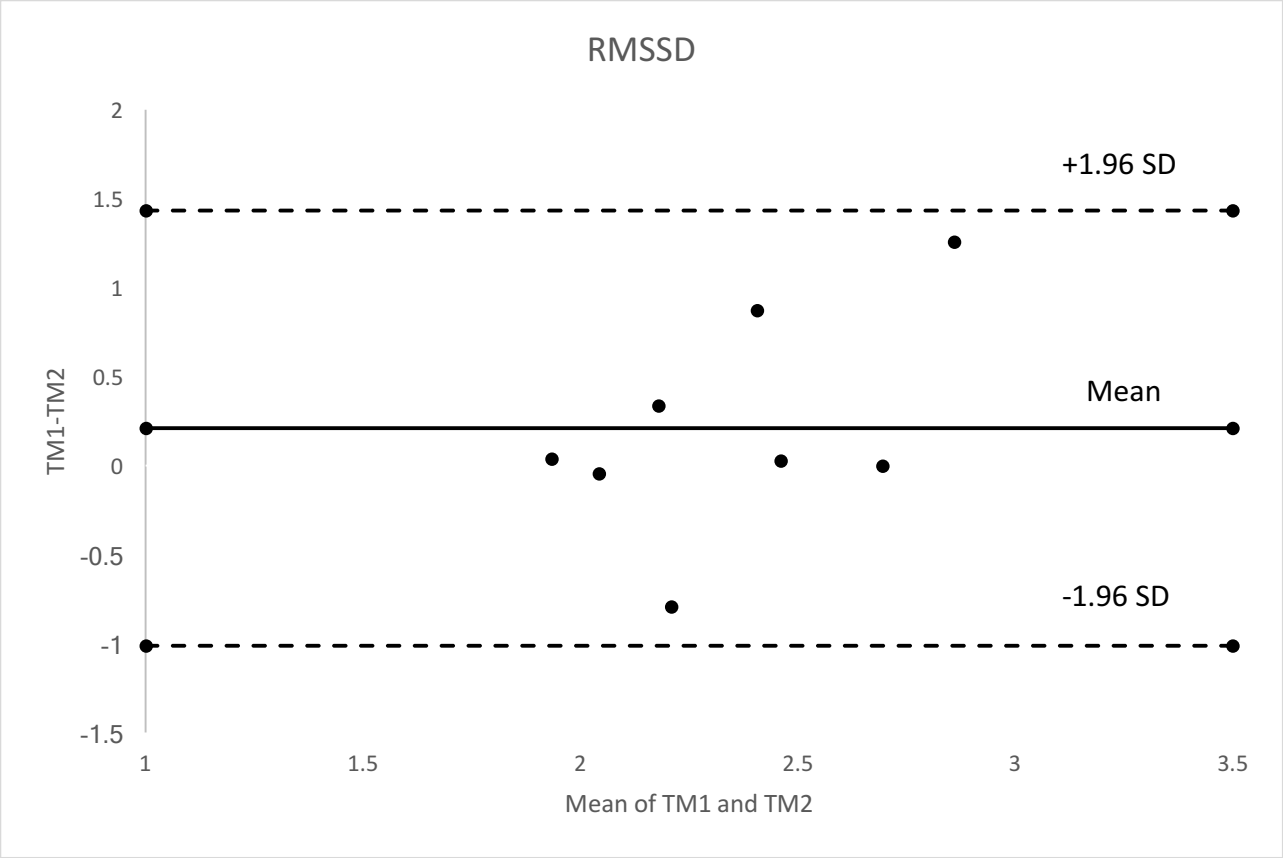

Figure 3. Bland-Altman displaying the average RMSSD occurrence between trials one and two versus the difference between the two trials for each subject. Dashed lines represent 1.96 standard deviations from the mean indicated by the solid black line.

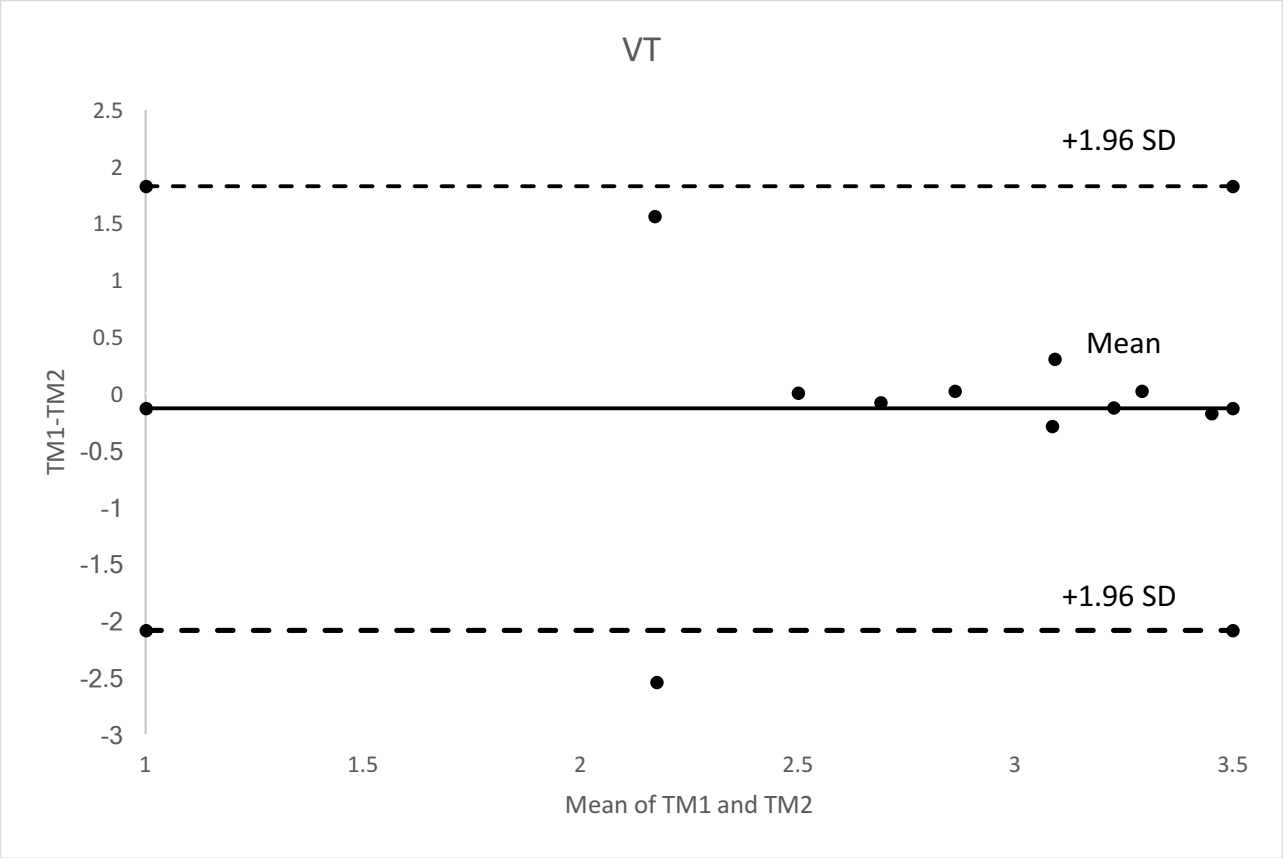

Figure 4. Bland-Altman displaying the average VT occurrence between trials one and two versus the difference between the two trials for each subject. Dashed lines represent 1.96 standard deviations from the mean indicated by the solid black line.

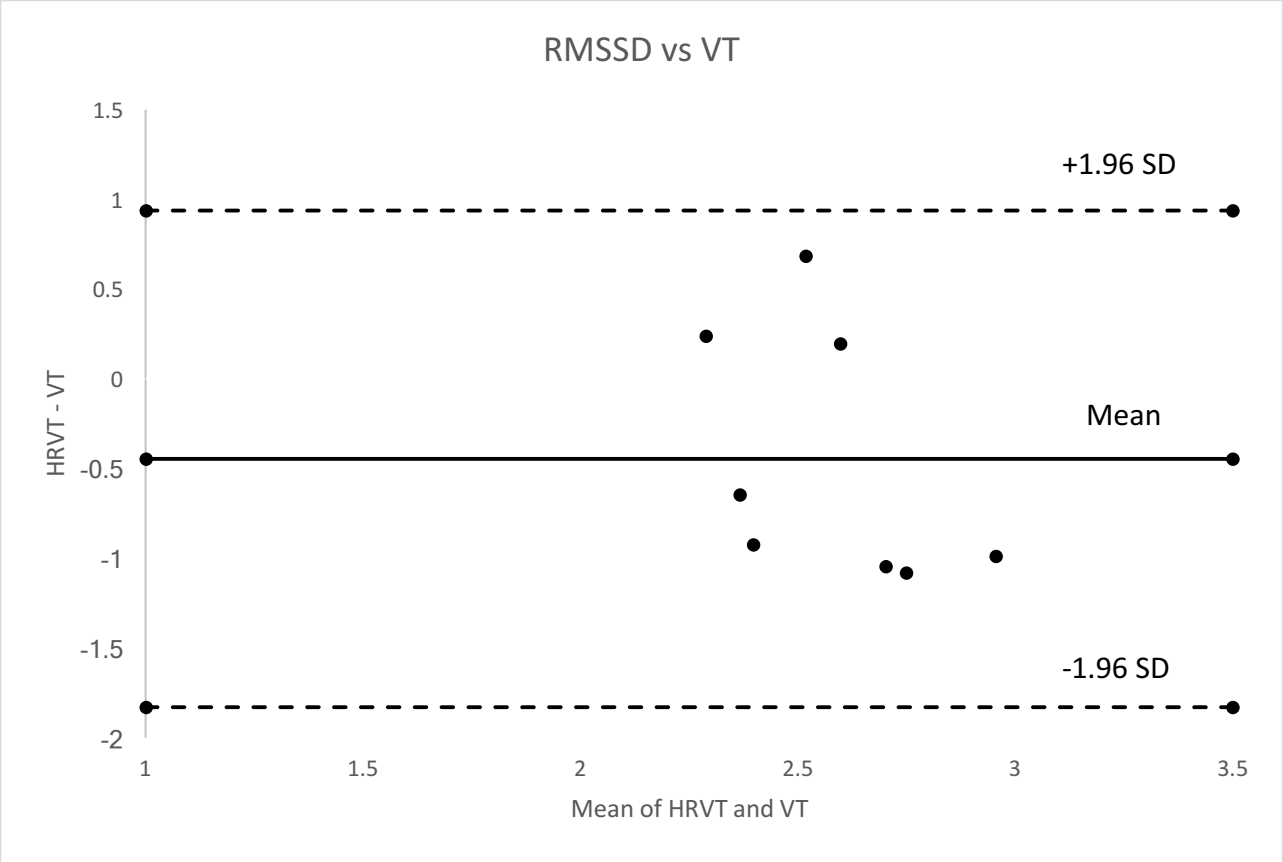

Figure 5. Bland-Altman displaying the average intensities of RMSSD and VT occurrence between trials one and two versus the difference between the two trials for each subject. Dashed lines represent 1.96 standard deviations from the mean indicated by the solid black line.

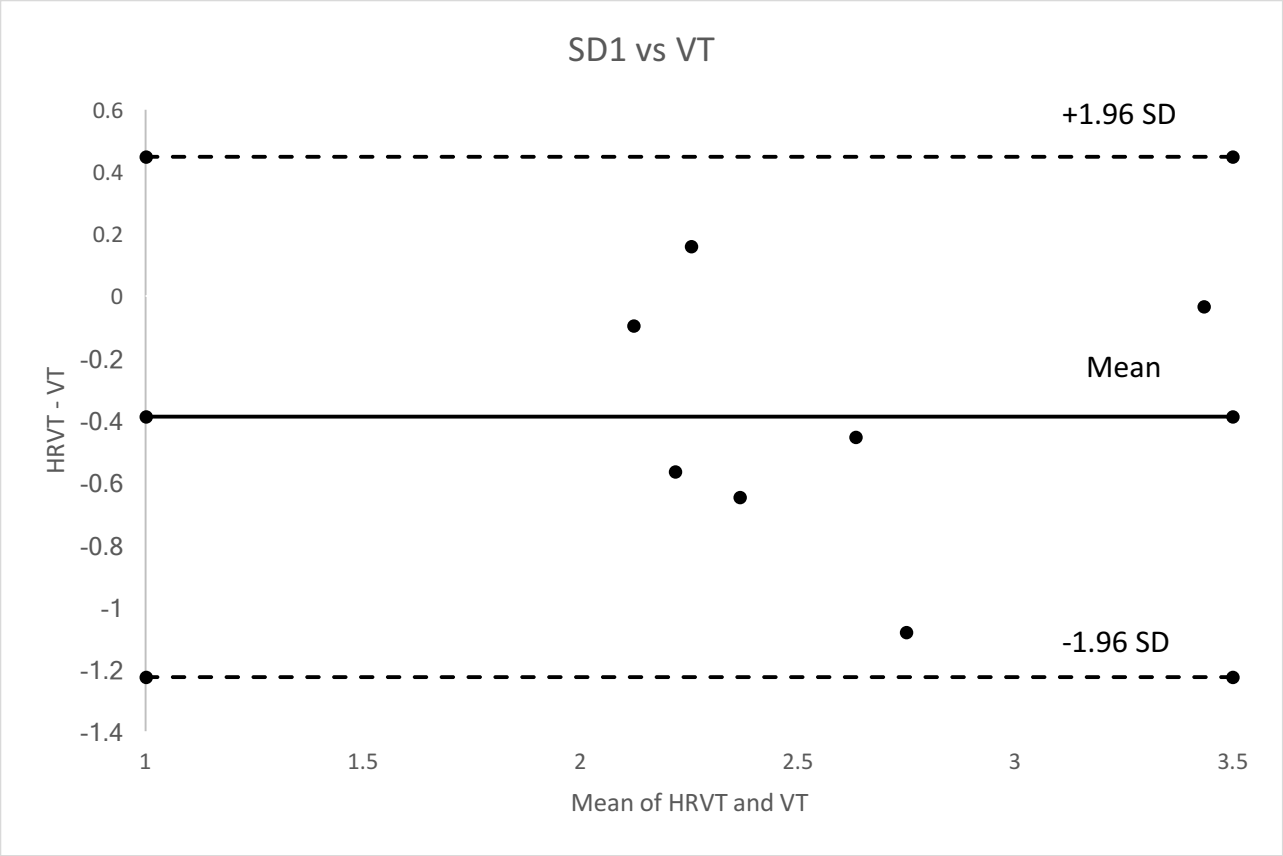

Figure 6. Bland-Altman displaying the average intensities of SD1 and VT occurrence between trials one and two versus the difference between the two trials for each subject. Dashed lines represent 1.96 standard deviations from the mean indicated by the solid black line.

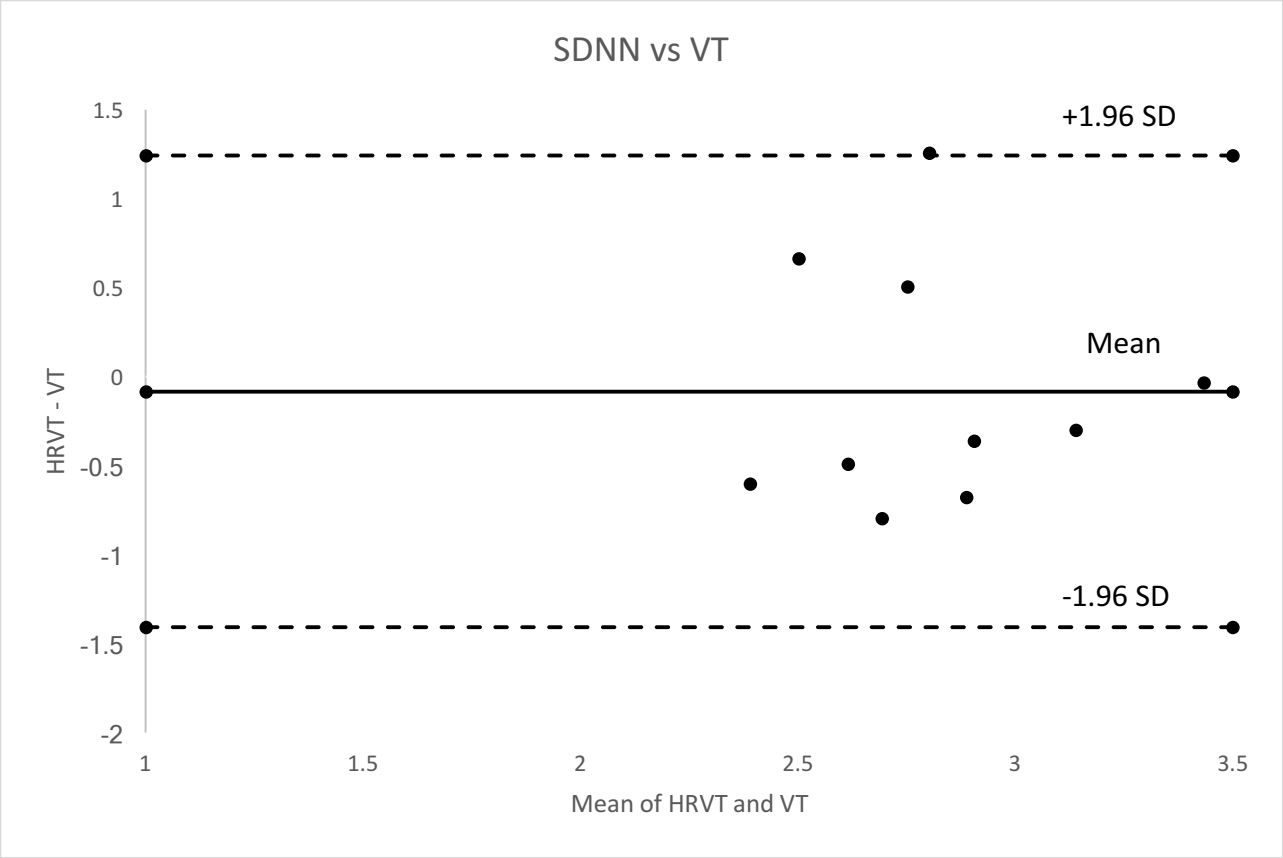

Figure 7. Bland-Altman displaying the average intensities of SDNN and VT occurrence between trials one and two versus the difference between the two trials for each subject. Dashed lines represent 1.96 standard deviations from the mean indicated by the solid black line.

Appendix A

Informed Consent

James Madison University
Department of Kinesiology
Informed Consent

Purpose

You are being asked to volunteer for a research study conducted by Dr. Trent Hargens and Shane Chambers from James Madison University entitled, "Heart Rate Variability Threshold Determination Across Exercise Trials".

The goal of this study is to examine whether a method for non-invasively measuring a measure of physiological function is repeatable on multiple days while exercising on a treadmill.

Experimental Procedures

You will be asked to visit the Human Performance Laboratory (HPL) in Godwin Hall 2 times over the course of about 1 – 2 weeks. Your total time commitment for participation in this study will be about 2 hours (each visit about an hour).

Upon completion of this informed consent, you will be asked to complete 2 short questionnaires, 1 that asks about your ability to participate in physical activity, and 1 that asks about your current level of physical activity participation. Each of these questionnaires should take about 5 minutes to complete.

You will then have your height and weight measured. Upon completion of this, you will be asked to complete a maximal treadmill exercise test. During the test, researchers will monitor your heart rate, blood pressure and your perceived exertion to the workload you will be doing. You will wear a strap on your skin on your torso, to allow us to monitor your heart rate. During the test, you will breathe only through a mouthpiece, with your nose clamped off, so that we can measure the amount of oxygen you use during exercise. The treadmill will start with a very slow speed and little grade, and will steadily increase in speed and grade every three minutes throughout the test. At the end of the test, it should be a best effort on your part. It may be as hard as any exercise you remember doing. At the end of each three minute stage, you will then dismount the treadmill and a drop of blood will be obtained through a finger lancet and analyzed for blood lactate. The exercise test will last about 12 – 18 minutes, requiring 4 - 6 blood samples. Prior to your arrival to the HPL that day, you will be asked to refrain from eating for 4 hours prior to your arrival, and to avoid caffeine and alcohol for that time period as well.

Upon completion of this visit, you will be asked to return to the HPL an additional time, a minimum of 48 hours later, and at the same time of day as the first visit. The remaining visit will consist of an additional maximal treadmill test, identical to your visit 1.

Risks

There is a risk of abnormal changes during the maximal exercise tests. These changes may include abnormal blood pressure, fainting, heart rhythm disorders, stroke, heart attack, and

death. The chance of serious heart problems during maximal exercise among adults is very small (less than 1/10,000 maximal exercise tests). Every effort will be made to minimize risks of an abnormal response by reviewing you health history and providing adequate supervision of the exercise test. All staff are certified by the American Heart Association in BLS (Basic Life Support), and all tests will be supervised by individuals certified by the American College of Sports Medicine. The risks associated with obtaining small samples of blood via finger-sticks are minimal but include bruising and discomfort for 24 to 48 hours and infection. The risk for infection is small and will be minimized by the use of sterile methods, including the use of sterile alcohol pads, sterile gauze, and band-aids.

Benefits

Participation may include knowledge about your health status. You will receive information on your cardiovascular fitness.

Inquiries

If you have any questions or concerns or you would like to receive a copy of the final aggregate results of this study, please contact Dr. Trent Hargens at hargenta@jmu.edu or (540) 568-5844.

Questions about Your Rights as a Research Subject
Dr. David Cockley
Chair, Institutional Review Board
James Madison University
(540) 568-2834
cocklede@jmu.edu

Confidentiality

All data and results will be kept confidential. You will be assigned an identification number and a pseudonym in place of your real name. At no time will your name be identified with your individual data. The researcher retains the right to use and publish non-identifiable data. All paper data will be kept secured in a locked cabinet in a locked office. All electronic data will be kept on a password-protected computer in encrypted file folders. Final aggregate results will be made available to participants upon request.

Freedom of Consent

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form I requested it. I certify that I am at least 18 years of age. By clicking "Yes" to the question below and submitting this confidential online survey, I am consenting to participate in this research.

Do you provide consent to participate in the research study entitled, "Heart Rate Variability Threshold Determination Across Exercise Trials"?

- o Yes
- o No

Please enter your name here

Please enter today's date

Appendix B

IPAQ

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Please input your name of a pet you've had and the name of the street you grew up on (example: Rover Main)

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling? (enter only the number of days in the blank)

How much time did you usually spend doing **vigorous** physical activities on one of those days? (only enter the number into each blank. If you don't know/are unsure, leave blank)

Hours Per Day: _____

Minutes Per Day: _____

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking. (Only enter the number into the blank)

How much time did you usually spend doing **moderate** physical activities on one of those days? (only enter the number into each blank. If you don't know/are unsure, leave blank)

Hours Per Day: _____

Minutes Per Day: _____

Think about the time you spent **walking** in the **last 7 days.** The includes at work and at home, walking to travel from palce to place, and any other walking that yo might do solely for recreation, sport, exercise, or leisure.

During the **last 7 days,** on how many days did you **walk** for at least 10 minutes at a time? (Only enter the number into the blank)

How much time did you usually spend doing **walking** physical activities on one of those days? (only enter the number into each blank. If you don't know/are unsure, leave blank)

Hours Per Day: _____

Minutes Per Day: _____

The last question is about the time you spent **sitting** on weekday during the **last 7 days.** Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

During the **last 7 days,** how much time did you spend **sitting** on a **week day**? (only enter the number into each blank. If you don't know/are unsure, leave blank)

Hours Per Day: _____

Minutes Per Day: _____

We thank you for your time spent taking this survey.
Your response has been recorded.

Appendix C

PARQ

Physical Activity Readiness Questionnaire (PAR-Q)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with your doctor before you start.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check **YES** or **NO**

Question	**Yes**	**No**
Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?		
Do you feel pain in your chest when you do physical activity?		
In the past month, have you had a chest pain when you were not doing physical activity?		
Do you lose your balance because of dizziness or do you ever lose consciousness?		
Do you have a bone or joint problem (for example, back, knee, or hip) that could be made worse by a change in your physical activity?		
Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition		
Do you have diabetes or a thyroid condition?		
Do you know of any other reasons why you should not do physical activity?		

Appendix D

Standardized Treadmill Protocol

Stage	Minutes	Speed (mph)	Grade (%)	METs
1	1-3	3.0	0	3.3
2	4-6	3.5	3.5	5.4
3	7-9	3.9	6.5	7.5
4	10-12	5.5	0.5	9.6
5	13-15	6.0	3.5	11.6
6	15-18	6.5	6.0	13.6
7	19-22	6.8	9.0	15.6

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