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The influence of autonomic nervous system (ANS) activity on the development and progression of cardiovascular disease (CVD) is widely acknowledged, as most common CVD risk factors (e.g., hypertension, impaired glucose management, obesity, low cardiovascular fitness) affect and/or are affected by ANS activity. Increased adiposity can be viewed as an amplifier of CVD development, as it has been shown to elevate the chances of developing hypertension and diabetes mellitus. Thus, adiposity may have direct and indirect effects on CVD development. In turn, it is important to determine if similar multidirectional relations can be observed between adiposity and altered ANS activity. Therefore, this dissertation addressed two main goals. The first was to evaluate the direct and indirect influence of adiposity on ANS activity through secondary data analyses employing structural equation modeling in a large, population-based cohort. The parasympathetic and sympathetic branches of the ANS were noninvasively assessed via heart rate variability [reflected by root mean square of successive differences between normal beats (RMSSD)] and the pre-ejection period of systole [(PEP); measured via impedance cardiography], respectively. The direct effects of adiposity on ANS activity were assessed by examining the magnitude of influence of waist circumference (WC) on RMSSD and PEP. Indirect effects were assessed via the impact of WC on the latent variables of glycemic impairment (GI; fasting blood glucose and insulin concentrations, hemoglobin glycosylation percentage) and cardiac stress (CS; heart rate, diastolic blood pressure) parameters. Adiposity had both significant direct ($\beta = 0.208$, $p = 0.018$) and indirect ($\beta = -.217$, $p=.041$) effects on PEP through GI. Adiposity displayed no significant direct effect on RMSSD. CS displayed a significant pathway ($\beta = -0.524$, $p = 0.035$) on RMSSD, but the indirect effect of

WC on RMSSD through CS was not significant. These results suggest that adiposity's relation to ANS activity is multifaceted, as increased central adiposity had opposing direct and indirect effects on markers of sympathetic activity in this population of older adults. The second goal of this dissertation was to determine if cardiovascular fitness, as determined through graded exercise testing, influenced the relation of adiposity and ANS activity beyond the role it plays in determining adiposity, *per se*. To accomplish this goal, ANS activity was examined in a small cohort of young males with a range of fitness and adiposity levels before and after maximal aerobic exercise. A mixed model analysis of covariance was employed to test the additional impact of cardiovascular fitness on the relations between adiposity and ANS activity at rest and following maximal exercise. Group stratification by waist circumference or body fat percentage (BF%) revealed no across-group differences in resting or post-maximal exercise HRV or PEP measurements. Accounting for weight-relative peak oxygen uptake (VO_{2peak}) resulted in significant between-group differences in the natural logarithm SDNN (ln-SDNN), RMSSD (ln-RMSSD)], and high frequency spectral power (ln-HF) at 3- and 5-minutes post-exercise when groups were stratified by BF%. However, these differences were no longer statistically significant following adjustments for lean body weight-relative VO_{2peak}, suggesting no effect of BF% on ANS activity. These results provide evidence that there may not be differences in resting ANS activity and post-maximal exercise ANS responsiveness across adiposity groups in apparently healthy males. Taken together, these two studies highlight the nuanced involvement of adiposity on physiological parameters that influence ANS activity. However, they do not support the notion that adiposity has a strong, independent influence on ANS activity.

INFLUENCE OF ADIPOSITY ON AUTONOMIC NERVOUS SYSTEM ACTIVITY

by

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CHAPTER I: INTRODUCTION

Cardiovascular disease (CVD) continues to be one of the leading causes of death in the United States. Heart disease alone caused or contributed to approximately 1 out of 4 deaths in 2016 (Heron, M., 2018). Given this, it is pertinent that the scientific community have a concrete understanding of the underling pathophysiology of CVD. Major risk factors for CVD include both lifestyle choices (i.e., physical inactivity, poor diet) and medical conditions (i.e. diabetes mellitus, hypertension, obesity) (Tzoulaki et al., 2016), both of which have been associated with altered autonomic nervous system (ANS) activity (Alam, Lewis, Morgan, & Baxter, 2009; Erdogan et al., 2011; Soares-Miranda et al., 2014; Tarvainen, Laitinen, Lipponen, Cornforth, & Jelinek, 2014).

ANS activity is comprised of output from the two branches of the ANS, the sympathetic and parasympathetic nervous system. The relation of these subsystems is both opposing and dynamic in nature. The sympathetic branch provides an overarching accelerator stimulus to the body, promoting metabolic substrate mobilization and utilization in addition to increasing heart rate and cardiac contractile force. In contrast, the parasympathetic branch is the overarching decelerator of the body, inducing metabolic substrate storage as well as promoting a decrease in heart rate (Thayer et al., 2010). The activity of these branches is stimulated by a myriad of external stimuli and is inhibited by the activity of the opposing branch. This continuous counterbalance of trigger and repression is brought about by daily activities and external stimuli and results in a dynamic fluctuation between the two branches. All target organs receive varying balance of input from both branches and the resulting action is a direct reflection of the ANS branch with the largest contribution related to a particular stimuli. Bodily functions are regulated by this balance and any prolonged disturbance or asymmetry of this relation in the ANS branches

can result in the development of several clinical and physiological conditions, including CVD (Pagani et al., 1988; Zhou et al., 2012).

Non-invasive evaluation of ANS activity can be assessed by multiple metrics but is most commonly gauged by indices of heart rate variability (HRV). HRV, or the variance among the intervals of consecutive cardiac cycles, is a widely used and is an accepted means of assessing ANS activity at the level of the heart (Malik et al., 1996). Various time and frequency domain HRV metrics can be used to estimate the level of parasympathetic output at the heart, including the root mean square of successive differences between normal (non-ectopic) beats (RMSSD) and frequency input observed in the range of 0.15-0.40 Hz, denoted as High Frequency (HF) (Michael, Graham, et al., 2017). Some HRV metrics have been suggested to estimate sympathetic activity (namely the low frequency spectral power component and the ratio of low frequency to high frequency spectral power components), but these values are inherently limited in evaluating sympathetic output given that they are also influenced by parasympathetic activity (Shaffer & Ginsberg, 2017). Thus, most HRV research assumes that if metrics of parasympathetic activity are high, sympathetic activity is most likely low, given their opposing nature. An alternative and more independent method of evaluating sympathetic activity at the level of the heart is to monitor systolic time intervals, i.e., the amount of time between electrical stimulation of the heart and its mechanical response. Specifically, the segment of time known as the pre-ejection period (PEP), an estimate of the electromechanical delay of the left ventricle (Tavakolian, 2016), can be used to accurately estimate sympathetic activity (Ahmed et al., 1972). Simultaneous utilization of PEP and HRV measurements would provide greater details of ANS activity at the level of the heart.

Irregular ANS activity has been evaluated for its association with CVD and mortality. Specifically, chronically reduced parasympathetic and elevated sympathetic activities are associated with increased CVD risk, including earlier mortality, increased risk for myocardial infarction, and development of diabetes mellitus and hypertension (Gerritsen et al., 2001; Kleiger et al., 1987; Tsuji et al., 1996). Furthermore, researchers have found significant associations between metrics of increased adiposity and altered ANS activity (Farah et al., 2013; Poirier et al., 2003; Windham et al., 2012).

Obesity is an independent factor for CVD development (Hubert et al., 1983) and is also a well-established risk factor for the development of comorbidities such as diabetes mellitus (Koh-Banerjee et al., 2004) and hypertension (Landsberg et al., 2013). This suggests that obesity (increased adiposity) has an amplified impact on CVD risk, as it has both direct and indirect routes of influence. It is unknown if this concept of amplified impact can be applied to the relation of ANS activity and increased adiposity.

Accounting for the physiological interaction of blood pressure and glucose metabolism makes it difficult to determine if the aforementioned associations with altered ANS activity are solely attributable to increased adiposity. The application of structural equation modeling can provide a holistic means to resolving this quandary. Structural equation modeling is an extension of regression analysis that models multiple paths of variable influence and represents the magnitude of each path with a regression weight. A path analysis based on structural equation modeling allows for detailed relations that have the capability to account for both observed and unobserved, or latent, variables (Anderson & Gerbing, 1988). As no single variable can accurately represent the concepts of glycemic impairment or cardiac stress parameters, this approach allows for the combined influence of multiple observed variables to be

treated as a single latent variable. Furthermore, this statistical approach allows the assessment of direct and indirect effects of observed variables and/or latent constructs on dependent variables, making it possible to more accurately account for the influences of both cardiac stress and glycemic impairment parameters while also teasing out the actual effects of adiposity on indictors of ANS activity.

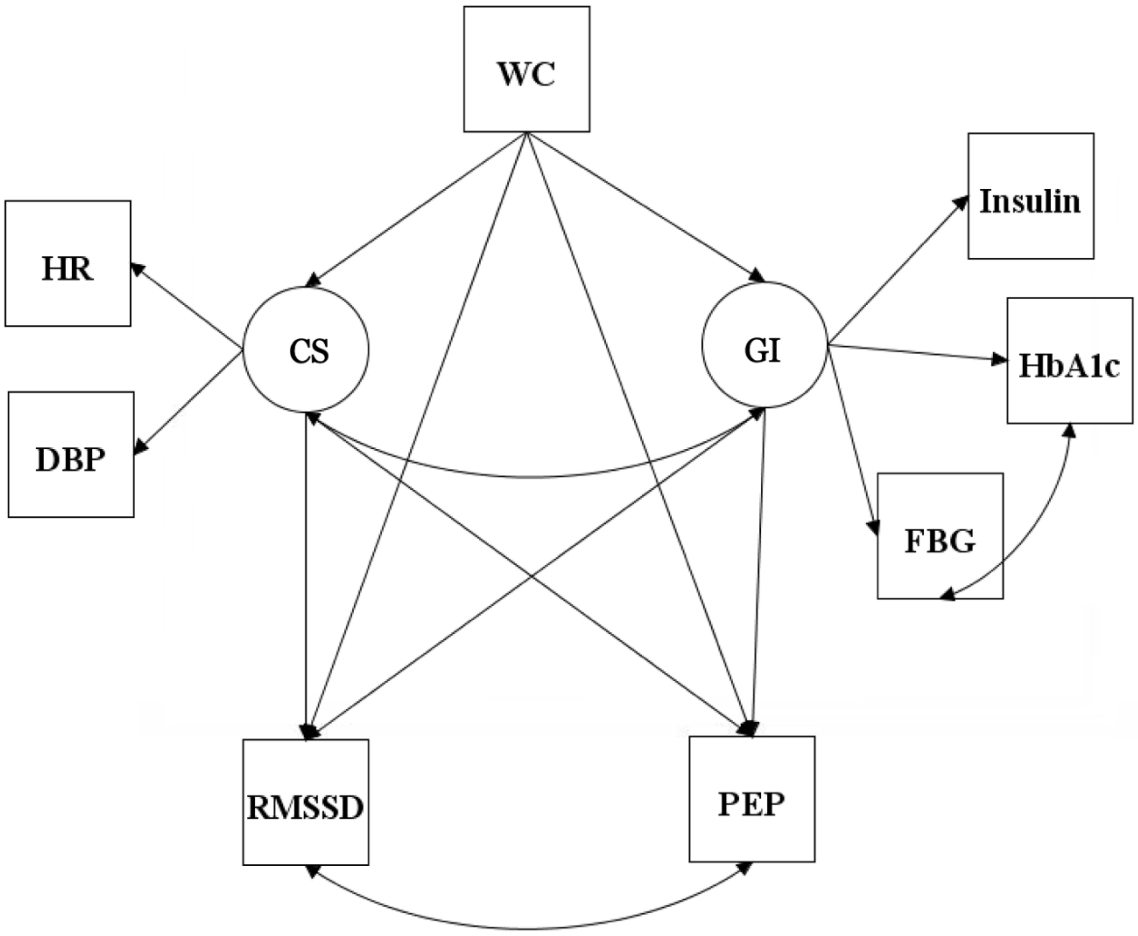
To further convolute this paradigm, the influence of cardiorespiratory fitness must be considered. Cardiorespiratory fitness has a natural inverse relation with measurements of adiposity, such that higher levels of cardiorespiratory fitness tend to be associated with lower levels of adiposity (Söğüt et al., 2019). Also, low cardiorespiratory fitness increases the risk of CVD mortality (Gupta et al., 2011), type II diabetes (Blair & Church, 2003), hypertension (Kokkinos, 2014), and an imbalance between sympathetic and parasympathetic activities (Thayer et al., 2010). Furthermore, it has been suggested that cardiorespiratory fitness may be a greater determinate of ANS activity than adiposity (Chen et al., 2018). Thus, the proposed amplified impact of adiposity may be an indirect function of the physiological effects of cardiorespiratory fitness.

Furthermore, the relation of cardiorespiratory fitness and ANS activity, specifically ANS activity post-exercise or ANS responsiveness and its representation of physical health, has long been documented (Blomqvist & Saltin, 1983; Smith et al., 1989). A faster heart rate recovery, the slowing of heart rate following exercise, is inversely related to cardiovascular disease incidence (Morshedi-Meibodi et al., 2002) and all-cause mortality (Jouven et al., 2005). The adjustment of heart rate (HR) before and after exercise is primarily due to modulations in ANS activity at the cardiovascular level in response to changing metabolic and physiological demands (L. R. B. E. Silva et al., 2017). HR increases in response to exercise due to parasympathetic

withdrawal and increased sympathetic drive, while the post-exercise decrease in HR is due to a reversal of this activity (Michael, Graham, et al., 2017). Evaluating alterations in ANS responsiveness to the cessation of exercise could yield valuable insights into the underlying influence of adiposity on ANS activity.

The proposed study had two purposes. The first was to evaluate the direct and indirect influences of adiposity on resting autonomic nervous system (ANS) activity. Utilizing the Atherosclerosis Risk in Communities (ARIC) dataset, activities of both the parasympathetic and sympathetic branches of the ANS were assessed via non-invasive methods. Specifically, parasympathetic activity was measured via heart rate variability (HRV) utilizing the measurement of the root mean square of successive differences between normal beats (RMSSD). Sympathetic activity was evaluated via impedance cardiography employing the systolic time interval known as the pre-ejection period (PEP). The direct effects of adiposity on ANS activity were assessed by the magnitude of influence that abdominal adiposity, reflected by waist circumference (WC), has on RMSSD and PEP. Indirect effects were assessed via the impact of adiposity on glycemic impairment and cardiac stress parameters. The proposed conceptual model (Figure 1.1) graphically outlines these direct and indirect effects on markers of ANS activity. Further details concerning the specific model are described in Chapter 3.

Figure 1-1. Proposed Model



Note: Theoretical model including observed variables (squares) depicting the proposed pathways for the direct and indirect effects of waist circumference on markers of ANS activity. Construction of the latent variables (circles) Cardiac Stress (CS) and Glycemic Impairment (GI) are depicted. WC, Waist Circumference; HR, Heart Rate; DBP, Diastolic Blood Pressure; Hb_{A1c}, Glycosylated Hemoglobin; FBG, Fasting Blood Glucose; RMSSD, Root Mean Square of Successive Differences between normal-to-normal beats; PEP, Pre-Ejection Period.

The second purpose was to establish if cardiorespiratory fitness influences the observed effects of adiposity on markers of ANS activity beyond the role it plays in determining adiposity. Data collected from a small cohort of apparently healthy, adult males was examined using

multiple mixed model analysis of covariance analyses to test the additional impact of cardiorespiratory fitness on the relation between two indices of adiposity and ANS activity. Specifically, parasympathetic activity was measured via multiple metrics of HRV while sympathetic activity was evaluated via impedance cardiography employing the systolic time interval of PEP. Characterizing adiposity was accomplished be the dualistic use of waist circumference (WC) and body fat percentage (BF%). A maximal exercise test was used to measure cardiorespiratory fitness with representative values used to determine if adjustments for cardiorespiratory fitness significantly influenced any observed differences across WC or BF% strata.

Specific Aims and Hypotheses

The following specific aims was tested:

Aim 1. To determine the direct and indirect influence of adiposity on ANS activity as evaluated through structural equation modeling.

Hypotheses: (1a) Adiposity (WC) will have a direct influence on resting ANS activity as reflecting inhibitory effect on parasympathetic activity (decreased RMSSD) and a stimulatory effect on sympathetic activity (decreased PEP). (1b) Adiposity (WC) will have significant indirect influences on marks of ANS activity through its impact on glycemic impairment and cardiac stress parameters.

Aim 2. To determine if cardiorespiratory fitness influences the observed effects of adiposity on markers of ANS activity beyond the role it plays in determining adiposity.

Hypotheses: (2a) Adjustments for cardiorespiratory fitness will mitigate the observed differences in resting ANS activity across WC and BF% strata. (2b) Adjustments for

cardiorespiratory fitness will mitigate the observed differences in ANS responsiveness post-maximal exercise across WC and BF% strata.

CHAPTER II: REVIEW OF THE LITERATURE

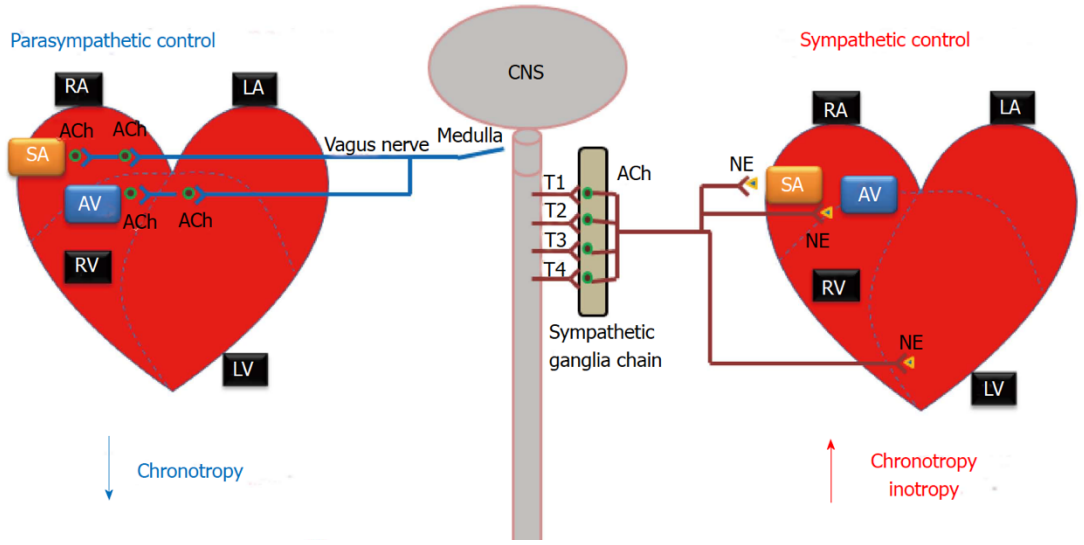
Overview

The contents of this literature review will commence by briefly outlining the effects of both branches of the ANS on chronotropic and ionotropic characteristics of the heart. While these specific mechanisms are not the central focus of the dissertation, a general understanding of the dual influence of the sympathetic and parasympathetic branches of the ANS on the heart is key to the implications of this dissertation. Following this summary, the common measurements of ANS activity will be discussed with special attention given to the most common non-invasive techniques of heart rate variability and systolic time intervals. Next, a synopsis of the mechanistic relation between the key influences on ANS activity related to this dissertation and the non-invasive measurements will be provided. These key influences include glycemic impairment, cardiac stress, adiposity, and cardiorespiratory fitness. Finally, a description of the proposed conceptual model and summary of the current literature describing the interrelated nature of the variables of interest will be offered.

Autonomic Nervous System Innervation of the Heart

At rest, the average adult's heart rate is between 60-80 beats per minute (Shaffer et al., 2014). This rate is much slower than the approximant auto-rhythmic rate of 100 bpm generated by the "pace making" sinoatrial node located lateral to the opening of the superior vena cava in the right atrium (Katona et al., 1982). The "braking" influence of the parasympathetic branch of the ANS is responsible for this lower resting rate, as it prevails over both the "accelerating" influence of the sympathetic branch of the ANS and the inherent pacing of the heart (Gordan et al., 2015).

Figure 2-1. Diagram of Cardiac Innervation.



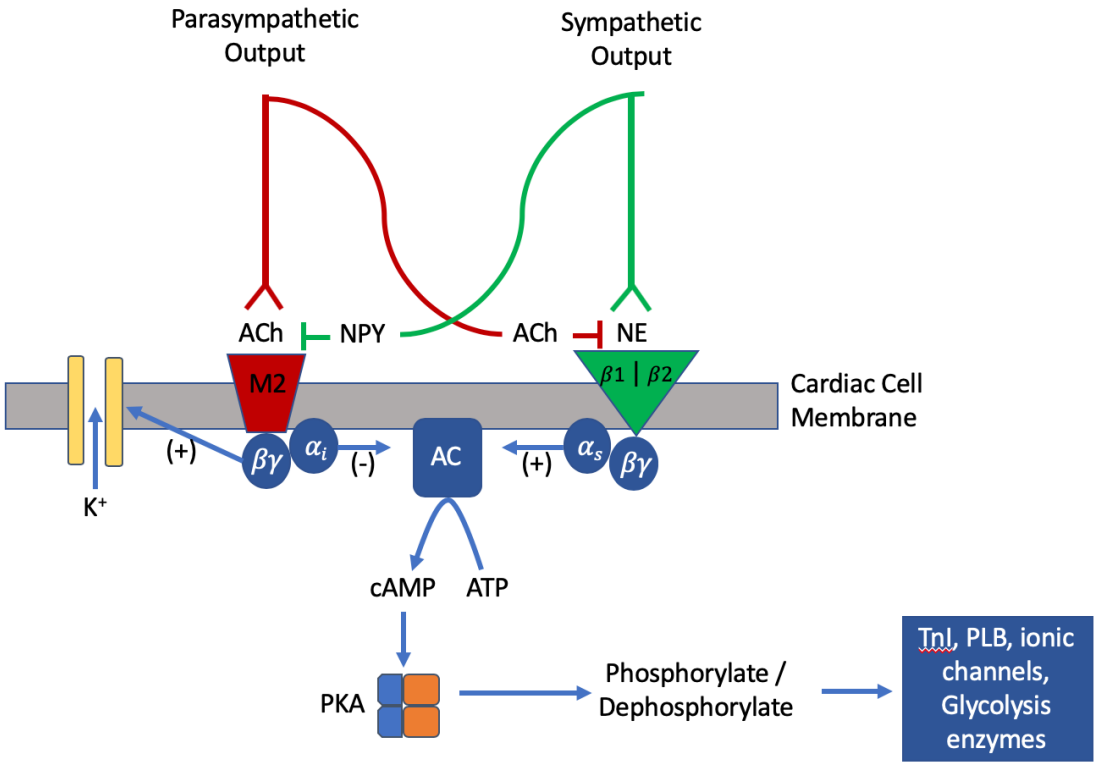
Note: CNS, Central Nervous System; T1-T4, Thoracic Segments 1-4; ACh, Acetylcholine; NE, Norepinephrine; SA, Sinoatrial Node; AV, Atrioventricular Node; RA, Right Atrium; LA, Left Atrium; RV, Right Ventricle; LV, Left Ventricle. (As illustrated by Gordan et al., 2015)

Above, Figure 2.1 provides a graphic representation of ANS innervation of the heart. Receiving input from the nucleus tractus solitarii (NTS), the medulla oblongata (located in the midbrain of the central nervous system) is the location of origin for both sympathetic and parasympathetic output. The caudal ventrolateral medulla and rostro ventrolateral medulla serve as the primary originators of sympathetic output while the nucleus ambiguous (NA) generates parasympathetic output (Michael, Graham, et al., 2017). Action potentials from the caudal ventrolateral medulla and rostro ventrolateral medulla travel down the spinal column to the thoracic region through preganglionic fibers to the sympathetic ganglia chain near the T1-T4 vertebra. At the ganglia chain, the neurotransmitter acetylcholine is released and stimulates action potentials on the postganglionic fibers that release norepinephrine (NE). At the end organ

level, the release of the neurotransmitter, NE, binds to adrenergic receptors on both the sinoatrial and atrioventricular nodes and ventricular cardiomyocytes. Once bound, norepinephrine will stimulate an increase in the chronotropic (rate) and inotropic (force) effects of the heart. In a similar fashion, parasympathetic output, initiated as action potentials from the NA, travel down the vagus nerve, stimulating the release of acetylcholine from preganglionic fibers that binds to the parasympathetic ganglia serving the heart, stimulating these ganglia to release acetylcholine at the heart. Some of the acetylcholine binds to muscarinic receptors on cardiac nodal tissue, directly causing a decrease in chronotropic effects while also influencing inotropic responses.

While both the sympathetic and parasympathetic branches originate from divisions of the medulla and provide constant input to the heart, the parasympathetic branch typically has the dominant influence during resting conditions. This is driven by inhibitory factors at both the neural and cell membrane levels as outlined in Figure 2.2. At the cardiac cellular membrane, parasympathetic stimulation occurs at an accelerated rate compared to sympathetic stimulation due to direct influence of specialized potassium ion channels regulated by G-protein subunits of the muscarinic receptor (Ivanova-Nikolova et al., 1998). The beta gamma subunit of the muscarinic receptor opens these specialized ion channels, allowing potassium to exit the cell, altering resting membrane potential and causing a delay in the ability to reach threshold and a subsequent action potential to induce contraction. The sympathetically-driven chronotropic effect requires activation of the second messenger cyclic adenosine monophosphate (cAMP). This additional step results in a comparatively slower effect. Thus, when present, parasympathetic influence dominates cardiac function at rest.

Figure 2.-2. Cardiac Cell Membrane Signaling.



Note: ACh, Acetylcholine; NE, Norepinephrine; NPY, Neural Peptide Y; M2, Muscarinic 2 Receptor; $\beta 1 \mid \beta 2$, Beta 1 and Beta 2 Adrenergic Receptors; $\beta\gamma$, Beta gamma subunit of G-protein; α_i, Inhibitory subunit of G-protein; α_s, Stimulatory subunit of G-protein; AC, Adenylyl Cyclase; ATP, Adenosine Triphosphate; cAMP, Cyclic Adenosine Monophosphate; PKA, Protein Kinase A; TnI, Troponin I; PLB, Phospholamban.

Additional mitigation of sympathetic influence is achieved by inhibition at the nerve level to inhibit norepinephrine release and within the membrane to alter signaling to the enzyme to influence cAMP formation. Conversely, increased sympathetic stimulation can result in a build-up of Neural Peptide Y (NPY), blocking ACH release, thus limiting ACH binding to muscarinic receptors. In addition, both muscarinic and adrenergic receptor activation alter the rate of adenylyl cyclase activation—muscarinic G-protein subunits are inhibitory, while adrenergic G-

protein subunits are stimulatory. Any increase in adenylyl cyclase activity will result in greater synthesis of cAMP. cAMP amplifies activation of protein kinase A which, in turn, causes the phosphorylation of several influential proteins, including troponin I, phospholamban, ionic channels and certain glycolysis-regulating enzymes. These proteins and regulatory factors increase the ionotropic and chronotropic effects on the heart primarily by increasing the concentration of intercellular calcium in the cardiomyocyte (Gordan et al., 2015). Thus, when sympathetic output is the predominate driver there is an increased rate of contraction (chronotropic effect) as well as an increase in contractile force (ionotropic effect). Conversely, the concentration of cAMP can be decreased via the inhibitory effect of the G-protein subunits of muscarinic receptors on adenylyl cyclase activity, thereby decreasing the signaling pathway of protein kinase A. This inhibitory action, coupled with the stimulation of acetylcholine sensitive potassium ion channels, exerts an overall decreased chronotropic effect on the heart. This chronotropic decrease happens without significant changes in the ionotropic effect given the limited appearance of muscarinic receptors on ventricle myocardium.

Although cardiac regulation is typically dominated by the parasympathetic nervous system at rest, autonomic imbalance can be created via a sympathetic-dominate state. This autonomic imbalance results in a chronic "accelerator" stimulation that manifests as an elevated resting heart rate and, potentially, increased blood pressure, raising the risk of idiopathic hypertension over time (Charkoudian & Rabbitts, 2009; Schroeder et al., 2003). Additionally, this long term increase in chronotropic and inotropic myocardial activity leads to an overall increase in the rate pressure product, an indirect reflection of myocardial work (Nelson et al., 1974). Furthermore, this prolonged imbalance has been found to be correlated with an increased risk of developing CVD (Thayer et al., 2010) and both the development and severity of diabetes

(Tarvainen, Laitinen, et al., 2014). Given these negative health implications, it is imperative that observations of ANS activity be made by accurate and valid means. The following section will outline common methods of evaluating ANS activity and defend the selection of metrics included in this study.

Assessment of Autonomic Nervous System Activity

Overview

ANS activity measurement techniques vary based on invasiveness, expense, and burden on the patient or research participant. While some techniques require chemical analysis of blood, electrode implantation in different tissues, or challenges of physiological reflexes and bodily functions, non-invasive techniques typically require little to no effort from the subject also exist. Measurement of catecholamine concentration in either blood or tissue samples is a classic laboratory method used to gage ANS activity in response to either varying stimuli or ANS blockade with various chemicals (Breuer et al., 1993). No technique is without limitation, as most of the methods discussed below primarily focus on evaluating the activity of the sympathetic nervous system and are highly invasive.

Neurotransmitters (primarily norepinephrine) can be measured in blood with some degree of laboratory proficiency. However, circulating concentration may not be a reliable indicator for neural activity at the end organ site as, it is estimated that circulating concentrations of norepinephrine represent only 5-10% of the total secreted amount of this neurotransmitter (Sinski et al., 2006). Additionally, acetylcholinesterase breaks down acetylcholine at an extremely accelerated rate, making it extremely difficult to collect and analyze a physiologically meaningful concentration of this neurotransmitter (Grassi & Esler, 1999). Thus, concentration analysis focuses exclusively on estimating sympathetic activity. Finally, venipuncture has the

potential to elicit a stress response that could alter the normal neurotransmitter milieu (Hoogerwerf et al., 2018).

Microneurographic measurements can be used with or in lieu of catecholamine concentration measurement. Traditionally used to observe sympathetic activity in peripheral skin and skeletal muscle tissue, microneurography requires extremely small needle electrodes to be inserted subcutaneously in a pinpointed peripheral nerve. Once placed, the electrode becomes a conductor of sympathetic neural traffic, allowing for the comprehensive recording of amplitude changes in stimulation (Neukirchen & Kienbaum, 2008). While this method can provide detailed information, it is limited by its inherent site-specific recording (peripheral skeletal muscle or skin), the production of motion artifact with minimal movement, and its invasive nature. Thus, this might not accurately reflect the sympathetic activity at the heart.

All of these measurement issues give rise to the use of physiological challenges and/or non-invasive techniques for observing ANS activity. Assessment of changes in heart rate or R wave to R wave intervals during physiological challenge such as the Valsalva maneuver, deep breathing, cold pressor test, orthostatic challenges, head-up tilt test, and/or various means of baroreflex stimulation (i.e. carotid massage), have been used regularly to assess ANS function (Zygmunt & Stanczyk, 2010). As ANS activity can vary depending on the organ or system observed, a battery of challenges is typical, with the Ewing Battery being most popular (Ryder & Hardisty, 1990). One of the earliest forms of heart beat interval evaluation (Malik et al., 1996), this battery provides only simple clinical markers of reflex engagement and lacks the ability to accurately tease apart the activities of individual ANS branches.

None of the metrics previously outlined can accurately account for specific parasympathetic engagement. Therefore, the need for a non-invasive, detailed measurement of

parasympathetic activity such as heart rate variability (HRV) is apparent. HRV coupled with the use of systolic time intervals (STI), a valid indicator of sympathetic activation, can potentially provide holistic insight into ANS activity at the same end-organ location. The following sections will provide a synopsis of both HRV and STI, with particular attention given to the metrics of interest to the proposed dissertation.

Heart Rate Variability

Since it was first employed by Hon and Lee in 1965 to detail preceding events of fetal death (Hon & Lee, 1965), HRV has been widely accepted as a non-invasive marker of cardiac-level ANS activity. While heart rate is merely the number of complete cardiac cycles in a minute, HRV can be described as the fluctuation in the time intervals between adjacent heartbeats (McCraty & Shaffer, 2015). Analyzing the variation of this interval pattern can provide an insight into autonomic function at the cardiac level. Given that cardiac contractions are under the influence of multiple sources, including both branches of the ANS, baroreceptors, peripheral chemoreceptors, and the auto-rhythmic pacing of the heart, the resulting signal or interval pattern is complex, or "regularly irregular". The degree of complexity and dysregulation of heart rate is reflective of the overall health of the cardiorespiratory system. In general, the degree of complexity that a biological system displays is thought to be linked to its function (Vaillancourt & Newell, 2002), *i.e.*, a system that is variable in nature and displays "organized variability" is healthy, while a less complex and more regular system coincides more with disease (Goldberger, 1997). Thus, a resting cardiac rhythm with greater variability is believed to be healthier than a rhythm that is more fixed and rigid. Various means of assessing a rhythm's degree of variability have been developed since 1965, including metrics that depict its time and frequency aspects. Although this dissertation acknowledges the existence and validity of multiple

non-linear HRV metrics (i.e. ApEn; approximate entropy), these indices are outside the scope of this dissertation. Thus, the remaining portion of this subsection will focus exclusively on the time and frequency domains of HRV.

The existence of numerous HRV monitoring nuances, including but not limited to, sampling rate and recording length, complicates the comparison of data across studies. A minimum sampling rate of 100Hz is suggested to ensure complete capture of the electrical signals (Malik et al., 1996), and this sampling rate is easily achieved by most modern electrocardiograms. Depending on the metric used, recording length can have a sizable impact on results depending on the metric used. Considered the gold-standard of clinical HRV assessment, a recording length of 24 hours provides the most detailed evaluation of ANS activity (Shaffer & Ginsberg, 2017). However, shorter recordings of approximately five minutes have been successfully used in numerous studies (Nunan et al., 2010). One limitation to a shorter recording is the magnified impact of baroreceptor activity on the measures of very low band and high band frequency analyses (Shaffer & Ginsberg, 2017). Additionally, length dependent time domain measurements, such as the standard deviation of normal beats (SDNN) are also weakened by shorter recording periods (Malik et al., 1996). It should be noted however, that when using short-term recordings, frequency domain measures are preferred since they are influenced to a lesser extent than most time domain measures (Malik et al., 1996). While not ideal, ultra-short recordings of 1-5 minutes have been used, typically when experimental design does not allow for longer periods of time. Finally, although the same equations and methods are used to calculate HRV metrics for recording of any length, it is viewed as inappropriate to compare values from short-term recordings to long-term recordings as they are inherently

affected by differing physiological variables and thus have varying biological interpretations (Stein & Pu, 2012).

Time Domain. While heart rate and the HRV time domain both employ units of time, they detail differing characteristics of the same recording. Stated previous, heart rate is merely the number of complete cardiac cycles in one minute. This metric only describes the average rate of cardiac contractions and does not give a detailed understanding as to the rhythm of the contractions in that same time frame. HRV reflects the fluctuations of the time intervals between adjacent heartbeats. The simplest and most commonly used time domain measurements of HRV are the standard deviation of normal to normal beats (SDNN) and the root mean square of successive differences between normal to normal beats (RMSSD) (Michael, Graham, et al., 2017; Thayer et al., 2010). Both metrics are valid and commonly used measurements in short-term recordings (Malik et al., 1996). The standard deviation of R to R waves (SDRR) is another commonly used measurement. SDNN excludes all abnormal cardiac cycles, while SDRR does not have such a restriction. Thus, SDRR has the inherent disadvantage of being influenced by non-sinoatrial node-induced (ectopic) contractions. These ectopic or premature beats may introduce noise or artifact into the recording and confound the true interpretation of SDRR (Shaffer & Ginsberg, 2017).

The SDNN and lesser valued SDRR can be used as overall indicators of variability (Michael, Graham, et al., 2017) but lack the capacity to discern the source of the variability. This is primarily due to the fact that both branches of the ANS have been shown to influence the measurement (McCraty & Shaffer, 2015). As previously mentioned, SDNN is highly susceptible to the length of the recording and as such, will be more tainted than other time-domain metrics in short-term recordings (Nunan et al., 2010). Furthermore, in short-term measurements, SDNN is

highly influenced by respiratory patterns and the subsequent activation of baroreceptors. These issues muddy the interpretation of SDNN in short-term recordings and strengthen the recommendation of frequency analyses in recording periods of less than 10 minutes.

On the contrary, RMSSD is not affected by the length of the recording. This is primarily a result of its statistical properties and inherent immunity to the influence of time on its computation (Malik et al., 1996). The value is calculated by squaring the time difference of each successive R-R interval, averaging all of those values, and then taking the square root of that overall average. The initial squaring of the R-R intervals and follow-up square root of the average interval length provides a mathematical correction for the recoding length. It has also been repeatedly shown to be a valid marker of parasympathetic activity in both short and long-term recordings (Nunan et al., 2010; Shaffer et al., 2014; Windham et al., 2012). Additionally, RMSSD highly correlates with frequency metrics of parasympathetic activity (high frequency band) during short-term recordings (Malik et al., 1996; Shaffer et al., 2014) and as such is one of the most commonly used time domain metrics.

Frequency Domain. Understanding the mechanisms of frequency domain metrics is not an inherently intuitive concept. In the simplest form, a mathematical algorithm is used to convert a time domain signal (i.e. ECG tracing) into its corresponding sinusoidal function that is comprised of multiple frequency components (Montano et al., 2009). A complex signal such as an ECG tracing is influenced by a plethora of stimuli including parasympathetic activity, sympathetic activity, the auto-rhythmic pacing of the sinoatrial node and other biological influences (i.e. hormones). It is appropriate to view it as the result of multiple signals that are providing influence over the electrical activity of the heart at various rates or frequency. If an ECG tracing (cardiac cycles over time) was to be processed using a mathematical algorithm, Fast

Fourier Transform being one of the most common (Shaffer & Ginsberg, 2017), the resulting data could be graphed as the power spectral density (area under the curve) on the y axis and frequency (Hz) on the x-axis. Any spike on this graph would identify a source component that is providing enough influence on the original signal to alter its overall appearance or shape.

Short term recordings, two to five minutes, of ECG tracings are classically composed of three frequency components; Very Low Frequency (VLF) observed between 0.0033-0.04 Hz, Low Frequency (LF) observed between 0.04-0.15 Hz, and High Frequency (HF) observed between (0.15-0.40 Hz) (Shaffer & Ginsberg, 2017). Though usually present, the meaning or cause of the VLF band in short-term tracings is not fully understood and the use of it should generally be avoided (Malik et al., 1996). The LF band was formally known as the baroreceptor range as alterations of baroreceptor signaling at rest provided the largest influence over LF values (McCraty & Shaffer, 2015). Although some studies try to infer sympathetic activity from the LF values, a large volume of evidence demonstrates that it is influenced by both parasympathetic and sympathetic activity (Billman, 2013; Eckberg, 1997). Additionally, some studies have observed little to no association with LF values and increased sympathetic activity assessed by epinephrine and norepinephrine concentration during exercise, suggesting that sympathetic activity and circulating catecholamines are not reflected by LF augmentation (Breuer et al., 1993).

Conversely, the HF band is broadly viewed as a reflection of parasympathetic activity (Malik et al., 1996; McCraty & Shaffer, 2015; Montano et al., 2009; Shaffer et al., 2014; Thayer et al., 2010; Zygmunt & Stanczyk, 2010). Researchers employing ANS blockade have shown HF to be one of the strongest non-invasive indices of parasympathetic activity (Cacioppo et al., 1994). The representation of parasympathetic activity occurring at a higher frequency or faster

rate per second is physiologically sound, given the ability of the parasympathetic nervous system to directly alter the chronotropic activity of the heart via acetylcholine sensitive potassium ion channels.

A ratio of LF to HF power has been suggested to be a indictor of "sympathovagal balance" (Malliani et al., 1997; Montano et al., 2009). With the true underlying physiological driver of the LF band still unclear, multiple sources have refuted this suggestion with findings of little to no association between the ratio of LF to HF power and validated markers of ANS activity (Billman, 2013; Breuer et al., 1993; Cacioppo et al., 1994; Michael, Graham, et al., 2017). Therefore, this metric will be reported for the sake of comparison to other research, but it will not be used for the statistical analyses or as a true reflection of ANS activity.

It should be stated that a fourth common frequency band, ultra-low frequency, does appear below the 0.0033Hz cut off of VLF, but this only emerges during recordings lasting 24 hours or longer (Malik et al., 1996). Given that this dissertation will focus primarily on data from short-term records (2-10 minutes), properties of this frequency band will not be assessed or discussed.

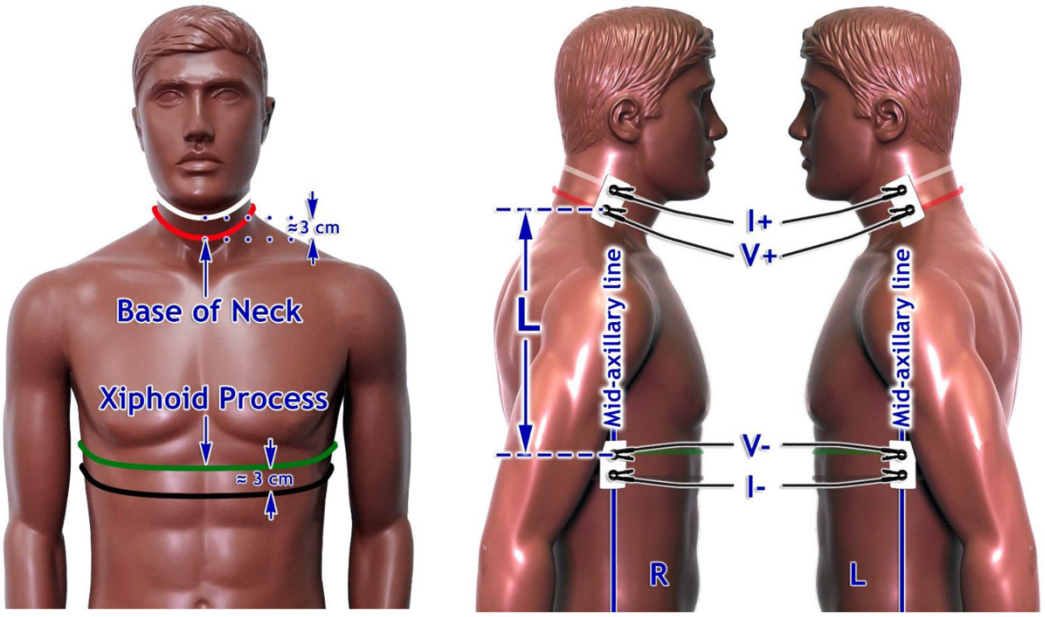
Systolic Time Intervals

Although there doesn't appear to be an agreed upon "gold standard" for measuring sympathetic activity (Grassi & Esler, 1999), systolic time intervals (STI) have been viewed as a non-invasive marker of cardiac performance since the 1940's (Benouar et al., 2018). Certain STI parameters have been repeatedly validated as indictors of sympathetic activity (Ablonskytė-Dūdonienė et al., 2012; Cacioppo et al., 1994; Michael, Graham, et al., 2017; Tavakolian, 2016). Originally collected by the simultaneous recording of an electrocardiogram, a carotid pulse tracing, and a phonocardiogram to estimate the movements of the aortic valve, STI provide a

non-invasive assessment of left ventricular function in regards to the amount of time required for the electrical activity of the heart to cause a mechanical effect (Lewis et al., 1977). Since it's conception, multiple methods of estimating this cause and effect relationship have been suggested in attempts to refine and simplify the data collection process. Recently, the use of esophageal doppler imaging, echocardiography, seismocardiography and impedance cardiography have all been suggested as alternative means of evaluating STI (Tavakolian, 2016). Compared to the other methods, impedance cardiography has the benefits of being one of the least invasive and most clinically repeatable measurements. Furthermore, the accuracy of impedance cardiography to estimate STI has been shown to be significantly correlated with STI measurements collected from highly invasive thermodilution techniques (Zoremba et al., 2007) and the less invasive (but still tedious) methods of echocardiography (Noda et al., 2017) and esophageal doppler monitoring (Lorne et al., 2014).

Impedance cardiography seeks to determine the timing of the opening and closing of the aortic valve by calculating the impedance of a small electrical current passing through the thorax. This is accomplished by the bilateral placement of paired electrodes at the base of the neck and around the chest at the level of the xiphoid process (Sherwood et al., 1990) with the intent to generate a small, imperceptible current and measure the voltage development across the thorax depicted in Figure 2.3.

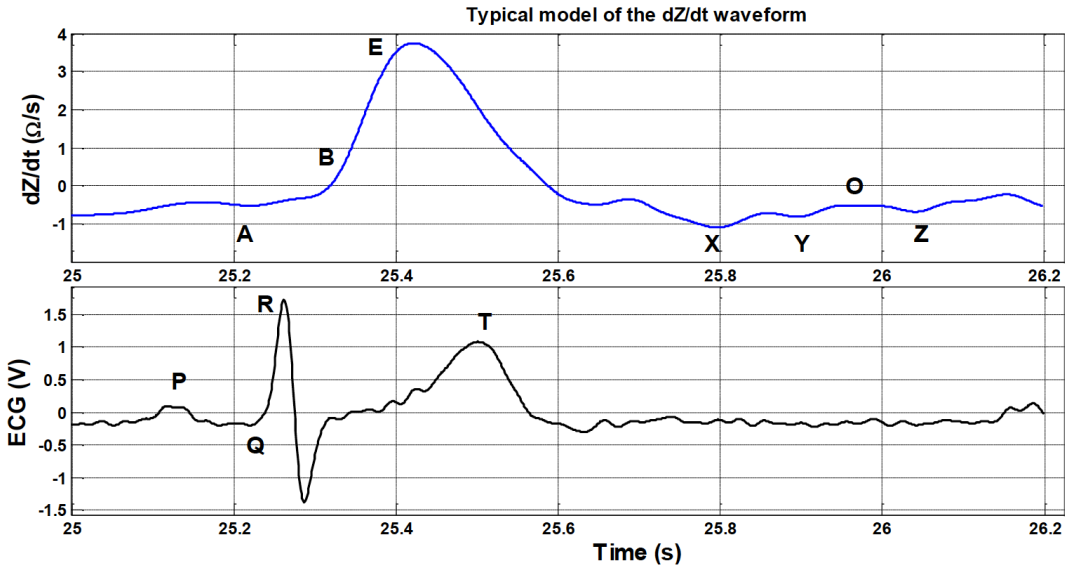
Figure 2-3. Typical Electrode Placement for Impedance Cardiograph.



Note: I, Current; V, Voltage. (As illustrated by Biopac Systems Inc.)

With this setup, the raw impedance value can be obtained and a calculated output of the change in impedance over change in time (dz/dt) measured in ohms per second can be generated. In order to calculate the desired STI, a simultaneous ECG tracing outlining the concomitant electrical activity is required since the impedance graph only specifies information regarding the mechanical cardiac events. A typical graphical product of both of these recordings is provided in Figure 2.4 with pertinent physiological events labeled on each tracing.

Figure 2-4. Diagram of typical Impedance Cardiography and ECG waveforms.



Note: (As illustrated by Benouar et al., 2018). See text for explanation. ICG points represent: A, atrial contraction; B, aortic valve opening; E, highest peak if dz/dt coinciding with maximal aortic flow; X, closing of aortic valve; Y, pulmonic point closing; O and Z, relating to mitral valve opening and possible regurgitation respectively.

Keeping in mind the inherent fluctuating nature of the electrical signal, the wave forms appearing in Figure 2.4 are typical morphology for both impedance and ECG voltage readings across the same section of time. The common ECG waveforms are labeled and presented on the lower section of Figure 2.4, with the P wave representing atrial depolarization, the QRS complex reflecting ventricular depolarization, and the T wave representing ventricular repolarization. The characteristics of impedance during a cardiac cycle are displayed on the same time scale. The A point signifies atrial contraction, followed shortly by the B point representing aortic valve opening. While not as clearly discernable as other specified wave forms, the location of the B point is typically defined as the point at which there is an initial rapid upstroke towards dZ/dt(max) or the highest peak of the dZ/dt signal. This apex or highest peak is labeled as the E

point (Sherwood et al., 1990). Physiologically, the E point, also referenced as the C point, is said to represent the maximal aortic flow (Benouar et al., 2018). The dZ/dt(max) precedes a sloping fall in impedance until the X point, the lowest point on the waveform, is reached. This low point represents the physical closing of the aortic valve and typically follows shortly after the completion of the T wave on the ECG in the same time series. The Y point reflects the pulmonic valve closing and is the minimum notch between the X point and the maximum impedance valve after the E point, known as the O point. The O and Z points respectively signify the opening of the mitral valve and any sign of mitral regurgitation if present (Benouar et al., 2018).

Metrics of Interest. Various physiological events and segments of time with deep functional value can be derived from the waveforms detailed above. Parameters such as cardiac output, stroke volume, left ventricular ejection time and the pre-ejection period as well as other factors can all be calculated using the combined recordings of ECG and impedance cardiography (Siedlecka et al., 2015). While it is possible to calculate stroke volume and thus cardiac output from the recordings, research has shown that the accuracy of impedance cardiography to observe these parameters should be questioned, with significant issues placed on the mathematical model employed in the analysis (Sherwood et al., 1990). However, the primary focus of this dissertation in regard to impedance cardiography is the estimation of the Pre-Ejection Period (PEP).

In terms of waveform analysis, the PEP is defined as the period of time between the Q wave on the ECG and the B point on the dZ/dt recording. The PEP is biologically defined as the interval of time from the initiation of left ventricle depolarization to the opening of the aortic value. The concept of the PEP is to estimate the amount of time required for the left ventricle to generate enough force to open the aortic valve once it has received the neural signal to contract.

Thus, the PEP can be used as an index of left ventricular contractility (Sherwood et al., 1990). Given that parasympathetic activity, while affecting HR, has minimal influence on contractility, an increase in contractility via neural stimulation can only be achieved by an increase in sympathetic activity. Thus, PEP is considered by most researchers as an acceptable marker of sympathetic activity (Cacioppo et al., 1994; Michael et al., 2017; Siedlecka et al., 2015).

Early studies on STI such as Ahmed et al. (1972), evaluated the validity of STI as a marker of myocardial performance and usefulness as a marker of left ventricular contractility. The study employed several invasive medical procedures including the placement of three cardiac catheters at the main pulmonary artery, left ventricular apex, and the aortic root used along with the coordinated injection of indicator dye in the left ventricle to directly measure left ventricular ejection fraction and cardiac output. Additionally, non-invasive recordings of ECG, oscilloscopic recordings, and an aortic pulse tracing were used to document pressure changes and STI parameters. After observing the cardiac function of 70 subjects, STI derived PEP positively correlated with direct measurements of cardiac pump function and exceeded several predetermined qualifications of a valid index of myocardial contractility (Ahmed et al., 1972).

Utilizing single and double autonomic blockade via metoprolol and atropine sulfate in 10 health males, Cacioppo et al. (1994) revealed that PEP derived from impedance cardiography and HF derived from short-term HRV were the only significantly reliable, non-invasive indices of sympathetic and parasympathetic nervous system activity respectively (Cacioppo et al., 1994). These findings echo those of Ahmed et al. (1972) in establishing the validity of PEP as a marker of myocardial contractility and sympathetic neural activity.

Since its origin, many studies have examined the properties of PEP and outlined the clinical significance of the parameter. In the late 1970s, critical reviews such as Lewis et al.

(1977), detailed the validity of utilizing PEP and other STI as clinical markers for various rhythm abnormalities and disease states (Lewis et al., 1977). Of more recent notoriety, researchers have suggested that PEP and other STI be used as non-invasive detectors of hypertension, cardiac insufficiency, and causes of acute dyspnea in addition to being used as an early stage risk factor for CVD development (DeMarzo, 2018; Siedlecka et al., 2015). Congruently, PEP has also been employed to evaluate ANS activity pre and post exercise in attempts to outline the initial disengagement and subsequent re-engagement of the parasympathetic nervous system (Michael, Graham, et al., 2017; Michael, Jay, et al., 2017a; Michael et al., 2018)

Table 2-1. Absolute Values for Short-term HRV and STI Measurements of Healthy Adults.

Measurement	Mean	SD	Range	Number of studies
[1]SDNN (ms)	50	16	32-93	27
[1]RMSSD (ms)	42	15	19-75	15
[1]LF (ms^2)	519	291	193-1,009	35
[1]LF (nu)	52	10	30-65	29
[1]HF (ms^2)	657	777	82-3,3630	36
[1]HF (nu)	40	10	16-60	30
[1]LF:HF	2.8	2.6	1.1-11.6	25
[2]PEP (ms)	n/a	n/a	50-120	n/a

Note: SD, standard deviation; SDNN, standard deviation of normal to normal beats; RMSSD, root mean square of successive differences between normal to normal beats; LF, low frequency power; nu, normalized units; HF, high frequency power; LF:HF, ratio of low frequency power to high frequency power; PEP, pre-ejection period. [1]As published by Nunan et al. (2010), [2]As published by Turner (2000).

Normative values for short-term recordings of HRV metrics of interest and PEP have been provided in Table 2.1 above. Given the widely agreed upon and followed guidelines of HRV recordings and analysis, these values are broadly accepted for recordings of 10 minutes or less in healthy adults. Such cohesion is not readily applicable to PEP values due to a lack of agreed upon measurement guidelines and the use of various methodological approaches to determining PEP. While methods previously discussed are significantly correlated with ICG derived PEP (Lorne et al., 2014; Noda et al., 2017; Zoremba et al., 2007), other methods such as phonocardiography and seismocardiography have been shown to produce significantly different PEP values compared to ICG (Dehkordi et al., 2019). Thus, staunch guidelines for PEP values are not fully available. The PEP range provided in Table 2.1 is based upon published clinical nursing guidelines for monitoring hemodynamics (Turner, 2000).

In sum, PEP can be used as a valid marker of sympathetic activity at the level of the heart. Coupled with various metrics of HRV, comprehensive observations of ANS activity can be achieved non-invasively. The remainder of this review of literature will focus on individual and compounding influences of various physiological variables on ANS activity by tracking changes in HRV and STI. The interrelated nature of these influences will be outlined in a conceptual model and give light to the rational for this dissertation.

Influencers of Autonomic Nervous System Activity

Glycemic Impairment

Overview. Type 2 diabetes mellitus (T2D) is the chronic disease state of poor glucose management and greatly effects the functionality of the ANS. This is primarily due to the metabolic imbalance associated with T2D and the resulting complication of cardiac autonomic neuropathy. Cardiac autonomic neuropathy is the impairment of cardiovascular autonomic

control due to nerve damage and affects 20-73% of T2D patients (Dimitropoulos, 2014; Fisher & Tahrani, 2017). Numerous studies have shown that altered ANS activity, characterized by reduced parasympathetic and elevated sympathetic activity, is associated with T2D and indices of glycemic impairment (Benichou et al., 2018; Seyd et al., 2008; Thayer et al., 2010; Wulsin et al., 2015). As the concept of glycemic impairment cannot be adequately evaluated by a single metric, this review will outline the relations of the most common indictors of glycemic impairment and ANS activity, as measured by HRV and STI. Glycemic impairment indictors include fasting blood glucose (FBG), insulin concentrations, and glycated hemoglobin (Hemoglobin A1c: HbA1c) concentration in the blood.

Fasting Blood Glucose. While the overall disease state of T2D has been associated with decreased parasympathetic and increased sympathetic activity, varying results have been found in regard to the relation between FBG concentration and altered ANS activity. FBG is used to evaluate current or transient blood glucose management status with a typical range being defined as less than 100 mg/dL (5.6 mmol/L). A concentration higher than 100mg/dL is viewed as a marker of poor glucose management, with concentrations between 100-126mg/dL termed as impaired FBG or pre-diabetic. Having FBG concentrations higher than 126 mg/dL on two separate occasions categorizes an individual as diabetic. Although FBG concentrations can be used to classify T2D status, it is inherently limited as a sole reflector of overall glucose management, given that concentrations constantly fluctuate. This fluctuation is caused by multiple factors including hormonal regulation, physical activity level, menstrual cycle, illness, stress and diet. The following discussion will summarize the general concepts of the relation between FBG concentration and ANS activity from both population and single cohort-based perspectives.

One of the first population based studies to examine the relation between FBG and ANS activity (Liao et al., 1995) focused on data from the Atherosclerosis Risk in Communities (ARIC) study. Using spectral HF power derived from two-minute ECG recordings, Liao and colleagues compared diabetic (n=154), defined as having a FBG greater than 140mg/dL, and non-diabetic (n=1,779) subjects while controlling for age, race, and gender. The diabetic group displayed significantly lower HF power compared to non-diabetic subjects [0.78 and 1.27 $(beat/min)^2$, respectively]. Further investigation of the non-diabetic group found a significant inverse linear trend between FBG and HF power when divided into quartiles. This relation lost statistical significance once the model was adjusted for age, race, and gender. It should be noted that only the highest quartile (>107mg/dL) did not fit the linear trend in the adjusted model, possibly due to the fact that FBG of this concentration already suggest glycemic impairment by present standards. In sum, it was concluded that non-diabetics display greater levels of parasympathetic activity as measured by HF power compared to diabetics. Moreover, FBG concentrations appear to have a negative relation with the amount of parasympathetic activity in non-diabetic subjects, specifically in individuals within the current recommended range (<100mg/dL) of FBG (Liao et al., 1995).

Similar results were found by Singh and colleagues (2000) utilizing data from the Framingham Heart Study. Groups from the offspring cohort stratified by FBG had ECG recordings completed between 1983-1987. A total of 1,919 subjects were used, with 1,779 classified as normal FBG (<110 mg/dL), 56 classified as impaired FBG (110-125mg/dL), and 84 classified as diabetic (≥126mg/dL). The natural log of SDNN, LF power, HF power, and LF/HF ratio derived from the first two hours of ambulatory activity were compared among the three groups. Additionally, a multivariable regression analysis was used to examine relations between

FBG and the same HRV metrics while controlling for age, sex, body mass index, heart rate, systolic and diastolic blood pressure, smoking, and the use of caffeine, alcohol, and cardiac medications. Group analyses revealed that the normal FBG group had significantly higher values of SDNN, LF and HF power, while the LF/HF ratio was only significantly lower in the diabetic group. The multivariate regression analysis demonstrated a significant inverse relation between FBG and SDNN, LF power, and HF power while accounting for the covariates. This association of higher FBG with lower HF values echoes the above findings of Liao et al., (1995). Taken together, these studies indicate that higher concentrations of FBG are associated with lower parasympathetic activity at the cardiac level.

A recent meta-analysis of over 25 studies, focusing on the relations between HRV indices observed in 24 hours ECG recordings and T2D status, was conducted by (Benichou et al., 2018). The analysis also examined the relations among multiple HRV indices and clinically relevant parameters of T2D status, including gender, age, FBG, HbA1c, time from diagnosis, systolic and diastolic blood pressure, and lipoprotein concentrations. Adults being treated with oral antidiabetic agents were classified as T2D. All studies used age and gender matched non-diabetic controls. It is important to note only one of these 25 studies controlled for body mass index (BMI), only two controlled for body weight, and only one controlled for blood pressure differences between groups. Overall, individuals with T2D (n= 1,356) presented with significantly lower SDNN, RMSSD, total spectral power, LF, and HF power compared to healthy controls (n = 1,576). These results collectively demonstrate low HRV in those with T2D, supporting the above findings of Singh et al. (2000) and Liao et al. (1995). Conversely, the meta regression analysis across the whole population revealed that FBG was positively associated LF power, HF power, RMSSD, and SDNN. The authors concluded that these results

may be due to the deleterious effects of altered glucose metabolism and suggest that an increase in FBG was associated with increases in both parasympathetic activity (as represented by RMSSD and HF power) *and* sympathetic activity (as represented by SDNN and LF power). While this dissertation does not support the use of SDNN and LF power as markers of sympathetic activity, it does acknowledge the interpretation that an increase in these specific markers would represent an increase in HRV components. These conflicting findings could be due to the lack of control for BMI, body weight, and blood pressure as previously noted. Elevations in BMI (Molfino et al., 2009) and blood pressure (Schroeder et al., 2003) have been found to be significantly associated with decreased HRV. Thus, a lack of control for these factors could muddy the findings of this meta-analysis. Additionally, the difference in recording length (24 hours vs two minutes) could play a major role in these findings, since the physiological interpretation of HRV metrics differ depending on the length of the recording.

The results of various smaller cohort studies are more diverse than those of the population-based findings, possibly due to the lack of universal control for assorted covariates as well as differing methods of observation. Cross-sectionally, Lutfi & Elhakeem (2016) evaluated the relations between FBG concentration and various HRV metrics derived from five-minute ECG recordings in 42 non-diabetic males free from disease. Individuals in the lowest quintile of FBG concentration (<73mg/dL) had significantly lower logarithmic HF power and normalized HF power and higher values of normalized LF power than individuals in the other four quintiles. BMI, mean arteriole blood pressure, mean heart rate, age, and gender did not significantly impact the relations between FBG and the HRV metrics as determined by linear regression analysis. No significant differences were found among the other four quintiles. These results contrast those of Perpiñan, Severeyn, Wong, & Altuve (2019), who found that healthy, sedentary, individuals with

an average FBG of 95 mg/dL had significantly lower values of normalized LF and significantly higher values of normalized HF compared to age-matched subjects with diagnosed metabolic syndrome. Both studies included individuals with normal FBG concentrations but found opposing results. This could be due to the effects of the considerably lower mean FBG concentrations reported in the study by Letfi & Elhakeem. While not considered hypoglycemic, 73mg/dL could be low enough to stimulate increases in the release of glucose regulatory hormones (e.g., glucagon, epinephrine, and cortisol) in an attempt to increase blood glucose concentrations, which would reflect less parasympathetic and greater sympathetic activity.

Multiple studies have also observed ANS activity in response to increased glucose concentrations during oral glucose tolerance testing (OGTT). Perpiñan et al. (2019) measured five-minute HRV recordings and blood glucose and insulin concentrations in 30-minute intervals before and 120 minutes after OGTT in 11 subjects with metabolic syndrome and five healthy age-matched controls. Compared to fasting values, those with metabolic syndrome displayed higher levels of SDNN at 30, 60, 90, and 120 minutes post OGTT, absolute LF power at 60, 90, and 120 minutes post OGTT, normalized LF power 120 minutes post OGTT, and lower normalized HF power 120 minutes post OGTT. The control group did not display significant changes in HRV parameters at any time point post OGTT compared to fasting (baseline) values. These results suggest that an increase in blood glucose concentration in those with poor glucose metabolism will result in ANS activity markers that reflect increased sympathetic drive and decreased parasympathetic tone while simultaneously displaying increased overall HRV (SDNN). Contrasting reactions in SDNN values following an OGTT were found by Perticone et al. (2016) in that subjects with normal glucose tolerance, impaired glucose tolerance, and T2D all displayed decreases in SDNN compared to fasting values. It was observed that the T2D

subjects had lower SDNN levels at two hours post OGTT compared to the normal glucose tolerance group. Additionally, LF and HF power values were not individually reported but the LF/HR ratio was noted to be elevated at all time pointes in the T2D group compared to the normal glucose tolerance group. In sum, an increase in blood glucose resulted in a decrease in overall HRV (SDNN) regardless of fasting glucose metabolism status and those individuals suffering from poor glucose metabolism displayed altered HRV metrics compared to health controls. Perticone et al. (2016) and Perpiñan et al. (2019) utilized different recording lengths (30 minutes and 5 minutes respectively) and both lacked control for obesity status or body composition.

Kanaley et al. (2007) provided data on ANS activity alteration before and after OGTT in females grouped by obesity and T2D status while accounting for body composition and cardiorespiratory fitness. The groups consisted of nine subjects with obesity and T2D, 22 non-T2D obese subjects, and 11 non-obese subject free from T2D, with all subjects completing an exercise test on a prior laboratory visit to determine cardiorespiratory fitness. HRV analyses derived from 10-minute supine ECG recordings and blood samples were taken before and after an OGTT. An up-right tilt test was done before and after the OGTT with HRV analyses and blood samples were taken on each occasion. Group by position (supine vs tilt) by glycemic load (pre vs post OGTT) statistical analysis revealed that all group displayed an increase in HF power following the OGTT. Interestingly, the only significant group difference was in the natural log of the LF/HF ratio, with the T2D group displaying significantly lower levels compare to both the obese and non-obese groups. Furthermore, neither fasting glucose or insulin concentrations were significantly correlated with any HRV metric. Notable differences in post OGTT data collection time were reported for the T2D group (1 hour) compared to both the obese and non-obese groups

(30 minutes) with the authors suggesting that these were the times that each group had the highest concentration of blood glucose. In spite of the lack of group differences, these results demonstrate that an increase in blood glucose is accompanied by an increase in a marker of parasympathetic activity (HF power) and provide yet another paradigm for the relation between ANS activity and blood glucose concentration.

PEP and its relation to FBG has been published to a lesser extent. Sykes, Wright, Malins, & Pentecost (1977) examined the changes in PEP in 19 diabetics undergoing treatment via diet modification (n=9) or sulphonylurea usage (n=10) compared to 27 healthy, age and weight matched controls over a three-month period. At baseline, all diabetic subjects had evaluated FBG concentrations compared to the controls. Additionally, PEP length corrected for resting HR was significantly shorter in all diabetic subjects compared to health controls, denoting an increase in sympathetic activity at the cardiac level in the diabetic subjects. After three months of treatment, both the diet modification and sulphonylurea groups presented with significantly decreased FBG concentrations, while the diet modification group still had significantly evaluated concentrations compared to the control group. Moreover, both treatment groups had markedly lengthened PEP measurements that were not significantly different than the control group. These results provide evidence that an improvement in glucose management represented by a decrease in FBG is related to a decrease in sympathetic activity at the cardiac level. Similar results were found more recently by Synowski, Kop, Warwick, & Waldstein, (2013) while investigating the effects of glucose ingestion on STI metrics before and after mental challenges in obese males. On two separate occasions, the subjects ingested a 20% solution of glucose at 1g of glucose per kilogram of body weight or a volume matched placebo of sugar-free Kool-Aid. Pre and 30 minutes post-ingestion impedance cardiography and ECG measurements were recorded for five

minutes. While changes in blood glucose were not confirmed, the ingestion of a glucose solution resulted in significant, prolonged shortening of PEP length for the remainder of the visit. This echoes the results of Sykes et al. (1977) in that an increase in blood glucose was related to an increase in sympathetic activity as represented by a decrease in PEP length.

Individually, each of the smaller cohort studies provide different pictures of ANS activity and acute blood glucose concertation. Holistically, blood glucose concentration is likely related to ANS activity, as individuals with glycemic impairment generally display indices of increased sympathetic and decreased parasympathetic activity. It has been suggested that periods of chronic hyperglycemia, in which glucose concentrations are evaluated for multiple months to years could have a more meaningful impact on ANS activity compared to acute fluctuations. Additionally, it is currently not possible to state whether this relation is caused by the acute effects of glucose on cardiac and neural tissue or if a simultaneous fluctuation in insulin concentration might have some effect. Furthermore, modifications of the hypothalamic-pituitary-adrenal (HPA) axis may play a mitigating role in the observed relation between blood glucose and ANS activity. Research into the relations of prolonged glycemic impairment and insulin concentrations with ANS activity will be presented in subsequent sections, followed by a brief outline of the HPA axis and its implications.

HbA1c. Given the evidence for a connection between ANS activity and acute glucose concentrations, it is logical that chronically elevated levels of glucose would have a magnified impact on ANS activity. Such observations have been conducted with particular attention given to glycosylated hemoglobin. Commonly used as an indicator of prolonged glucose homeostasis, glycosylated hemoglobin or Hemoglobin A1c, (HbA1c) is also used to categorize diabatic status. Having a HbA1c level of 6.5% or higher on two separate occasions indicates diabetic status, with

percentages between 6.5 and 5.7 denoting pre-diabetes and non-diabetic status residing below 5.7%.

Jaiswal and colleagues evaluated the relations of multiple HRV parameters and various physiological characteristics of 530 age matched youths with and without (control group) type I diabetes (Jaiswal et al., 2013). Compared to the control group, the diabetic group displayed significantly lower SDNN, RMSSD, and normalized HF power alongside elevated normalized LF power. Additional analysis was conducted on the diabetic group by subdivision based on HbA1c level (above and below 7.5%) and revealed that all HRV metrics were further reduced in those with HbA1c greater than 7.5% compared to the control group. Interestingly, following the subdivision, those diabetics with HbA1c less than 7.5% were no longer significantly different from the control group. These findings support the view that prolonged glycemic impairment is related to altered ANS activity characterized by reduced parasympathetic activity. Similar results were discovered analyzing information collected in the Data from an Epidemiology Study on Insulin Resistance syndrome (DESIR) Study. A significant linear trend between HbA1c percentage and SDNN derived from five-minute ECG recordings among quartiles of diabetic status denoted a reduction in SDNN was associated with an increase in HbA1c percentage (Valensi et al., 2011).

Though studying different recording lengths, Boer-Martins et al (2011) found the same inverse association between SDNN and HbA1c levels in hypertension patients with and without T2D. Calculated from the 24-hour ECG recording, total group analysis determined that SDNN was negatively correlated with HbA1c levels ($r = -0.58$, $p < 0.000$). The meta-analysis by Benichou et al. (2018) on 24-hour ECG recordings harmonizes with these findings reporting that HbA1c percentage was significantly associated with decreased HRV as defined by a decrease in

R-R intervals. Furthermore, time from diagnosis of diabetes was inversely related to total spectral power in diabetics. Together, these results support the notion that altered ANS activity intensifies with sustained glucose mismanagement.

Conversely, the findings outlined above by Kanaley et al (2007) do not fully agree with this paradigm. While ANS activity differed between the groups stratified by obesity and diabetic status, there was no significant group difference in regard to HbA1c levels. This suggests that the increase in parasympathetic activity post glucose consumption is not further influenced by imbalances in prolonged glucose homeostasis beyond HbA1c's relation to diabetic status determined by FBG. Moreover, it has been suggested that baseline HbA1c is not significantly associated with observed decreases in PEP length post insulin treatment in diabetics (Dungan et al., 2013). Beyond agreeing with the lack of impact found by Kanaley et al (2007), this implies that insulin could have an influence on ANS activity regardless of HbA1c status. The following section will review this concept and confirm the relation between insulin concentration and ANS activity.

Insulin. It is apparent that insulin concentrations are fundamental in the relation between ANS activity and glycemic impairment, as insulin is both a key regulatory hormone for glucose homeostasis and insulin secretion is sensitive to neural output. Insulin is the principle hormonal stimulator of glucose uptake from the circulation, hepatic glycogenesis, lipogenesis in the adipose tissue, and stimulates cellular growth. Release of Insulin from pancreatic beta cell islets is stimulated by both elevated blood glucose concentrations and parasympathetic stimulation from the vagus nerve. Conversely, lowered blood glucose concentrations, sympathetic stimulation from the lower thoracic and upper lumbar preganglionic sympathetic neurons, and sympathetically driven hormones (epinephrine, norepinephrine, and glucagon) all inhibit the

release of insulin. Thus, insulin is an intertwined aspect of the relation between ANS activity and glycemic impairment.

Research on insulin concentrations and markers of ANS activity has been conducted independently and congruent with glucose concentrations. In the above study by Liao et al. (1995), the relation between insulin concentration and HRV metrics derived from two-minute ECG recordings was evaluated using the Atherosclerosis Risk in Communities dataset. Quartile comparisons revealed a significant inverse linear trend between insulin concentration and HF power in non-diabetic subjects, with the lowest quartile of insulin presenting with the highest value of HF power (1.34 beats/min^2) while the highest quartile of insulin presented the lowest value of HF power (1.14 beats/min^2). This suggests that higher concentrations of insulin are associated with lower parasympathetic activity in non-diabetic individuals.

A similar inverse trend was found by Saito et al. (2015) examining the Toon Health Study. Quartiles of SDNN values derived from five-minute ECG recording were taken from 1,899 individuals not currently taking diabetic medication. Quartile comparison displayed a significant inverse relation with insulin concentrations, in that the lowest quartile of SDNN had the highest concentration of insulin (36.0mmol/L) and the highest quartile of SDNN had the lowest concentration of insulin (32.4mmol/L). The study compared frequency component HRV analyses to insulin sensitivity via the Gutt's insulin sensitivity index, rather than insulin concentration, per se. Similarly, Festa, D'Agostino, Hales, Mykkanen, & Haffner (2000) observed that heart rate had a significant relation with fasting insulin (r=0.20) as well as an acute insulin response (r=0.18) to a glucose load in 1,000 nondiabetic subjects. These relations remained significant after accounting for age, sex, ethnicity, smoking status, and glucose tolerance status. Taken together, these studies provide evidence that HRV decreases and heart

rate increases as insulin concentrations increase, suggesting a predominance of sympathetic activity.

The notion of increased sympathetic activity is not shared by Quilliot, Zannad, & Ziegler (2005), who observed an increase in normalized HF power in groups with varying BMI values and insulin resistance status after an OGTT. Groups consisting of non-obese (BMI 18-24.9kg/m^2) without insulin resistance, overweight (BMI 25-29.9kg/m^2) with, class I and II obesity (BMI 30-39.9kg/m^2) and class III obese (BMI $\geq$ 40.0kg/m^2) were evaluated before and after an OGTT using 10-minute heart rate recordings. Based on group averages, both obesity groups were classified as having insulin resistance. All groups displayed increased LF power, and normalized LF and HF power following the OGTT, with the two obesity groups having the highest area under the curve for normalized HF power and lowest LF power post OGTT. The obesity groups also displayed the highest concentrations of insulin and blood glucose, suggesting that these concentrations are associated with the altered ANS activity post OGTT. While providing opposing results to research pervious presented, these findings could not delineate the individual effects of increased blood glucose and insulin concentrations. Furthermore, this study utilized finger photophlethysmography to monitor heart rate and calculate HRV alterations instead of a standard ECG recording. These severe differences in methodology could introduce a potential source of error.

More in-line with the large cohort studies, Perciaccante, Fiorentini, Paris, Serra, & Tubani (2006) presented data on 24 hour ECG recordings across groups of varying insulin resistance status and age matched controls. HRV was calculated during nighttime and daytime hours as well as total 24-hour period. Total SDNN was significantly decreased in all insulin resistance groups compared to the normal glucose tolerance controls, suggesting a decrease in

HRV with an increase in insulin concentrations. Additionally, total normalized LF power was significantly elevated while normalized HF power was significantly decreased in all insulin resistance groups compared to the control group. Those with the most severe status of insulin resistance (T2D) had the lowest values of normalized HF and LF power compared to the other insulin resistance groups. Taken together, these data support the notion that an increase in insulin concentration is associated with a decrease in parasympathetic activity accompanied by an increase in sympathetic activity resulting in an overall decrease in HRV in individuals with glycemic impairment.

Individuals with normal glucose management have been shown to have different ANS reactions with alterations in insulin concentrations. Stockhorst, Huenig, Ziegler, & Scherbaum (2011) evaluated the effects of insulin, glucose, or placebo injections on HRV metrics in apparently healthy, normal weight individuals. HF power, insulin, and glucose were assessed at regular time intervals post injections. Both injections of insulin and glucose resulted in large increases in insulin concentrations that aligned with spikes in the total change of HF power values compared to the placebo injection of volume matched saline solution. These results demonstrate that in individuals with healthy glucose management, a direct increase in insulin concentration or increased stimulation of insulin secretion via increase in blood glucose is associated with increased parasympathetic activity. Thus, it appears that glycemic impairment status alters the ANS response to insulin concentration fluctuations.

From a medical treatment standpoint, most research supports the concept that insulin infusions alter ANS activity, typically in a sympathetically predominant manner. An infusion of a glucose, insulin, and potassium cocktail has been used for decades to increase cardiac parameters in cardiovascular patients (Klein & Visser, 2010). Postulated to be caused by

calcium dependent and independent pathways (von Lewinski et al., 2005), insulin infusions have been shown to exert positive inotropic effects on the heart, including an increase in cardiac index and coronary blood flow (Klein & Visser, 2010).

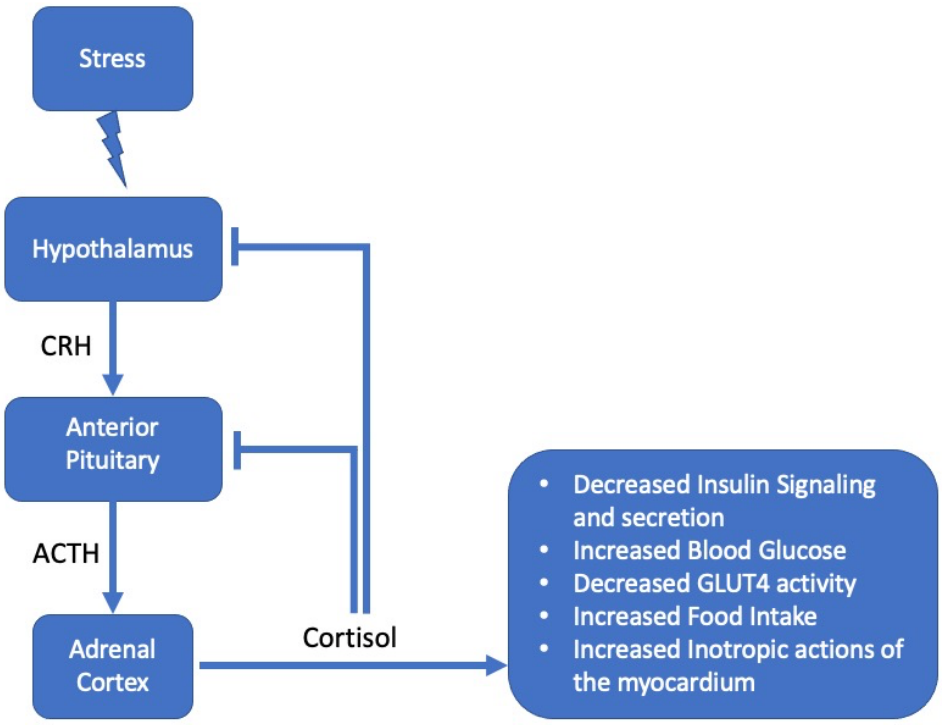
Injections of insulin alone have been shown to increase heart rate, pulse pressure, double product, and mean arterial pressure, suggesting an increase in sympathetic activity at the cardiac level (Rowe et al., 1981). Additionally, insulin infusions in hospitalized diabetic patients resulted in decreased PEP length at six hours and 24 hours post administration, further representing an increase in sympathetic activity (Dungan et al., 2013). This relation of increased insulin concentration and accompanying decrease in PEP length is also supported by Ochoa et al. (2015) who investigated the association between insulin resistance and ANS activity in 731 non-diabetic individuals. Quartile comparison based on homeostasis model assessment of insulin resistance scores demonstrated that increasing the degree of insulin resistance (and subsequent concentration of insulin) was associated with a shorting of PEP length regardless of BMI and blood pressure status.

In summary, it appears that insulin's influence on ANS activity is dependent on multiple factors including disease status. As outlined above, most of the sympathetic drive effects of insulin on the cardiac tissue are more pronounced in individuals with some degree of disease status. This could possibly be due to the concept that it is difficult to improve myocardial function in tissue that is currently performing adequately (Klein & Visser, 2010). Even in disease-free individuals, the sympathetic effects of insulin appear to manifest most in those that are approaching disease status i.e. pre-diabetic (Ochoa et al., 2015). Furthermore, there are site specific differences in the ANS reaction to insulin binding. In the above studies, the effects of insulin administration focused primarily on the myocardial outcomes. However, insulin

injections into cerebral ventricles can stimulate areas of the hypothalamus resulting in increased parasympathetic activity to the liver and white adipose tissue. This action causes reduced hepatic gluconeogenesis and increased lipogenesis (Ruud et al., 2017). This postulates the interaction of insulin and glucose metabolism and the HPA axis. The following section will highlight the main concepts of the HPA axis and its likely involvement in glycemic impairment.

HPA Axis. While the mechanistic effects of the HPA axis on glycemic impairment and ANS activity goes beyond the scope of this dissertation, a general description of how the two are related is warranted. As its name implies, the HPA axis reflects how a physiological or mental stressor stimulates a cascading effect among the hypothalamus, anterior pituitary, and the adrenal glands. Graphically outlined below in Figure 2.5, acute stress stimulates the release of corticotrophin-releasing hormone (CRH) from the hypothalamus, which stimulates the anterior pituitary gland to release adrenocorticotrophic hormone (ACTH). ACTH then travels through circulation to the adrenal cortex and stimulates the release of cortisol. Following its release, cortisol provides negative feedback to the hypothalamus and anterior pituitary gland, inhibiting the release of CRH and ACTH respectively. Additionally, multiple downstream effects are caused by cortisol, including alterations in glucose metabolism and cardiac performance.

Figure 2-5. Diagram of HPA Axis



Note: CRH, corticotrophin-releasing hormone; ACTH, adrenocorticotrophic hormone

In terms of glucose management, glucocorticoids such as cortisol act in various ways to increase concentrations of blood glucose including impairing peripheral insulin signaling resulting in insulin resistance (Rafacho et al., 2014), decreasing the functionality of glucose transporters in skeletal muscle (Weinstein et al., 1995), and increasing food intake (Torrezan et al., 2019). Additionally, cortisol is associated with a decrease in insulin secretion (Kamba et al., 2016), compounding its effects on rising blood glucose concentration. All of these actions support the concept that dysregulated HPA axis activity is related to the pathophysiology of T2D (Chan et al., 2005; John & Buckingham, 2010; Torrezan et al., 2019).

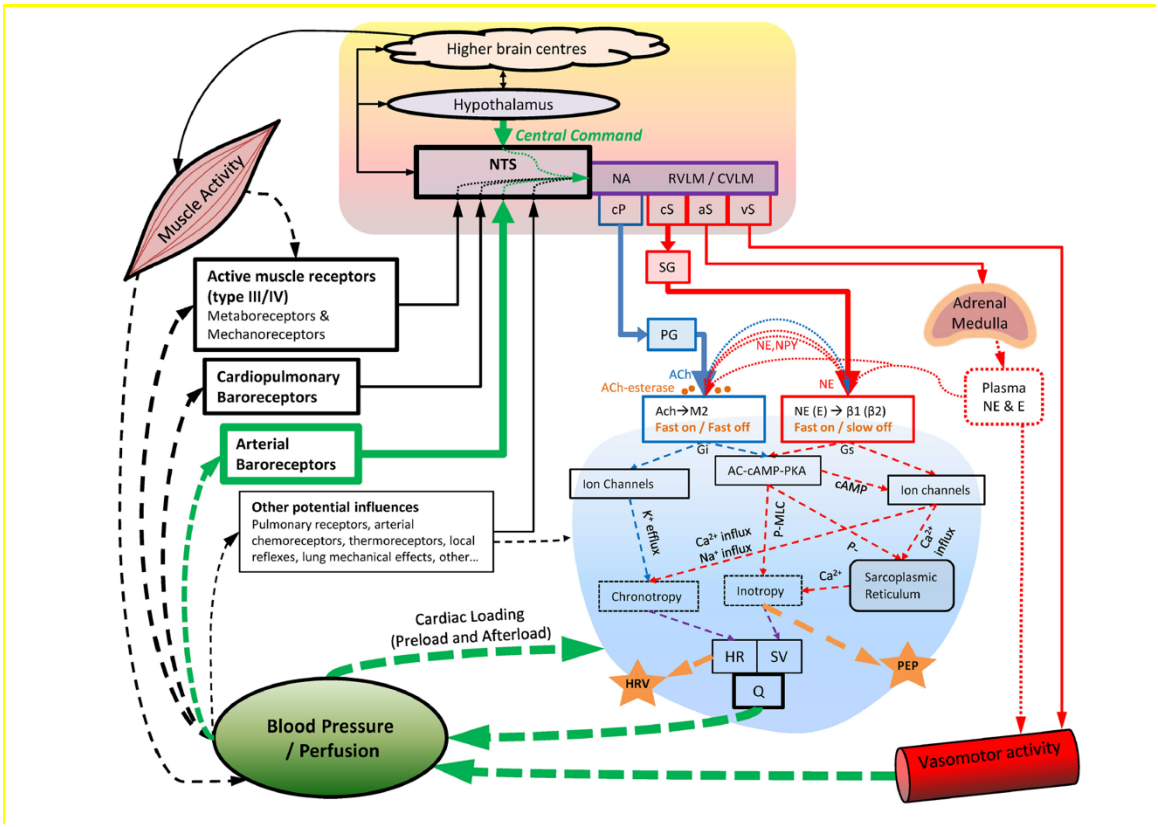
Acutely, glucocorticoids stimulate an inotropic effect in cardiomyocytes (Penefsky & Kahn, 1971), such that the binding of cortisol to the glucocorticoid receptor on cardiac muscle

tissue will cause an increase in contractile force. Additionally, cortisol plays a role in inhibiting the apoptotic effects of ischemia in cardiomyocytes (Oakley & Cidlowski, 2015). Both of these mechanisms are critical for short-term reactions to physiological or environmental stressors and are fundamental for the natural development and normal function of the heart. However, chronically highly levels of cortisol, like those seen in Cushing's Syndrome, are associated with the development of cardiomyocyte hypertrophy, hypertension, dyslipidemia, obesity, and increased risk of cardiovascular morbidity and mortality (Whitworth et al., 2005). Given this, it is apparent that a dysregulation in HPA axis activity resulting in prolonged cortisol secretion will have drastic effects on cardiovascular function. The impact of these cardiovascular dysfunctions, specifically hypertension, and their relation to ANS activity will be outlined in the following section.

Cardiac Stress Parameters

This dissertation has previously outlined the mechanisms by which the ANS directly effects chronotropic and inotropic cardiac function, as well as the indirect influence produced by altered ANS activity via dysregulated HPA axis activity. The resulting feedback loops created by those changes in cardiac functionality have yet to be addressed. Figure 2.6 provides an exceptional depiction of the interrelated nature of ANS innervation and the cardiovascular system as illustrated by Michael et al. (2017).

Figure 2-6. Regulatory Pathways of Blood Pressure and ANS Innervation.



Note: AC-cAMP-PKA, adenylate-cyclase/cyclic-AMP/Protein-kinase-A cascade; ACh, acetylcholine; aS, sympathetic outflow to adrenal medulla; β1 (β2), Beta1 (Beta2) adrenergic receptors; Ca2+, calcium ions; cP, cardiac parasympathetic outflow; cS, cardiac sympathetic outflow; CVLM, caudal ventrolateral medulla; E, epinephrine; Gi, G-protein inhibitory subunit; Gs, G-protein stimulatory subunit; HR, heart rate; HRV, heart rate variability; K+, potassium ions; M2, M2muscarinic receptor; MLC, myosin light chain; NA, nucleus ambiguus; Na+, sodium ions; NE, norepinephrine; NPY, neuropeptide Y; NTS, Nucleus Tractus Solitarii; P-, phosphorylation; PEP, pre-ejection period; PG, parasympathetic ganglia; Q, cardiac output; RVLM, rostro ventrolateral medulla; SG, sympathetic ganglia; SV, stroke volume; vS, vascular sympathetic outflow. (As illustrated by Michael et al., 2017)

The neural activity from the hypothalamus, through the nucleus tractus solitarius, nucleus ambiguous, and medulla, traveling to the heart via their corresponding ganglia, and their respective effects on the ionic and cellular activity was discussed in an earlier section. These actions culminated with alterations to chronotropic and inotropic heart function, presented as a change in heart rate (HR) and myocardial contractility. Oscillations in HR will directly affect and provide observable changes in HRV metrics. Additionally, fluctuations in myocardial contractility are reflected by variations in the length of PEP. The subsequent effects of chronotropic and inotropic modifications on stroke volume, cardiac output (Q), and pathways of neural feedback are further detailed.

Alterations in heart rate and stroke volume will result in a fluctuation in cardiac output (Q). These oscillations in Q will influence blood pressure and the rate of prefusion. This is enhanced by vasomotor activity in peripheral blood vessels that are sensitive to sympathetic activation via NE and E released from the nervous and adrenal systems. The adjustments in pressure and perfusion will stimulate various receptors located in the aorta, cardio-pulmonary circuit, and active muscles. These receptors translate changes in blood pressure and perfusion into neural signals that create a feedback loop of neural activity that is received and processed by the NTS to modulate ANS output. Thus, Figure 2.6 keenly illustrates the need to account for the influence of HR and blood pressure on measurements of ANS activity. Current views on these influences are outlined in the following subsections.

Heart Rate. It is widely accepted that most HRV metrics are significantly influenced by HR (Billman, Huikuri, Sacha, & Trimmel, 2015; Bolea, Pueyo, Orini, & Bailón, 2016; Gasior et al., 2015; McCraty & Shaffer, 2015; Monfredi et al., 2014; Sacha, Barabach, et al., 2013; Sacha, Sobon, Sacha, & Barabach, 2013; Shaffer et al., 2014). This is due in part to the physiological

link between HR and HRV, as previously outlined, and the mathematical fact that HR has a non-linear, inverse relation to R-R intervals, in that an increase in HR results in an unequal decrease in R-R intervals (Sacha, 2013). An explanation of this relation and its accompanying ceiling effect has been presented since the 1980's (Akselrod et al., 1985). While the existence of this mathematical linkage is agreed upon, the means to account for it is still being debated. One of the prevailing methods is to normalize the individual HRV metrics (de Geus et al., 2019; Laborde et al., 2017). A popular approach to normalize the data is to divide each HRV metric by the mean R-R interval for the time epoch raised to a corresponding power (Sacha, 2013; Sacha, Barabach, et al., 2013; Sacha, Sobon, et al., 2013). The time domain metrics of SDNN and RMSSD would each be divided by the mean R-R interval, while the power spectral density measurements of the frequency domain would be divided by the mean R-R interval squared (de Geus et al., 2019; Sacha, Barabach, et al., 2013). More complex corrections have been suggested but this simple normalization process is supported by a majority of researchers (Billman et al., 2015; de Geus et al., 2019; Gasior et al., 2015; L. E. V. Silva et al., 2017; van Roon et al., 2016). Some researchers believe that normalization of the HRV metrics may still not be enough to account for the full influence of HR, given that HR is the inherent sampling rate of the HRV signal (Bolea et al., 2016). Nevertheless, normalization of HRV metrics to mean R-R intervals is a valid data processing method to use during a stationary period. In data analytics, the principle of stationarity expresses the requirement that the statistical properties (e.g. mean and variance) of time series data does not change over the length of the recording. This does not mean that the series does not change over time, just that the rate or magnitude of change is consistent and does not follow a trend. If this statistical principle is violated, as it is during exercise recovery HR

recordings, the data will need to be detrended to account for the downward trend in HR before HRV metrics are calculated (Tarvainen et al., 2002).

A similar relation between HR and PEP has long been suggested and described in an inverse, linear manner (Weissler, 1983; Weissler et al., 1968). This linear decrease in PEP length with an increase in HR appears logical in that an increase in the frequency of contractions would decrease the amount of time available to cause said contraction. This relation has be observed and supported by additional works (Cokkinos et al., 1976; Cybulski, Niewiadomski, Strasz, Laskowska, & Gąsiorowska, 2009; Warrington, Weerasuriya, & Burgess, 1988). Correction equations based on population studies have been used to correct for this underlying relation when using PEP as a diagnostic tool for cardiovascular health, with the most frequently used being $PEP_{corrected} = PEP + 0.40(HR)$ (Warrington et al., 1988; Weissler et al., 1969). These findings are not universally shared in that other research groups have found no significant relation between HR and PEP (Ferro et al., 1980; Mertens et al., 1981; Rousson et al., 1987; Sundberg, 1986). These studies suggest that PEP should not be corrected for mean HR during a steady state. The majority of these studies were conducted on small sample sizes, while studies by Weissler and colleagues' utilized population-based observations. The most robust defense for not correcting PEP for mean HR comes from Cacioppo et al. (1994), who found that corrected PEP was a less accurate marker of sympathetic activity than uncorrected PEP during varying autonomic blockades. Recent studies use this research to justify not correcting PEP values for mean HR during a given time epoch (Michael et al., 2018; Michael, Jay, et al., 2017b). It should be noted that an alternative line of thought suggests that the true nature of the HR and PEP relation is based on the linkage of PEP and R-R intervals of the given time period (Van der Hoeven et al., 1977; Wolf et al., 1978). Thus, instead of correcting for mean HR, PEP

measurements should be corrected or normalized by mean R-R intervals. It has been further recommended that superior methods of correction for cases of repeat observations or group comparisons would be to use the Theil-Sen regression equation, PEP = 70.3 + 0.0367(RRI), or an analysis of covariance respectively (Puri & Sen, 1971; Theil, 1992; Wolf & Belz, 1981). Given that only 5-10 consecutive cardiac cycles are needed to calculate PEP, there is no current recommendation to detrend raw signals before conducting STI calculations.

Blood Pressure. It has been suggested that the link between ANS activity and hypertension may be a large factor for the relation of ANS activity and CVD risk (Thayer et al., 2010). While Figure 2.6 provides a mechanistic explanation of how short-term adjustments in ANS activity are related to changes in blood pressure, population-based studies have found data on the long-term effects of this relation. Baseline group comparisons and three-year preceptive analysis revealed a strong link between baseline ANS activity and hypertension status and its development in data from the ARIC study (Liao et al., 1996). At baseline, hypertensive subjects (n = 650), defined as those with systolic blood pressure of at least 140mmHg, diastolic blood pressure of at least 90mmHg, or who were taking medication for hypertension, were found to have significantly lower HF power, LF power, and SDNN compared to 1,411 normotensive subjects. This suggests that lower HRV is related to a hypertensive state. Odds ratios for the development of hypertension were calculated from the quartile comparisons of baseline HRV values and hypertension status at a three-year follow-up. Of the 1,411 normotensive subjects, 173 developed untreated-hypertension and another 477 were being newly treated for hypertension. Baseline HF power had a significant inverse relation with the development of hypertension, with those in the lowest quartile having a 2.44 odds ratio compared to the highest quartile. SDNN displayed a similar relation with the lowest quartile having a 1.74 odds ratio for

the development of hypertension compared to the highest quartile. Both relations suggest that lower levels of HRV, specifically lower parasympathetic activity as measured by HF power, increase the risk of hypertension. This notion was further confirmed by the findings of Schroeder et al. (2003) who examined nine years of data from the ARIC study. As with Liao et al. (1996), hypertensive subjects were found to have lower indices of HRV (RMSSD and SDNN) at baseline compared to normotensive subjects. Moreover, quartile comparison of baseline SDNN values revealed that those normotensive subjects with the lowest value of HRV had a hazard ratio of 1.24 for the development of hypertension over a nine-year span. Quartiles based on baseline RMSSD values had a similar trend in that the normotensive subjects with the lowest RMSSD value had a hazard ratio of 1.36 for the development of hypertension. This further supports the relation between ANS activity and hypertension. While the ARIC dataset utilized short, two minute records of HRV analysis, similar relations have been investigated over two hour records from the Framingham Heart Study (Singh et al., 1998). Hypertensive subjects (n = 245) displayed significantly lower SDNN, RMSSD, HF power, and LF power at baseline compared to normotensive subjects (n=686). However, only baseline LF power was a significant predictor of hypertension development in males at a four-year follow-up visit.

Similar cross-sectional findings are presented by Fagard, Pardaens, & Staessen (2001), who observed subjects receiving treatment for hypertension (n=84) having significantly lower absolute LF and HF power compared to normotensive subjects (n=337) during resting conditions. However, these relations were not significant during comparisons of 24-hour ambulatory blood pressure measurements. Correlation analysis over the entire population revealed that normalized LF and absolute HF power were significantly correlated with conventional systolic (r = 0.14 and -0.11, respectively) and diastolic (r= 0.18 and -0.15,

respectively) blood pressure measurements at rest. Only normalized LF power was weakly associated with diastolic blood pressure ($r = 0.10$) over the 24-hour ambulatory blood pressure measurements. This stronger association of diastolic pressure and ANS activity has been previous examined with the suggestion that it has a more potent influence on baroreceptors than systolic pressure (Sanders & Ferguson, 1989). Nevertheless, these results suggest that short-term measurements of HRV are related to hypertensive status as determined by acute resting measurements but provide little influence in 24-hour ambulatory measurements of blood pressure. Comparable results examining short-term HRV measurements and 24-hour blood pressure monitoring have been found, with 24-hour pulse-pressure having no significant relation to any short-term metric of HRV after correcting for age and sex (Virtanen et al., 2004). Furthermore, Virtanen et al (2004) showed that 24-hour diastolic blood pressure was a significant determinate of baroreflex sensitivity, providing additional support to the notion that diastolic pressure is closely related to the ANS signaling.

The notion of sex as a moderating factor in the relation between HRV measurements and blood pressure has been explored by multiple studies. HRV metrics derived from 24-hour recordings were evaluated in 87 subjects with and without a family history of hypertension. SDNN, RMSSD, and HF power values were found to be significantly lower only in males with a family history of hypertension compared to males without a family history of hypertension and females regardless of a family history of hypertension. No significant differences were found between females with and without a family history of hypertension (Pitzalis et al., 2001). Its suggested that this sex influence is related to the notation that females have greater parasympathetic influence on R-R intervals and less sympathetic activity compared to males (Barnett et al., 1999).

While it has been suggested that parasympathetic neurons are more sensitive to baroreceptor input compared to sympathetic neurons (Kollai & Koizumi, 1989), markers of sympathetic activity, such as PEP have been found to be related to blood pressure. Shah & Slodki (1964) noticed that hypertensive patients presented with a prolonged QS2 (the total amount of time of electromechanical activity during systole) which was primarily due to a prolonged PEP. A landmark study published by Weissler and colleagues outlined that systolic time intervals collected from 27 subjects with heart failure revealed a strong association between diastolic pressure and PEP corrected for heart rate (r=0.60), with diastolic pressures about 90mmHg having a stronger relation with an increase in PEP (Weissler et al., 1968). Manipulation of various aortic and ventricular pressures in dogs supports this notion, finding that a small increase in diastolic pressure caused a notable increase in PEP (Talley et al., 1971). Further investigations on the correlation of blood pressures and STI found that diastolic pressure above 90mmHg was positively associated with PEP (r=0.44) and PEP corrected for HR (r=0.47) (Shaw et al., 1973). This relation of diastolic blood pressure and PEP has not been found in all studies. Montoye, Willis, Howard, & Keller (1971) failed to find any significant correlation between PEP and any measure of blood pressure in males and females in varying age ranges. Given that they failed to report the blood pressure ranges, it is unknown if the subjects were under the 90mmHg threshold for hypertension. Their study noted that the testers, testing clinics, and data analysis teams were blinded to the health status of any subject. A more recent study conducted by Wong and colleagues only found a significant relation between systolic blood pressure and PEP (Wong et al., 2011). It should be noted that their study utilized "cuffless" pulse arrival time to estimate blood pressure readings.

Adiposity

While the true nature of the relation is multifaceted, an increase in sympathetic tone is one of the mechanisms by which obesity is linked to increased CVD risk (López-Jiménez & Cortés-Bergoderi, 2011). The connection between an increase in adiposity and increased sympathetic activity is believed to be through obesity's influence on insulin signaling (Landsberg, 1986), gut hormone secretions (Guarino et al., 2017), and blood pressure alterations via baroreceptor impairment and increased renin-angiotensin-aldosterone system activity (Seravalle & Grassi, 2016). Furthermore, the location of the adipose tissue has a strong influence on the relation between adiposity and sympathetic tone, in that deposits of visceral adipose tissue have a greater association with increased sympathetic activity than subcutaneous adipose tissue (Seravalle & Grassi, 2016). Most studies that examine the relation between adiposity and sympathetic activity utilize microneurography, chemical analysis, or inference from HRV metrics. While limited, some studies have employed the use of PEP measurements. The following paragraphs will outline the current understandings of this relation with attention given to those studies using HRV metrics or PEP.

Similar to glucose metabolism, multiple markers of adiposity have been examined in relation to HRV metrics. In general, an increase in adiposity has been observed by an increase in overall body weight, body mass index (BMI), waist circumference (WC), or body fat percentage (BF%). A majority of studies have found that an increase in adiposity is linked to a decrease in markers of overall HRV, reflective of a decrease in parasympathetic and an increase in sympathetic activity. Indeed, while observing the effects of weight change in seven male subjects, Hirsch, Leibel, Mackintosh, & Aguirre (1991) noted that a 10% increase in body weight was accompanied by a decrease in HF power and an increase in mean resting heart rate observed

during 4-5 minute ECG recordings. Group comparisons of 52 obese subjects seeking assistance with weight reduction and 28 lean control subjects were made before and after differing weight loss interventions using 24 hour ECG recordings (Hirsch et al., 1991). At baseline, the obese subjects presented with lower HF power compared to the lean subjects. At a one-year follow-up, those subjects in the obese group that received weight-reducing gastric surgery lost a mean body weight of 28%, while those who received conventional dietary recommendations for weight loss did not have any significant change in adiposity. The weight-reducing gastric surgery subjects displayed improved levels of HF power compared to those subjects that received dietary recommendations. While the weight loss cannot claim full responsibility for these improvements in HF power, it can be inferred that the reduction in body weight was a contributing factor based on baseline comparisons with the lean control group.

The same inverse nature of the HF component of HRV and adiposity has been observed with measurements of BMI. HRV metrics from 24-hour ECG recordings and BMI were examined in 25 male and female adults without hypertension (Molfino et al., 2009). While body weight was not significantly correlated with HF power, BMI displayed an inverse association with normalized HF power ($r=-0.50$, $p<0.01$). Group stratification based on BMI values revealed that only those with a BMI < 20kg/m^2 had significantly different (higher) values of normalized HF power compared to the BMI 20-25kg/m^2 and the BMI >25kg/m^2 groups. These results suggest that BMI may be a stronger marker for changes in HRV metrics in those with average or lower than average BMI values. Similar results were found with five minute ECG recordings in 59 apparently healthy adults (Koenig et al., 2014). BMI was significantly correlated with RMSSD ($r=-.279$, $p=0.039$), suggesting an inverse association between adiposity and

parasympathetic activity. There was no group difference in RMSSD when stratified by BMI values.

Analysis of data from a larger sample size has suggested that WC may have a stronger association with markers of parasympathetic activity than BMI (Windham et al., 2012). The association of both BMI and WC with SDNN and RMSSD calculated from 24-hour ECG recordings were examined in 214 subjects from the Baltimore Longitudinal Study of Aging. While controlling for the covariates of age, sex, race, hypertension, glucose tolerance, and physical activity, no significant association between BMI and SDNN or RMSSD was found in any age group. An increase in WC was found to be significantly associated with a decrease in RMSSD and SDNN in younger (~45 years old) subjects. These results could be indicative of the greater association of sympathetic tone with centralized adiposity. While WC was not found to be associated with any HRV metric in a study by Yadav et al. (2017), the waist to hip ratio was found to be significantly correlated with absolute HF power ($r=-0.374$, $p=-.042$) and normalized HF and LF power ($r=-0.478$, $p=0.008$; $r=0.478$, $p=0.008$, respectively) in 30 obese subjects. The study compared HRV metrics derived from five-minute ECG recordings in obese (BMI 30-35 kg/m^2) and non-obese (BMI 18-24kg/m^2) subjects. While the obese group had significantly lower SDNN, RMSSD, and absolute HF power, neither BMI or WC were found to be correlated with any HRV metric in the obese group (Yadav et al., 2017). The opposing findings of these two studies could be due to the differing anatomical location used for the WC measurement. Windham et al. (2012) defined the WC as one inch above the anterior superior iliac crest while Yadav et al. (2017) measured at the natural waist or one finger width below the umbilicus.

Poliakova et al. (2012) sought to determine the value of differing measurements of adiposity and the influence of body fat distribution on HRV metrics in 97 adult males. All

subjects were non-diabetic and not taking blood pressure medication and completed a duel energy x-ray absorptiometry scan, computed axial tomography evaluation, and a 24-hour ECG recording. Visceral adipose tissue volume was derived from the computed axial tomography evaluation and found to be significantly correlated with SDNN (r=-0.27), RMSSD (r=-0.21), HF power (r=-0.21) and LF power (r=-0.26). Thus, as the amount of visceral adiposity increased, overall HRV (SDNN) and parasympathetic activity (RMSSD and HF power) decreased. This further supports the positive relation between central adiposity and sympathetic activity. Additionally, BMI, WC, and BF% calculated from the duel energy x-ray absorptiometry scan were significantly correlated with visceral adipose tissue volume (r = 0.41, 0.66, 0.48, respectively), but none of these measurements were found to be significantly related to any HRV metric.

While sympathetic activity can be loosely inferred from HRV data, multiple studies have sought to directly evaluate the relation of adiposity and sympathetic activity by observing differences in PEP. Hu, Lamers, Hiles, Penninx, & de Geus, (2016) examined the cross-sectional and longitudinal relation between ANS activity and components of metabolic syndrome, including WC, in 1,922 adults. PEP was used as the primary index of sympathetic activity and was found to have a negative relation with WC ($r^2 = 0.277$, $p < 0.001$) in cross-sectional analysis. Furthermore, shorter baseline PEP was a significant predictor of increased WC at a four year follow up appointment ($r^2 = 0.081$, $p=0.015$). Both of these findings suggest greater levels of sympathetic activity with increased adiposity. Licht et al. (2010) observed the same negative relation between PEP and WC in 1,883 adults (β =-0.143, $p < .001$). Given that WC is considered a clinical marker of centralized adiposity (Windham et al., 2012), the notion

that it has a significant relation with PEP is congruent with the idea that visceral adipose tissue has a greater association with sympathetic activity.

Another study failed to find a similar cross-sectional relation between PEP length and the waist to hip ratio in 1,540 children aged 5-6 years old (Vrijkotte et al., 2015). This study did not report WC; thus, a true comparison cannot be made. Moreover, few studies have found completely opposite results, suggesting a decrease in sympathetic activity with elevated levels of adiposity. Evaluation of STI and cardiac function in 17 severely obese subjects free from hypertension, diabetes mellitus, or other cardiorespiratory diseases were compared to 16 age matched control subjects. The researchers discovered that PEP and rate-corrected PEP (PEPI) were significantly lengthened in the severely obese subjects (Romano et al., 1986). Correlational analysis in the severely obese group revealed that both PEP and PEPI were significantly associated with the amount of adiposity ($r = 0.49$ and 0.59, respectively). These data suggest that as the degree of adiposity increases, so does the length of the PEP, representing a decrease in sympathetic activity.

Compounding Influences

As implied by Figure 1 on page 12, ANS activity is related to multiple metabolic and physiological factors. This review of literature has outlined data concerning the influence of glucose metabolism, cardiac control parameters, and adiposity on ANS activity. Additionally, it has been suggested that these three factors have a strong interrelated nature; with adiposity being an amplifier for the development of hypertension (Landsberg, 2001; Landsberg et al., 2013) and diabetes mellites (Koh-Banerjee et al., 2004), while poor glucose metabolism and hypertension share common metabolic pathways (Cheung & Li, 2012). The compounding influence of these variables has traditionally been addressed by means of group stratification, covariate assignment,

or exclusion of diseased populations (i.e. non-hypertension or non-diabetic subjects). While these methods provide some control for confounding variables, they do not address the indirect effects of adiposity on ANS activity. Given its magnifying ability on glycemic impairment and cardiac control parameters, it is evident that adiposity exerts its influence on ANS activity through both direct and indirect pathways.

Finally, this paradigm may be further complicated by the influence of cardiorespiratory fitness. Improvements in adiposity (Söğüt et al., 2019), decreased risk for type II diabetes (Blair & Church, 2003), reduced risk of hypertension (Kokkinos, 2014), and alter resting ANS activity that promotes a decrease in resting sympathetic activity (Thayer et al., 2010) have all been linked to cardiorespiratory fitness. Recent statistical modeling has shown that cardiorespiratory fitness may be a stronger determinant of HRV metrics than adiposity as defined by WC or BMI (Chen et al., 2018). Thus, the suggested amplified influence of adiposity may be an indirect function of the physiological effects on cardiorespiratory fitness

CHAPTER III: MANUSCRIPT I

Direct and Indirect Effects of Adiposity on Markers of Autonomic Nervous System Activity.

Abstract

Several cardiovascular disease (CVD) risk factors (e.g., hypertension, poor glycemic impairment) affect and/or are affected by autonomic nervous system (ANS) activity. Since excess adiposity can influence CVD development through its effect on hypertension and diabetes mellitus, it is important to determine how adiposity and altered ANS activity are related. The present study employed structural equation modeling to investigate the relation between adiposity and ANS activity both directly, and indirectly through biological variables typically associated with glycemic impairment and cardiac stress. Utilizing the Atherosclerosis Risk in Communities (ARIC) dataset, 1,145 non-smoking adults (74±4.8 yrs, 62.8% female) free from known CVD, hypertension, myocardial infraction, and diabetes and not currently taking beta-blockers were evaluated for fasting glucose (FBG), insulin, and Hb_{A1c} concentrations, waist circumference (WC), blood pressure (BP), and markers of ANS activity. Resting 2-minute electrocardiograph recordings and pulse wave velocity data were used to assess the root mean square of successive differences in RR intervals (RMSSD) and the pre-ejection period (PEP), markers of parasympathetic and sympathetic activity, respectively. FBG, insulin, and Hb_{A1c} inferred a latent variable termed glycemic impairment (GI), whereas heart rate and diastolic BP inferred a latent variable termed cardiac stress (CS). The structural equation model fit was acceptable [root mean square error of approximation = 0.050 (90% CI = .036, .066), comparative fit index = .970, Tucker Lewis Index = 0.929], with adiposity having both significant direct (β = 0.208, p = 0.018) and indirect (β = -.217, p=.041) effects on PEP through GI. Adiposity

displayed no significant direct effect on RMSSD. CS displayed a significant pathway (β = -0.524, p = 0.035) on RMSSD, but the indirect effect of WC on RMSSD through CS was not significant. These results suggest that the true nature of adiposity's relation to ANS activity is multifaceted, as increased central adiposity had opposing direct and indirect effects on markers of sympathetic activity in this population of older adults.

Introduction

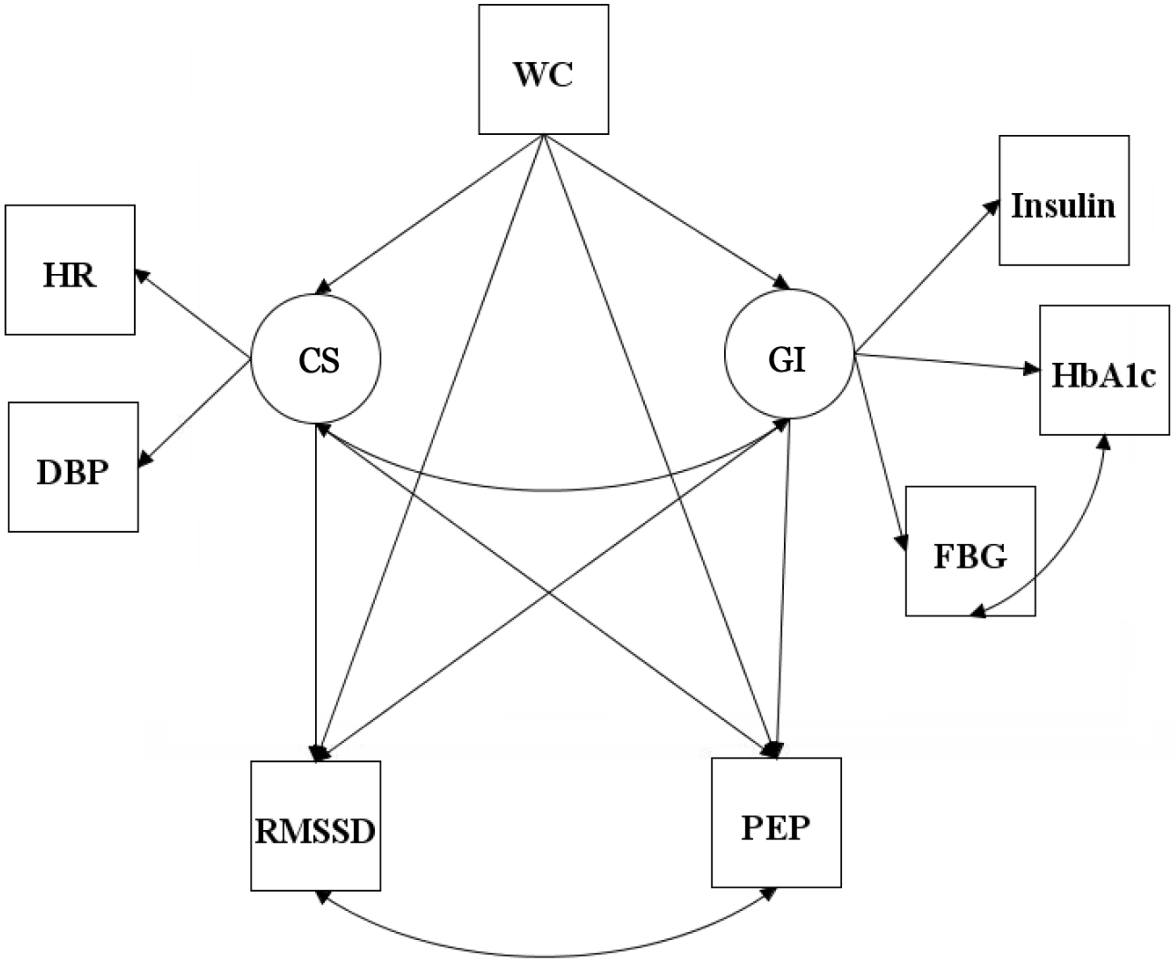
Major risk factors for cardiovascular disease (CVD) include both lifestyle choices (i.e., physical inactivity, poor diet) and physiological conditions (i.e. diabetes mellitus, hypertension, obesity) (Tzoulaki et al., 2016). These physiological conditions have been associated with altered autonomic nervous system (ANS) activity (Alam, Lewis, Morgan, & Baxter, 2009; Erdogan et al., 2011; Soares-Miranda et al., 2014; Tarvainen, Laitinen, Lipponen, Cornforth, & Jelinek, 2014). ANS activity reflects a balance between the sympathetic and parasympathetic branches, which oppose one another but are flexible and dynamic in nature. The sympathetic branch provides an overarching accelerating stimulus, promoting metabolic substrate mobilization and utilization in addition to increasing heart rate and cardiac contractile force. In contrast, the parasympathetic branch is the overarching decelerator of the body, promoting substrate storage and decreased heart rate (Thayer et al., 2010). Bodily functions are regulated by the balance between these opposing systems and any prolonged disturbance or asymmetry in activity can result in the development of several clinical and physiological conditions (Pagani et al., 1988; Zhou et al., 2012).

ANS activity is often evaluated indirectly through indices of heart rate variability (HRV). HRV, or the time variance among intervals of consecutive cardiac cycles, is a widely used and accepted means of assessing ANS activity at the cardiac level (Malik et al., 1996). Irregular

ANS activity as assessed by HRV has been heavily evaluated for its association with CVD and mortality. Specifically, chronically reduced parasympathetic and elevated sympathetic activities are associated with increased CVD risk, including premature mortality, myocardial infarction, and development of type II diabetes mellitus and hypertension (Gerritsen et al., 2001; Kleiger et al., 1987; Tsuji et al., 1996). Various time and frequency domain HRV metrics can be used to estimate the level of parasympathetic output at the cardiac level (Michael, Graham, et al., 2017). The root mean square of successive differences between normal (non-ectopic) beats (RMSSD) is the primary time domain metric used to reflect vagally mediated changes, with an increase in RMSSD reflecting an increase in parasympathetic activity (Shaffer et al., 2014) . Some HRV metrics have been suggested to estimate sympathetic activity (namely the low frequency spectral power component and the ratio of low frequency to high frequency spectral power components), but these values are inherently limited in evaluating sympathetic output given that they are also influenced by parasympathetic activity (Shaffer & Ginsberg, 2017). An alternative and more independent method of evaluating sympathetic activity at the cardiac level is to monitor systolic time intervals, i.e., the amount of time between electrical stimulation of the heart and its mechanical response. Specifically, the segment of time known as the pre-ejection period (PEP), an estimate of the electromechanical delay of the left ventricle (Tavakolian, 2016), can be used to accurately estimate sympathetic activity (Ahmed et al., 1972). PEP has been shown to have a pronounced inverse relation with sympathetic activity in that sympathetic neural blockade stimulates an increase in PEP length, while parasympathetic blockade results in no change in PEP (Cacioppo et al., 1994). Used concurrently, PEP and HRV measurements can provide holistic details of ANS activity at the cardiac level.

While increased adiposity is an independent risk factor for CVD development (Hubert et al., 1983), it is also a well-established risk factor for CVD comorbidities such as diabetes mellitus (Koh-Banerjee et al., 2004) and hypertension (Landsberg et al., 2013). While significant associations between metrics of increased adiposity and altered ANS activity have been reported (Farah et al., 2013; Poirier et al., 2003; Windham et al., 2012), it is unclear if increased adiposity has a multifaceted relation to ANS activity (i.e. direct and indirect influence). However, evaluating the influence of adiposity on ANS activity through its risk for diabetes mellitus and hypertension development is a complex undertaking, as there is no single metric that can accurately reflect diabetic or hypertensive risk. Latent variable modeling constructs an unobserved variable that reflects the combined influence of multiple measured variables. Applying this methodology to reflect relations within our conceptual model (Figure 3.1) provides a more comprehensive assessment of the influences of adiposity on ANS activity. In this model, adiposity is reflected by waist circumference (WC) and has direct effects on RMSSD and PEP (markers of parasympathetic and sympathetic nervous system activities, respectively). Our model also suggests that adiposity has indirect effects on ANS activity through latent constructs representing glycemic impairment (GI) and cardiac stress (CS). Utilizing a large previously collected dataset, we tested the direct and indirect influences of adiposity on ANS activity.

Figure 3-1. Proposed Model



Note. *A priori* model including observed variables (squares) depicting the proposed pathways for the direct and indirect effects of waist circumference on markers of ANS activity. Construction of the latent variables (circles) Cardiac Stress (CS) and Glycemic Impairment (GI) are depicted. WC, Waist Circumference; HR, Heart Rate; DBP, Diastolic Blood Pressure; Hb_{A1c}, Glycosylated Hemoglobin; FBG, Fasting Blood Glucose; RMSSD, Root Mean Square of Successive Differences between normal-to-normal beats; PEP, Pre-Ejection Period.

Methods

Study Population

The data used in the present study was obtained from the Atherosclerosis Risk in Communities (ARIC) study. The ARIC dataset consists of 15,792 participants recruited from four different field centers throughout the United States of America (Minneapolis, Minnesota; Washington County, Maryland; Forsyth County, North Carolina; and Jackson, Mississippi). These participants were given a comprehensive physical examination at baseline between the years of 1987-1989 and were reevaluated every three years. Detailed methods about data collection and study design for ARIC have been published ("The Atherosclerosis Risk in Communities (ARIC) Study," 1989). Data for the present study was derived from a fifth cycle of visits that was conducted with 5,900 participants between 2011 and 2013. For the analyses outlined here, individuals with known cardiac disease, history of myocardial infraction or diabetes mellitus, those who identified as a smoker or that reported smoking in the last 6 months, those with a diastolic blood pressure ≥90 mm Hg or systolic blood pressure ≥140 mm Hg, and those taking beta-blockers at the time of evaluation were excluded. Furthermore, those deemed as statistical multivariate outlier based on a mahalanobis distance analysis were excluded from the study. The resulting participant pool (n=1145) was 61% female and 39% male, aged 74.6 ± 4.8 years. The majority (89%) of subjects self-identified as "white" with 11% self-identifying as "black". Further sample characteristics are provided in Table 3.1.

Study Variables

Overall, latent variables were constructed based on the proposed model and potential markers available from the ARIC dataset that aligned with each latent variable.

Glycemic Impairment. Multiple biomarkers and indices can be used to provide a holistic evaluation of an individual's glucose handling capacity. Thus, the variables of fasting blood glucose (FBG), glycosylated hemoglobin (Hb_{A1c}), and insulin were used to construct a "Glycemic Impairment" (GI) latent variable. FBG concentration provides a short-term approximation of glucose tolerance (~8-12 hours) while Hb_{A1c} concentrations provide a marker of long-term glucose homeostasis (~3-4 months). Furthermore, the inclusion of concurrent insulin concentration provides information on possible insulin resistance.

Cardiac Stress – Two resting cardiac parameters were used to reflect the functional status of the cardiac system. The overall function of the heart is reflected through heart rate (HR) and contractility. HR is regulated by a multitude of factors and is tightly related to ANS output. Although it is intuitive to employ systolic blood pressure (SBP) as a marker of contractility, previous research with the ARIC dataset showed diastolic blood pressure (DBP) to have greater associations with HRV metrics in normotensive subjects (Landsberg, 2001; Landsberg et al., 2013). Additionally, DBP has a greater influence on mean arterial pressure at rest. Of interest, various models were tested using various permutations and combinations of mean atrial pressure, SBP, and DBP, with all other models having weaker goodness of fit indices compared to the current model. Given these factors, DBP appears to be a better metric for this concept. Thus, the observed variables of HR and DBP were used to construct a "Cardiac Stress" (CS) latent variable.

ANS Activity Markers

Resting electrocardiogram and pulse wave velocity data were collected in a supine position following at least eight hours of fasting. A 2-minute electrocardiogram recording was used to calculate short term HRV metrics. The root mean square of successive differences in RR

intervals (RMSSD) was employed to reflect parasympathetic nervous system activity (Nunan et al., 2010; Shaffer et al., 2014; Windham et al., 2012). The pre-ejection period (PEP), calculated from the pulse wave velocity measurement (Omron BP-203RPEIII, Omron Healthcare co. Ltd, Kyoto, Japan), was used to indicate sympathetic nervous system activity (Cacioppo et al., 1994; Michael, Graham, et al., 2017; Siedlecka et al., 2015).

Model Construction

Central adiposity, more than overall adiposity, is strongly related to the development of cardiometabolic diseases (Seravalle & Grassi, 2016). As such, the present model explored the effects of waist circumference (WC) on RMSSD (parasympathetic) and PEP (sympathetic), both directly and through the latent variables GI and CS. Given the inherent nature of FBG and Hb_{A1c} and their strong relation ($r=0.61$, $p<.001$) in the current dataset, the model allowed for a covariance between the error terms of the two variables. While Hb_{A1c} may possibly be related to postprandial glucose concentration as much or more than FBG (Ketema & Kibret, 2015), the use of FBG and Hb_{A1c} as markers of short-term (~4-6 hours) and chronic (~3-6 months) GI is theoretically sound. Similarly, the error terms of the latent variables of GI and CS were allowed to covary given their strong correlation ($r=0.78$, $p<.001$) and their shared relation to CVD through similar metabolic pathways (Cheung & Li, 2012). Since structural equation model calculations automatically assume that the error terms of the outcome variables in a latent variable analysis have a shared covariance, RMSSD and PEP were allowed to covary in the present analysis, even though their relation was not statistically significant (Table 3.2).

Statistical Analysis

Means, standard deviations, and Pearson's correlation coefficients for the observed variables were calculated using SPSS Statistics Version 28. The structural equation model was

gauged for goodness of fit using multiple fit indices including the root mean square error of approximation (RMSEA), comparative fit index (CFI: criterion value of 0.95), Tucker Lewis index (TLI: criterion value of 0.95), and the standardized root mean square residual (SRMR: criterion value of 0.08) (L. Hu & Bentler, 1999). The direct and indirect relations outlined by the proposed model were conducted in Mplus Version 8.7 with statistical significance set at α=0.05.

Results

Descriptive Statistics

Anthropometric and cardiometabolic characteristics are provided in Table 3.1. The mean waist circumference (WC) for the total cohort was 96.6 cm, with the average for females (94.1 cm) and males (100.4 cm) indicating borderline risk of CVD. The mean values of Glycemic Impairment (GI) metrics (FBG, insulin, and Hb_{A1c}) were lower than clinical cutoffs for diabetes mellitus, though the mean FBG of 104.79 $mg{\cdot}dL^{-1}$ falls in the pre-diabetes range. Mean values for cardiac stress (CS; HR and DBP) were within normal ranges. The values for RMSSD and PEP are similar to the normal ranges presented in Table 2.1.

Pearson's correlation coefficients of all observed variables are provided in Table 3.2. Relations among GI variables were fair to moderate (r=0.19-0.61) while the CS variables, HR and DBP were weakly but significantly related (r=0.11). Waist circumference (WC) displayed fair relations (r=0.28-0.49) with all GI metrics and was weakly but significantly related to DBP (r=0.18). WC was weakly but significantly related to RMSSD (r=0.06) and was not significantly related to PEP.

Table 3-1. Characteristics of Study Population

	Mean	Standard Deviation	Range (min, max)
N = 1,145			
Age (years)	74.6	4.8	67, 88
Mass (kg)	73.4	15.1	37.7, 145.2
Height (cm)	165.6	9.5	141, 195
Body Mass Index ($kg \cdot m^{-2}$)	26.67	4.6	15.89, 57.43
Waist Circumference (cm)	96.6	12.5	59, 155
Females (N=696)	94.1	13.1	59,155
Males (N=449)	100.4	10.4	73, 137
FBG ($mg \cdot dL^{-1}$)	104.79	15.85	65.01, 185.03
Insulin ($\mu U \cdot mL^{-1}$)	10.86	7.97	0.20, 70.02
Hb_{A1c} (%)	5.66	0.48	4.2, 8.6
DBP (mmHg)	66.4	9.3	40, 89
HR (bpm)	66	9.9	41, 110
RMSSD (msec)	23.52	23.14	1.22, 166.98
PEP (msec)	94.7	18.6	4.0, 173.5

Table 3-2. Preliminary Correlations Matrix (Pearson's r)

	FBG	Hb_{A1c}	Insulin	DBP	HR	PEP	RMSSD	WC
FBG	--							
Hb_{A1c}	0.61**	--						
Insulin	0.31**	0.19**	--					
DBP	0.06	0.01	0.11**	**--**				
HR	0.13**	0.14**	0.18**	0.11**	--			
PEP	-0.04	0.02	-0.02	0.07*	0.05	--		
RMSSD	-0.05	-0.03	0	-0.05	-0.21**	-0.02	--	
WC	0.28**	0.19**	0.49**	0.18**	0.05	0.05	0.06*	--

Note: *p<.05, **p<.001

Model Analyses

The structural equation model displayed an adequate fit for the sample population with significant goodness of fit indices (χ_M^2= 47.013, p=.000, RMSEA = 0.050 90% CI [0.036-0.066],

SRMR = 0.028, CFI = 0.970, TLI = 0.929). While the Chi squared value was elevated and significant, this is most likely reflective of the large sample size. Additionally, the other fit indices are well within acceptable ranges (L. Hu & Bentler, 1999). Both latent variables (GI and CS) displayed significant factor loadings with their indicator variables (Table 3.3), suggesting a solid construct. Direct variances and covariance parameter estimates for the entire model are listed in Table 3.4. Most of the hypothesized relations specified by the conceptual model were statistically significant while the resulting direct and indirect effects displaced mixed outcomes

Table 3-3. Latent Variable Parameter Estimates

	Parameter	Unstandardized	SE	Standardized
Glycemic Impairment (GI)	FBG	1	-	0.431**
	Insulin	14.964	1.418	0.711**
	Hb_{A1c}	0.36	0.036	0.285**
Cardiac Stress (CS)	HR	1	-	0.519**
	DBP	0.385	0.1	0.213**

Note: SE, standard error; **p<.001

Table 3-4. Variance and Covariance Estimates

	Parameter	Unstandardized	SE	Standardized
RMSSD	GI	17.605	13.228	0.288
	CS	-2.352	1.117	-0.524*
	WC	-0.091	0.215	-0.049
PEP	GI	-15.573	7.252	-0.318*
	CS	0.874	0.51	0.243
	WC	0.308	0.131	0.208*
Glycemic Impairment (GI)	WC	0.021	0.002	0.682**
Cardiac Stress (CS)	WC	0.074	0.022	0.178**
Covariances	FBG~~ Hb_{A1c}	0.206	0.013	0.566**
	GI~~CS	0.796	0.141	0.566**
	RMSSD~~PEP	22.51	22.7	0.06

Note: SE, standard error; *p<.05; **p<.001

Direct Effects

The direct, indirect, and total effects of WC on RMSSD and PEP are provided in Table 3.5. WC failed to have a significant direct effect on RMSSD but had a significant direct effect on PEP ($\beta = 0.209$, p = .018) with a one standard deviation increase in WC being related to a 0.208 standard deviation increase in PEP

Table 3-5. Standardized Parameter Estimates of Direct, Indirect, and Total Effects of Waist Circumference on RMSSD and PEP

	Path	Estimate	S.E.	P-value
RMSSD				
	Direct	-0.049	0.108	0.647
	Indirect: WC›GI	0.196	0.147	0.181
	Indirect: WC›CS	-0.094	0.063	0.137
	Total Indirect	0.103	0.105	0.329
	Total	0.053	0.029	0.07
PEP				
	Direct	0.208	0.088	0.018*
	Indirect: WC›GI	-0.217	0.106	0.041*
	Indirect: WC›CS	0.043	0.034	0.205
	Total Indirect	-0.173	0.083	0.038*
	Total	0.034	0.03	0.247

*Statistically significant at $\alpha = 0.05$

Indirect and Total Effects

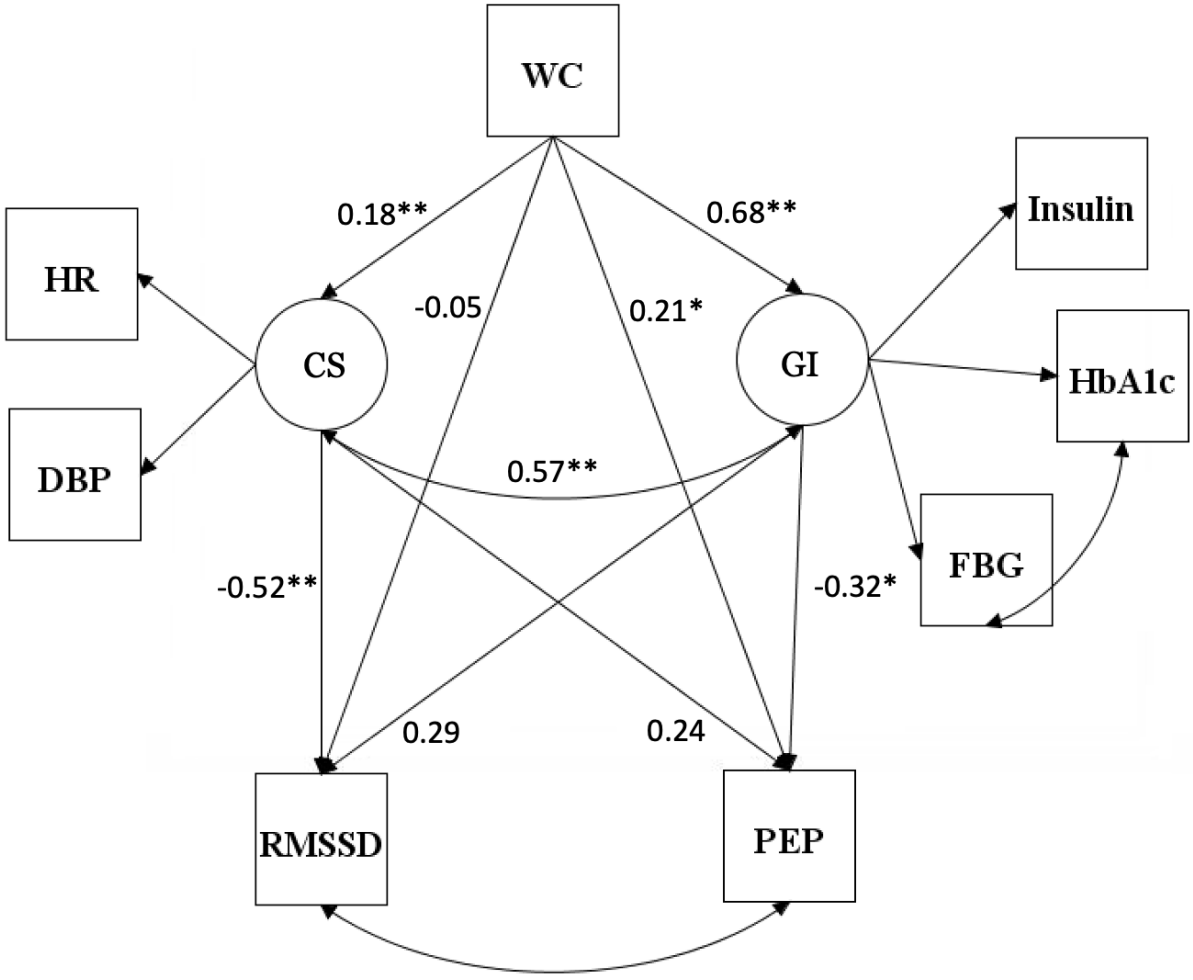
In this model, WC failed to have a significant indirect effect on RMSSD through the latent variables of GI or CS, resulting in the lack of a significant total indirect effect (the sum of all indirect effects on the depended variable). The Total effect (direct effect + Total indirect effect) of WC on RMSSD approached significance (p = 0.070). The indirect effect of WC on PEP through CS was not significant. The indirect effect of WC through GI had a significant

effect on PEP in that a one standard deviation increase in WC was associated with a 0.217 standard deviation decrease in PEP. Furthermore, the total indirect effect of WC on PEP was significant with a one standard deviation increase in WC being related to a 0.173 standard deviation decrease in PEP. However, the total effect of WC on PEP was not significant.

Other Significant Pathways

WC had significant effects on GI and CS with a one standard deviation increase in WC being associated with 0.682 and 0.178 standard deviation increases in GI and CS, respectively. CS had a significant effect on RMSSD in that a one standard deviation increase in CS would elicit a 0.524 standard deviation decrease in RMSSD. However, CS failed to have a significant effect on PEP. GI had a significant effect on PEP with a one standard deviation increase in GI being associated with a 0.318 standard deviation decrease in PEP. GI did not have a significant effect on RMSSD.

Table 3-6. Conceptual Model



Note: Conceptual model including standardized weights, both direct and indirect effects of waist circumference on markers of RMSSD and PEP. Cardiac Stress, CS; Glycemic Impairment, GI; WC, Waist Circumference; HR, Heart Rate; DBP, Diastolic Blood Pressure; Hb_{A1c}, Glycosylated Hemoglobin; FBG, Fasting Blood Glucose; RMSSD, Root Mean Square of Successive Differences between normal-to-normal beats; PEP, Pre-Ejection Period. Note: SE, standard error; *p<.05; **p<.001

Discussion

The results of this study support the concept that central adiposity, as measured by WC, has significant direct and indirect effects on markers of ANS activity. Specifically, WC displayed robust associations with markers of sympathetic nervous system activity, while having little influence on parasympathetic nervous system activity when accounting for the impact of glycemic impairment markers and indices of cardiac stress. This highlights the need to directly evaluate both branches of the autonomic nervous system when discussing the relation of ANS activity and adiposity. Additionally, there is evidence that the impact of central adiposity goes beyond direct effects and that nuanced mechanisms may influence autonomic health.

In this model, WC failed to have a significant direct, indirect, or total effect on RMSSD. These findings differ from earlier studies that have demonstrated direct relations between adiposity and metrics of parasympathetic activity. Windham et al. (2012) found that an increase in WC was significantly associated with a decrease in RMSSD and SDNN in younger (~45 years old), but not older (~85 years old), participants when controlling for age, sex, race, hypertension, glucose tolerance, and physical activity. Our findings suggested that the direct effect of WC on RMSSD had a similar inverse nature as seen in the younger group, though it was not statistically significant. The age difference between the Windham et al. younger cohort and the present study could be a contributing factor for this difference in statistical magnitude as it has been shown that the age-related decrease in time domain metrics of HRV subsides after 60 years of age in apparently healthy individuals (Geovanini et al., 2020).

Our results displayed a significant direct effect of WC on PEP, suggesting a decrease in sympathetic activity. However, the total effect of WC on PEP was neutralized through the negative influence of GI. This model suggests that increases in central adiposity (WC) might

have a blunting effect on sympathetic activity (direct effect) while simultaneously increasing sympathetic drive by increasing (worsening) indices of glycemic impairment (indirect effect). These opposing actions appear to mitigate observable physiological changes in PEP, as reflected by the lack of a significant total effect of WC on PEP. Simultaneous examinations of both the direct and indirect effects of adiposity on sympathetic activity in the previous literature are sparse, limiting comparison with the current findings. However, the individual (direct) effect of WC on sympathetic activity as well as the individual effect of markers of GI (major portion of the significant indirect effect) have been documented (Esler et al., 2018; Seravalle & Grassi, 2016).

The direct effect of WC on PEP, suggesting a decrease in sympathetic activity with an increase in WC, seems counterintuitive, but is biologically plausible. Aging is often accompanied by an increase in sympathetic nervous system activity (Balasubramanian et al., 2018), particularly due to impaired ability of baroreflexes to buffer changes in blood pressure (Monahan, 2007). However, limited evidence suggests that sympathetic activity at the level of the heart is normal or lower in obese individuals compared to normal weight individuals in elderly populations with hypertension, lending credence to the concept that central adiposity could decrease sympathetic activity (Esler et al., 2018). While those diagnosed with hypertension were excluded from the current study, this observation of lower and/or normal sympathetic activity in obese individuals coincides with the observed significant direct effect and the general lack of a total effect of WC on PEP.

The observed indirect effect of increased WC being related to increased sympathetic activity (decrease in PEP) via an increase in GI was expected. The latent variable GI reflects the combined influence of FBG, Hb_{A1c}, and insulin, with any increase in these metrics resulting in

increases in the GI variable. Insulin had the highest factor loading for the GI construct and is likely the primary driver for the observed effects. Elevated serum insulin concentrations are postulated as one of the major pathways linking increased adiposity (specifically central adiposity) to sympathetic nervous system overactivity (Seravalle & Grassi, 2016). In addition, direct exposure of cardiac tissue to insulin can induce positive inotropic effects through calcium dependent and independent mechanisms (von Lewinski et al., 2005). The present study also showed FBG and Hb_{A1c} to have significant factor loadings for the GI construct. Previous research has demonstrated increases in FBG (Perpiñan et al., 2019; Sykes et al., 1977; Synowski et al., 2013) and Hb_{A1c} (Benichou et al., 2018; Boer-Martins et al., 2011; Jaiswal et al., 2013) with increases in markers of sympathetic activity. Furthermore, ANS activity reflective of cardiac autonomic neuropathy complications have been associated with diabetes mellitus (Dimitropoulos, 2014).

Given that HRV metrics have been shown to be altered with diabetic status and impaired glycemic control (Nganou-Gnindjio et al., 2018; Ribeiro et al., 2017; Schroeder et al., 2005), those previously diagnosed with diabetes mellitus were excluded from the current study. However, the sample population in this cohort had a mean fasting blood glucose of 104.79±15.85 $mg \cdot dL^{-1}$, suggesting that a large section of the sample could be classified as pre-diabetic. Reports of prediabetics showing signs of cardiac autonomic neuropathy have been increasing (Williams et al., 2019), suggesting that prediabetics may have altered ANS activity. Similarly, blood pressure between the normal and hypertensive ranges is associated with autonomic disfunction (Erdogan et al., 2011). While the mean diastolic blood pressure of the sample population did not classify as hypertensive (66.4±9.3 mmHg), there were 102 participants (roughly 9% of the sample population) that had a DBP of ≥80 mmHg, demonstrating

that those at risk for but not formally diagnosed with hypertension were included in the sample. Therefore, despite the exclusion of those diagnosed with diabetes and/or hypertension, the ranges of glycemic and cardiovascular parameters in the present data set are likely adequate for the investigation of their influence on autonomic function.

The choice of WC as our marker of adiposity over other population-based metrics could have influenced the observed results. WC reflects the amount of visceral fat (Ross et al., 2020), which is more metabolically deleterious and a greater predictor of mortality than subcutaneous fat (Ibrahim, 2010). Increases in visceral fat depots as measured by computed axial tomography have been shown to have significant inverse relations with RMSSD in the absence of a significant correlation between WC and RMSSD, despite WC and visceral fat area being positively related (Poliakova et al., 2012). Still, studies with larger cohorts (n=8,538) suggest that WC has a stronger inverse relation to HRV metrics, specifically RMSSD, than body mass index or waist to hip ratio in apparently healthy populations (Koenig et al., 2015). These previously observed decreases in markers of parasympathetic activity, including RMSSD, were attributed to an increase in sympathetic activity due to an increase in adiposity, which the current study observed in the significant indirect effects of WC on PEP via GI. As a whole, the results of our study suggest that a greater association of sympathetic tone with centralized adiposity occurs without direct alterations to parasympathetic activity.

The ARIC study utilized pulse wave velocity data to calculate PEP. Studies have used this methodology to provide various markers of CVD risk (Koji et al., 2004; Tomiyama et al., 2016) and monitor disease progression (Ato & Sawayama, 2017). This method has been shown to be a good alternative to measure systolic time intervals in the absences of more precise methods such as echocardiogram (Su et al., 2013), giving credence to the accuracy and validity

of the PEP measurements used for the current study. HRV results were derived from a 2-minute ECG rhythm strip, which depending on the metric used, could have a sizable impact on results. Considered the gold-standard of clinical HRV assessment, a recording length of 24 hours provides the most detailed evaluation of ANS activity (Shaffer & Ginsberg, 2017). However, shorter recordings of approximately five minutes have been successfully used in numerous studies (Nunan et al., 2010). Frequency domain measures are preferred since they are influenced to a lesser extent by shorter recording lengths than most time domain measures (Malik et al., 1996), but only time domain metrics were available in the ARIC dataset.

Our results are strengthened by the inclusions of validated indices of both branches of the ANS. This provides an accurate, holistic representation of ANS activity without relying on the inferences from a single branch as is a common practice when solely using HRV results. Additionally, our cohort included both male and female participants. While this choice could possibly introduce confounding effects of sex differences, HRV differences between the sexes has been shown to be reduced after approximately 60 years of age (Voss et al., 2015). However, given that there are differences in the physiological manifestations of hypertension (Gillis & Sullivan, 2016) and diabetes mellitus (G Duarte et al., 2019) between the sexes, evaluation of the proposed model should be conducted in sex specific samples. Such comparisons utilizing the structural equation model were not conducted in the present study due to a limited sample size for males. The current study is not without its limitations. The cross-sectional design of our study diminishes inferences of causality in the observed relations and the directions of the effects beyond those suggested in the model development. Given that only 11% of the sample self-identified as "black", the ability of our results to be generalized to a more diverse population is limited. Furthermore, the use of a single adiposity marker, WC, limits our ability to assess the

potential effect of overall adiposity on ANS activity. The use of dual-energy x-ray absorptiometry to assess body composition and/or the use of computed axial tomography to more accurately quantify visceral fat could strengthen the observed effects in this model and explain more of the variance in ANS activity.

Interestingly, similar studies have found that adding markers of cardiovascular fitness could improve model fit parameters and be a stronger determiner of HRV indices of ANS activity (Chen et al., 2018). The current model did not account for cardiovascular fitness or physical activity status even though both have direct effects on all the study's variables. Specifically, an increase in cardiovascular fitness will have large physiological implications for adiposity, influence glycemic management capabilities, directly alter cardiac stress parameters, and have strong influences on ANS activity. Investigations into how cardiovascular fitness may mitigate or abolish the observed effects of adiposity on ANS activity are warranted.

In summary, our results provide support for a theoretical and statistically sound model for describing the relation of adiposity and ANS activity while accounting for the interactions of contributing physiological parameters. In a population of older, apparently healthy adults, increased central adiposity displayed opposing direct and indirect effects on markers of sympathetic activity, resulting in unobservable changes in PEP. Moreover, WC presented no significant direct or indirect effects on an index of parasympathetic activity. These results suggest that the true nature of adiposity's relation to ANS activity is multifaceted. Using a latent path analysis, such as the purpose model, to uncover the complex interrelations of multiple risk factors may better illustrate the biological control systems behind cardiometabolic risk factors and CVD.

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CHAPTER IV: MANUSCRIPT II

Effects of Adiposity and Cardiorespiratory Fitness on Autonomic Nervous System Activity Following Maximal Exercise.

Abstract

In addition to directly contributing to the development of cardiovascular disease, excess adiposity has been shown to be related to altered autonomic nervous system (ANS) activity that further exacerbates health complications. Cardiovascular fitness is related to both adiposity and ANS activity and may, therefore, affect the relation between the two. The purpose of this study was to determine if adiposity influences the markers of ANS activity at rest and following maximal exercise, with and without accounting for fitness. Twenty-six apparently health males, were assessed for body composition and then underwent telemetric electrocardiogram and impedance cardiography recordings while resting in a supine position for 10-minutes and while seated in a semi-recumbent position for 10-minutes before and immediately after a maximal exercise test. Markers of ANS activity were derived from time and frequency domains of heart rate variability (HRV) as well as systolic time intervals, namely the pre-ejection period (PEP). Group stratification by waist circumference or body fat percentage (BF%) revealed no among-group differences in resting or post-maximal exercise HRV or PEP measurements. Accounting for peak oxygen uptake (VO_{2peak}) resulted in a significant between-group difference in the natural logarithm of time domain parameters [standard deviation ofnormal-to-normal beats (ln-SDNN) and the root mean square of successive differences between normal-to-normal beats (ln-RMSSD)] as well as high frequency spectral power (ln-HF) at 3- and 5-minutes post-exercise, based on BF% stratification. However, statistical significance was lost following adjustments for peak oxygen uptake standardized by lean body mass (Lean VO_{2peak}), suggesting no difference in

HRV across BF% groups. These results provide evidence that there may not be differences in resting ANS activity and post-maximal exercise ANS responsiveness across groups of differing levels of adiposity in young, apparently healthy males. Adjusting for cardiovascular fitness minimally effected the observed relation of adiposity and ANS activity post-maximal exercise.

Introduction

The relation between adiposity and autonomic nervous system (ANS) activity is multifaceted and complex. Increased sympathetic tone is considered one of the main mechanisms by which obesity is linked to increased risk of cardiovascular disease (CVD) (López-Jiménez & Cortés-Bergoderi, 2011). The connection between adiposity and sympathetic activity is believed to occur through obesity's influence on insulin signaling (Landsberg, 1986), gut hormone secretions (Guarino et al., 2017), baroreceptor impairment, and increased renin-angiotensin-aldosterone system activity (Seravalle & Grassi, 2016). Furthermore, visceral adipose tissue has a greater association with increased sympathetic activity than does subcutaneous fat (Seravalle & Grassi, 2016). Most studies that examine the relation between adiposity and ANS activity utilize microneurography, chemical analysis, or inference from heart rate variability (HRV) metrics. Numerous studies indicate that increased adiposity is linked to altered HRV indices, reflecting a decrease in parasympathetic and an increase in sympathetic activity (Hirsch et al., 1991; Molfino et al., 2009; Thayer et al., 2010; Windham et al., 2012). While limited, some studies have employed the use of systolic time intervals, namely the pre-ejection period (PEP) to non-invasively measure sympathetic activity (M. X. Hu et al., 2016; Licht et al., 2010; Romano et al., 1986).

The relation of cardiorespiratory fitness and ANS activity has long been documented (Blomqvist & Saltin, 1983; Smith et al., 1989). Low cardiorespiratory fitness has been

associated with an imbalance of ANS activity (*i.e.*, increased sympathetic and decreased parasympathetic activity) at rest (Thayer et al., 2010) in addition to an increased risk of CVD mortality (Gupta et al., 2011) . Since cardiorespiratory fitness is inversely related to adiposity (Söğüt et al., 2019), its influence on the relation between adiposity and ANS activity must be considered. Moreover, it has been suggested that cardiorespiratory fitness may be a greater determinate of ANS activity than adiposity (Chen et al., 2018). Thus, the proposed impact of adiposity may be an indirect function of the physiological effects of cardiorespiratory fitness. Furthermore, a faster heart rate recovery, the slowing of heart rate following exercise, is inversely related to cardiovascular disease incidence (Morshedi-Meibodi et al., 2002) and all-cause mortality (Jouven et al., 2005). The adjustment of heart rate (HR) before and after exercise is primarily due to modulations in ANS activity at the cardiovascular level in response to changing metabolic and physiological demands (Silva et al., 2017). HR increases in response to exercise due to parasympathetic withdraw and increased sympathetic drive, while the post-exercise decrease in HR is due to a reversal of this activity (Michael, Graham, et al., 2017). Evaluating alterations in ANS responsiveness to the cessation of exercise could yield valuable insights into the underlying influence of adiposity on ANS activity.

The purpose of this study was to evaluate the influences of adiposity on the parasympathetic and sympathetic branches of the ANS before and after exercise. Specifically, parasympathetic, and sympathetic activities were assessed via HRV and PEP, respectively. Central adiposity was characterized by waist circumference (WC), while overall adiposity was characterized by body fat percentage (BF%). We hypothesized that increased adiposity reflects a blunted ANS response after maximal exercise through a delayed and decreased magnitude of parasympathetic activity (lower HRV) accompanied by prolonged and increased intensity of

sympathetic activity (lower PEP). Our secondary hypothesis was that differences in post-exercise ANS activity across the adiposity continuum would be partially explained by differences in cardiorespiratory fitness.

Methods

Participants

To limit the potential influence of hormonal variation (Koenig & Thayer, 2016) and age (Antelmi et al., 2004) on ANS activity, only males between the ages of 18-35 years old were recruited. A total of 26 participants were evaluated and found to meet the inclusion criteria of no use of tobacco or vaping products for at least the past 6 months, having a diastolic blood pressure <90 mm Hg and a systolic blood pressure <140 mm Hg, no history of cardiovascular disease, myocardial infraction, and/or diabetes, and not currently taking medications known to affect heart rate and/or blood pressure. Additionally, to limit the influence of circadian rhythm on ANS activity (Sammito et al., 2016), only participants that regularly rise by 0900 hours were included.

Experimental Protocol

Participants were instructed to fast for a minimum of 8 hours and to refrain from exercising 24 hours prior to testing. Upon arrival to the laboratory, participants completed the Aerobic Center Longitudinal Study Physical Activity Questionnaire (Kohl et al., 1988). Resting blood pressure was then assessed using an automated cuff (HEM-907XL, Omron Healthcare Inc., Kyoto, Japan) following ≥5 minutes of quiet sitting.

To provide comprehensive assessment of adiposity, multiple anthropometric measurements were collected. Waist circumference was reported as the mean of two measurements taken at the narrowest part of the torso located above the umbilicus and below the xiphoid process. Body mass index was calculated as weight (kg) divided by height (m) squared.

Estimation of fat and lean body mass was accomplished via air displacement plethysmography (BODPOD, COSMED, Chicago, Illinois).

Participants were then prepared for ANS activity measurement (see below) and then rested quietly in a supine position for 10 minutes in an isolated exam room while ANS activity was recorded. This 10-minute session was taken to assess true resting ANS activity. Next, the participants again had their ANS activity monitored for an additional 10 minutes, this time while resting in semi-recumbent position. This second resting position was used to limit the influence of postural changes on ANS activity during pre-post-exercise comparisons (Carnethon et al., 2002). At the end of the semi-recumbent resting session, blood was sampled via venipuncture for determination of glucose management parameters (see below). Following a maximal graded exercise test (see below), participants rested in the semi-recumbent position for 10 minutes while post-exercise ANS activity was assessed.

Assessment of Autonomic Nervous System Activity

Activities of the parasympathetic and sympathetic nervous systems were assessed via heart rate variability (HRV) and systolic time intervals (STI), respectively. HRV was monitored through standard 3-electrode ECG. All ECG recordings were automatically corrected for signal artifact and cardiac dysrhythmia vis Kubios HRV Premium software (Tarvainen, Niskanen, et al., 2014) confirmed by follow-up visually inspected. For classification purposes, time domain parameters [natural logarithm of the standard deviation of normal-to-normal beats (SDNN) and the root mean square of successive differences between normal-to-normal beats (RMSSD)] were calculated across the entire 10-minute supine rest period. For pre-to-post exercise comparisons, ln-SDNN and ln-RMSSD were calculated for 1-minute segments during the 10^{th} minute of semi-recumbent rest pre-exercise and during the 1^{st}, 3^{rd}, 5^{th}, and 10^{th} minutes post-exercise rest periods.

Frequency domain parameters [natural logarithm of low frequency (ln-LF) assessed between 0.04-0.15 Hz and high frequency (ln-HF) assessed between 0.15-0.40 Hz] were also calculated across the 10-minutes of supine rest. Recordings in the semi-recumbent position were used for pre-post exercise test comparison and were conducted over two-minute epochs per the minimal suggested requirement (Malik et al., 1996). These time epochs align with those utilized in the time-domain analyses in attempts to best reflect ANS activity during the chosen time points. As such, ln-LF and ln-HF analysis were conducted during the last two minutes of semi-recumbent rest pre-exercise and during minutes 0-2, 2-4, 4-6, and 8-10 post-exercise.

Following bilateral placement of paired electrodes at the base of the neck and around the chest at the level of the xiphoid process (Sherwood et al., 1990), the pre-ejection period (PEP) was calculated via impedance cardiography (BioNomadix, BIOPAC system Inc, Goleta, CA). PEP represents the time from the origination of ventricular depolarization (beginning of QRS complex) to the opening of the aortic valve (B-point, the point of inflection directly before the large amplitude jump in the wave, or the point of maximum rate change) on the simultaneous impedance recording. PEP was calculated during the last minute of the 10-minute supine recording and at the same semi-recumbent pre-post exercise epochs as the ln-SDNN and ln-RMSSD metrics, utilizing an ensemble average for each epoch.

Blood Sampling and Analyses

Glycosylated hemoglobin (Hb_{A1c}) was assessed immediately following the blood draw (Polymer Technology Systems Inc., Indianapolis, IN). The remaining blood sample was centrifuged at 3500 rpm for 10-15 minutes and serum was aliquoted into coded tubes and immediately stored at -80°C. Samples were analyzed for glucose and insulin concentrations via

a colorimetric assay (Cayman Chemical, Ann Arbor, MI) and an enzyme linked immunosorbent assay (Mercodia, Winston Salem, NC), respectively.

Exercise Testing

To account for the impact of cardiorespiratory fitness on ANS activity, participants completed a maximal graded exercise test. All tests were conducted on a treadmill utilizing a protocol that increased in intensity every 2 minutes. Oxygen consumption (VO_2) was calculated through the measurement of expired gas composition and volume (Parvo, Salt Lake City, UT). Participants were instructed to give maximal effort and preformed the test to volitional fatigue. Immediately following the test termination, the mouthpiece and head gear were removed and the participants rested as described above. The start time for the recovery period began as soon as the participants assumed the semi-recumbent position, approximately 30-45 seconds following test termination.

Statistical Analyses

To assess the influence of various aspects of adiposity on ANS activity, WC and BF% were used as grouping factors. Given that most WC measures were classified as low risk for cardiometabolic diseases, the participants were stratified into three groups derived from ordered racking of WC values. Naturally occurring breaks at 78cm and 88cm provided stratification boundaries resulting in groupings of "Low" (WC $\leq$78cm), "Mid" (78cm$\leq$WC $\leq$88cm), and "High" (WC $\geq$88cm) WC values. BF% varied considerably among the participants and natural breaks in the data. Post hoc cluster analyses supported the use of these cutoff values as grouping determinants, despite resulting in unbalanced groups. These groups represented "Low" (BF%<16%), "Mid" (20<BF%<30), and "High" (BF%>30%) body composition. For both BF% and WC groupings, baseline characteristics and supine ANS activity metrics of the groups were

compared using a one-way ANOVA while pre-post maximal exercise alterations in ANS activity were evaluated using a factorial ANOVA with repeated measures. Baseline ANCOVA and a factorial ANCOVA with repeated measures utilized VO_{2peak} as a covariate to assess the additive impact of cardiorespiratory fitness on the dependent variables. It has been suggested that the traditional practice to evaluate cardiorespiratory fitness by standardizing VO_2 values by overall body mass (mL·kg body mass^{-1}·min^{-1}) underestimates cardiorespiratory fitness in obese populations (Krachler et al., 2015; Savonen et al., 2012). Thus, to further delineate the independent effects of adiposity on ANS performance accounting for cardiorespiratory fitness, the above analyses were repeated with "Lean VO_{2peak}" (mL·kg lean body mass^{-1}·min^{-1}) as a covariate. Main effects and post hoc pairwise comparison results using a Bonferroni correction are reported. Statistical significance was predetermined at $\alpha = 0.05$.

Results

Waist Circumference (Unadjusted)

The baseline characteristics of the WC groups are provided in Table 4.1. The Low and Mid groups had narrow WC ranges with a mean and standard deviation of 74.9±2.6cm and 83.3±3.4cm respectively, while the range of the High group varied largely at 97.8± 12.1cm. The High group had significantly higher anthropometric measures than the other two groups, but no significant intergroup differences were present among the other cardiometabolic parameters, VO_{2peak} (mL·kg body mass^{-1}·min^{-1}), or weekly leisure-time physical activity. In addition, the three groups did not differ in any supine ANS metric before or after adjustment for VO_{2peak} (mL·kg body mass^{-1}·min^{-1}) (Table 4.2) and these metrics were similar to the typical resting values outlined in Table 2.1.

Table 4-1. Baseline Characteristics for the WC Groups

Group	Low	Mid	High	Overall
N	9	9	8	26
Age	23(5)	27(4)	25(5)	25(5)
Height (cm)	174(8.0)	174.5(6.3)	181.5(4.5)	176.5(7.1)
Weight (kg)	69.79(7.7)*	76.32(5.22)*	104.94(18.45)	82.86(18.87)
Bodpod (BF%)	11.88(3.36)*	17.87(7.91)*	27.64(9.27)	18.80(9.50)
WC (cm)	74.9(2.6)*	83.3(3.4)*	97.8(12.1)	84.9(11.7)
BMI ($kg \cdot m^{-2}$)	22.94(0.88)*	25.09(2.08)*	31.86(5.61)	26.43(4.99)
Lean mass/Fat mass	8.19(2.93)*	5.96(3.34)	2.97(1.32)	5.81(3.38)
Resting SBP (mmHg)	125(5)	124(10)	129(8)	126(8)
Resting DBP (mmHg)	75(8)	72(5)	79(10)	75(8)
Resting Heart Rate (bpm)	56(7)	57(10)	61(8)	59(10)
Hb_{A1c} (%)	5.1(0.5)	4.9(0.2)	4.9(0.2)	5.0(0.3)
Glucose ($mg \cdot dL^{-1}$)	47.61(9.33)	57.14(14.67)	54.94(8.82)	53.16(11.68)
Insulin ($\mu U \cdot mL^{-1}$)	3.48(1.42)	2.86(0.80)	4.13(1.60)	3.47(1.36)
VO_{2peak} ($mL \cdot kg$ body $mass^{-1} \cdot min^{-1}$)	51.81(7.42)	49.76(9.12)	43.61(11.34)	48.58(9.62)
Lean VO_{2peak} ($mL \cdot kg$ lean body $mass^{-1} \cdot min^{-1}$)	58.62(6.90)	60.27(8.29)	59.72(11.69)	59.53(8.72)
HR_{peak}	194(10)	186(10)	193(10)	191(10)
$APHR_{max}$%	100.9(4.1)	98.6(4.7)	101.9(4.2)	100.4(4.4)
RER_{peak}	1.08(.04)	1.07(.05)	1.07(.04)	1.08(.04)
RPE_{peak}	19(1)	19(1)	19(1)	19(1)
MET·h/wk	101(39)	81(49)	76(65)	86(51)

Note: Mean(stdev); $APHR_{max}$%, Percentage of age predicted maximal heart rate achieved;

* significantly different than High group

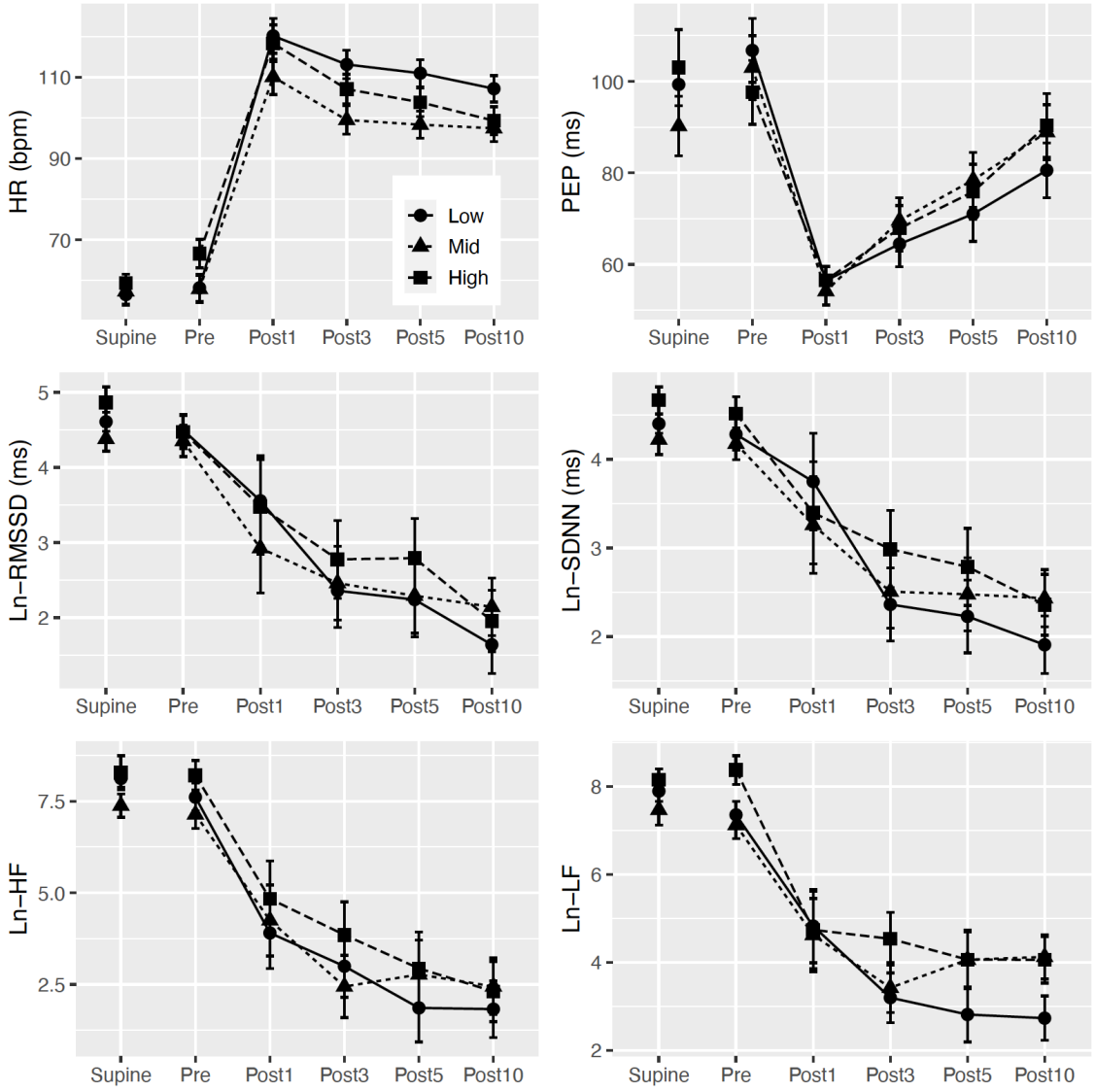
The results of the one-way ANOVA for supine measurements and factorial ANOVA with repeated measures appear in Figure 4.1. All variables displayed significant changes across time. HR remained significantly higher and the HRV metrics of Ln-HF, ln-LF, ln-RMSSD, and ln-

SDNN remained significantly decreased following maximal exercise and remained so for the entire 10-minute recovery. PEP significantly decreased at the onset of semi-recumbent recovery and remained so for all groups until 10 minutes post-exercise cessation. There were no meaningful between-group differences in any variable amongst the three groups.

Table 4-2. Supine ANS Measurements by WC Grouping by Model

Model	Group	HR	ln-RMSSD	ln-SDNN (msec)	PEP (msec)	ln-HF ($msec^2$)	ln-LF ($msec^2$)
WC ANOVA	Low	56(8)	4.60(0.38)	4.40(0.32)	99.3(14.0)	8.20(0.74)	7.89(0.70)
	Mid	57(10)	4.38(0.49)	4.22(0.51)	90.2(19.5)	7.38(0.96)	7.47(1.04)
	High	59(6)	4.86(0.59)	4.66(0.42)	103(23.6)	8.28(1.32)	8.15(0.71)
	p value	0.754	0.15	0.118	0.38	0.168	0.257
WC adjusted for VO_{2peak}							
	Low	57(8)	4.61(0.40)	4.41(0.34)	96.5(14.2)	8.16(0.74)	7.90(0.70)
	Mid	57(10)	4.38(0.53)	4.2(0.53)	89.2(20.6)	7.40(0.96)	7.47(1.04)
	High	59(8)	4.86(0.59)	4.65(0.42)	107.4(23.6)	8.22(1.32)	8.14(0.71)
	p value	0.91	0.159	0.246	0.275	0.203	0.301

Figure 4-1. Pre-Post ANS metrics for WC Groups



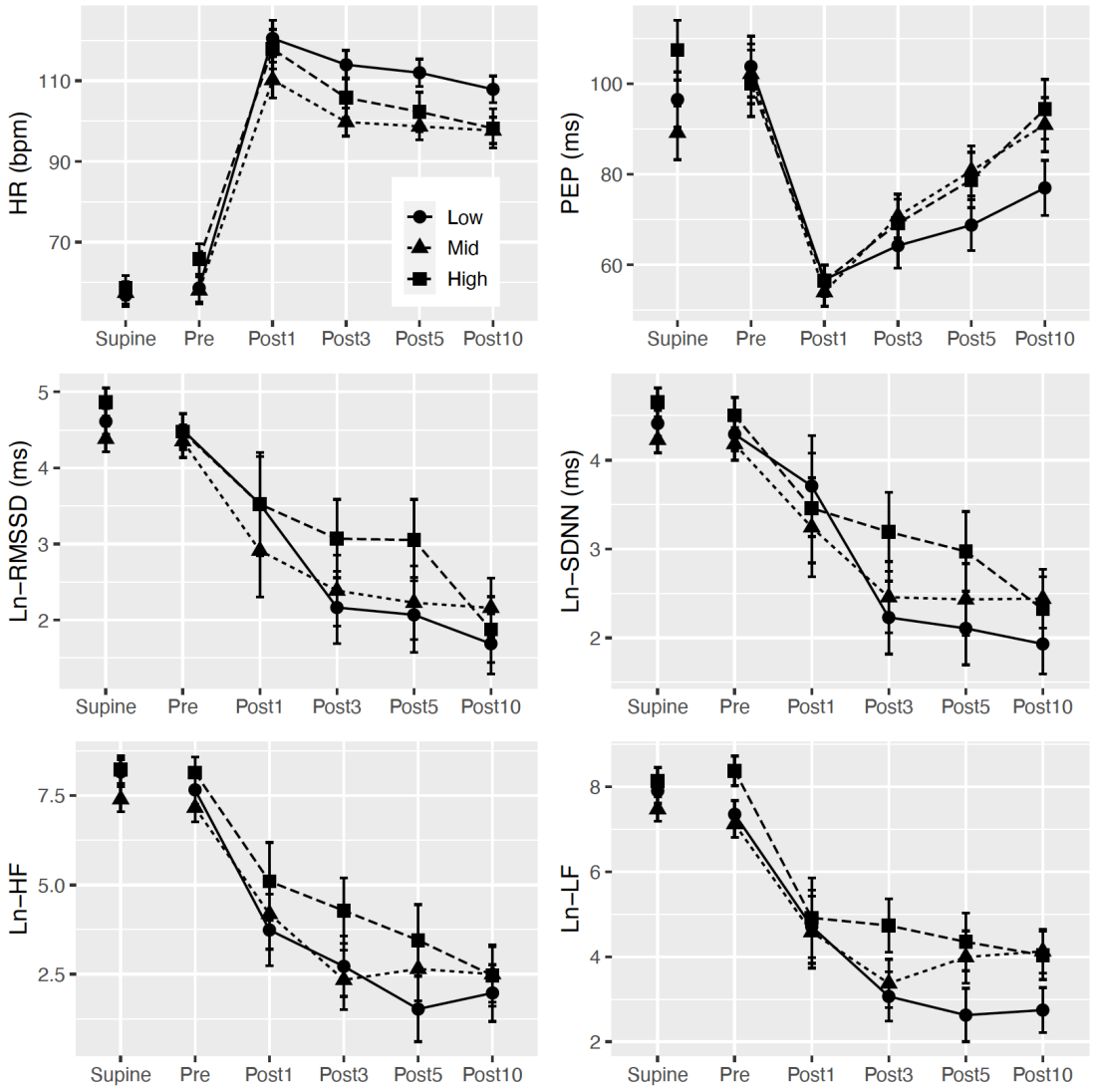
Note. Data are means ± standard error for each variable from the 10 minutes supine, semi-recumbent position pre- and post- 1,3,5, and 10 minutes maximal exercise. Supine values were not included in the repeated measures analyses and were compared with one-way ANOVA. Groups represent the Low (74.9±2.6cm), Mid (83.3±3.4cm), and High (97.8±12.1cm) WC groups respectively. Ln-HF, Natural logarithm of high frequency power; ln-LF, Natural logarithm of low frequency power; ln-RMSSD, Natural logarithm of the root mean squared of

successive differences; ln-SDNN, Natural logarithm of stand deviation of normal-to-normal beats.

Waist Circumference Adjusted for VO_{2peak}

As shown in Table 4.2, including VO_{2peak} as a covariate did not result in appreciable adjustments of the means of supine measurements. Still, there were no significant intergroup differences at baseline after adjusting for an average VO_{2peak} of 48.58 mL·kg body mass^{-1}·min^{-1}. The results from the factorial ANCOVA with repeated measures are displayed in Figure 4.2. All variables trended in a similar manner following exercise cessation, regardless of whether or not they were adjusted for VO_{2peak}. Ln-HF, ln-LF, and ln-RMSSD displayed significant main effects across time while PEP and ln-SDNN did not. There were no significant between-group main effect differences in any variable among all three groups when adjusting for VO_{2peak}. Additional factorial ANCOVA with repeated measures analyses were conducted for each dependent variable while controlling for an average Lean VO_{2peak} of 59.53mL·kg lean body mass^{-1}·min^{-1} and there were still no between-group differences (data not shown).

Figure 4-2. Pre-Post ANS metrics for WC Groups Accounting for $VO2_{peak}$



Note. Data are adjusted means ± standard error accounting for an average VO_{2peak} of 48.58 ml/kg/min for each variable from the 10 minutes supine, semi-recumbent position pre- and post- 1,3,5, and 10 minutes maximal exercise. Supine values were not included in the repeated measures analyses and were compared with one-way ANOVAs. Groups represent the Low (74.9±2.6cm), Mid (83.3±3.4cm), and High (97.8±12.1cm) WC groups respectively. Ln-HF, Natural logarithm of high frequency power; ln-LF, Natural logarithm of low frequency power;

ln-RMSSD, Natural logarithm of the root mean squared of successive differences; ln-SDNN, Natural logarithm of stand deviation of normal-to-normal beats.

Body Fat Percentage (Unadjusted)

The participants were grouped by Low (BF%<16%), Mid (20<BF%<30), and High (BF%>30%) BF%. As opposed to WC stratification, this grouping resulted in differences across several cardiometabolic variables (Table 4.3). The Low group had significantly lower body weight, WC, BMI, BF%, fat mass, resting DBP, and insulin in addition to significantly higher VO_{2peak} and MET-h/wk compared to the High group. The Mid group was significantly lower compared to the High group in terms of fasting insulin concertation, BF%, and fat mass. The Mid group only significantly higher compared to the Low group in terms of BF% and fat mass(kg).

The results of the one-way ANOVA for resting supine values (Table 4.4) revealed no significant intergroups differences for any ANS activity marker. The repeated measures ANOVA for BF% groups (Figure 4.3) displayed trends similar to those of the WC strata. HR significantly increased for all groups immediately post maximal exercise and remained significantly elevated for the entire recovery period. All groups displayed the same steep decrease in frequency-domain and gradual decline in time-domain HRV metrics post-exercise like WC group comparisons. Similar alterations in PEP were observed post exercise, with a general steep decrease immediately upon resting and a marked increase following post-1 minute recovery. There were no significant differences between main effects for any variable across the three groups.

Table 4-3. Sample Characteristics for BF% Groups

Group	Low	Mid	High	Total
N	15	7	4	26
Age	23(4)	28(5)	27(4)	25(5)
Height (cm)	174.6(7.7)	180.0(6.5)	177.6(4.2)	176.5(7.1)
Weight (kg)	73.34(8.49)*	90.29(12.97)	105.60(31.97)	82.86(18.87)
Body Fat (%)	12.12(3.23)†	23.54(2.77)†	35.55(6.20)†	18.80(9.50)
Fat Mass (kg)	8.80(2.733) †	21.43(4.17) †	38.97(18.31) †	16.84(13.10)
Lean Mass (kg)	64.68(7.45)	69.03(9.90)	66.77(14.61)	66.17(9.16)
Lean mass/Fat mass	8.05(2.69)	3.27(0.47)**	1.87(0.46)**	5.81(3.38)
Waist Circumference (cm)	78.5(5.4)*	90.0(7.1)	100.1(18.3)	84.9(11.7)
BMI ($kg \cdot m^{-2}$)	24.00(2.02) *	27.80(2.83)	33.15(8.85)	26.43(4.99)
Resting SBP (mmHg)	123(8)	128(5)	132(9)	126(8)
Resting DBP (mmHg)	73(7)*	75(4)	85(11)	75(8)
Resting Heart Rate	58(9)	57(8)	71(14)	59(10)
Hb_{A1c} (%)	5.1(0.4)	4.9(0.1)	4.8(0.3)	5.0(0.3)
Glucose ($mg.dL^{-1}$)	53.85(12.67)	50.51(12.55)	55.22(6.84)	53.16(11.68)
Insulin (μU/mL)	3.12(1.23)*	3.21(1.06)*	5.21(1.17)	3.47(1.36)
VO_{2peak} ($mL \cdot kg$ body $mass^{-1} \cdot min^{-1}$)	51.92(7.71)*	48.59(8.07)	36.03(9.86)	48.58(9.62)
Lean VO_{2peak} ($mL \cdot kg$ lean body $mass^{-1} \cdot min^{-1}$)	58.83(7.56)	63.49(9.10)	55.22(11.74)	59.53(8.72)
HR_{peak}	192(10)	190(12)	190(12)	191(10)
$APHR_{max}$%	100.2(4.1)	100.7(4.9)	100.5(5.7)	100.4(4.4)
RER_{peak}	1.07(0.05)	1.08(0.05)	1.08(0.03)	108(0.04)
RPE_{peak}	19(1)	19(0)	19(1)	19(1)
MET·h/wk	101.0(41.2)*	86.5(64.0)	31.6(13.3)	86(51)

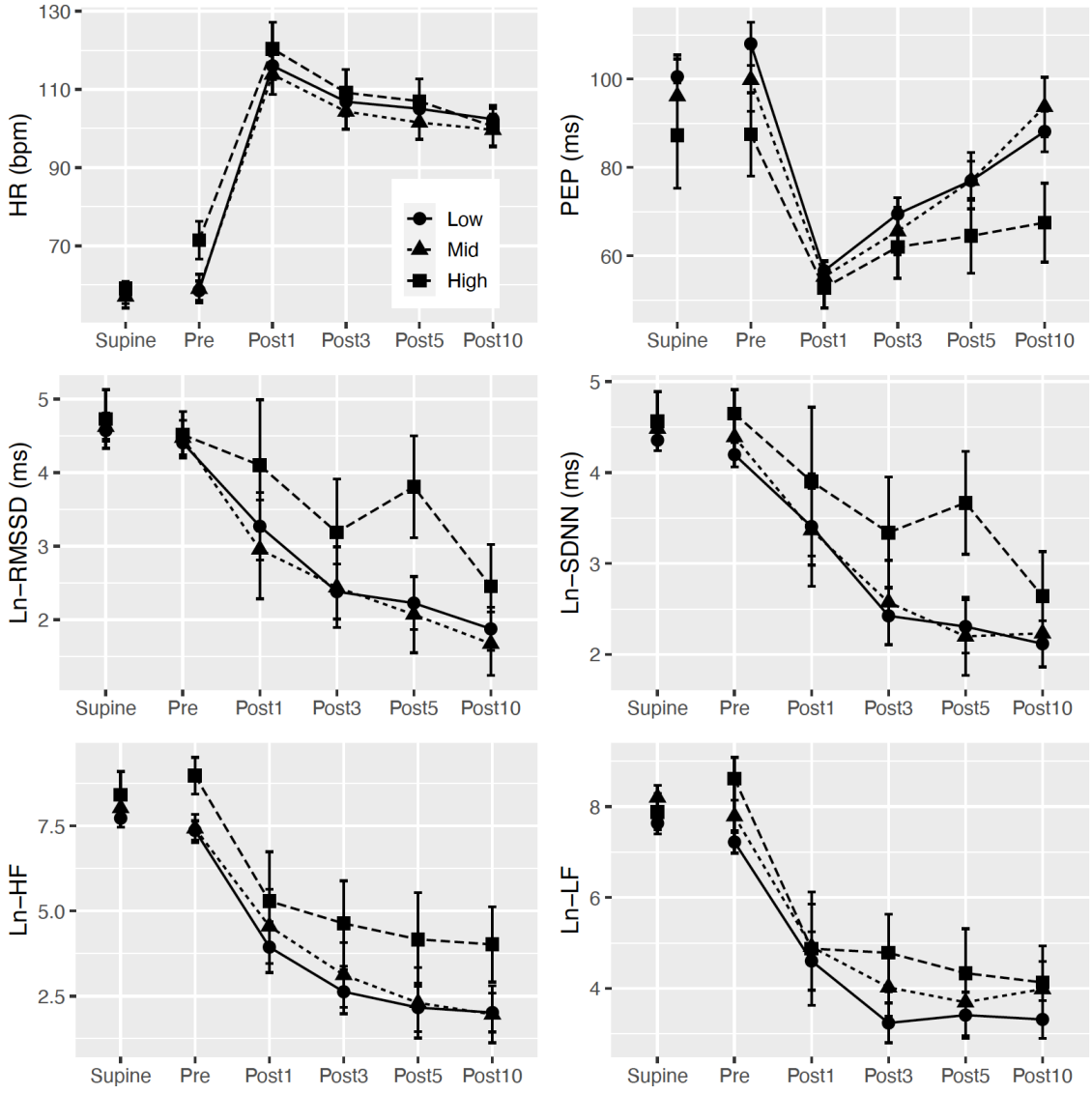
Note. Mean(stdev); Groups representative of BF% values at Low (BF%<16%), Mid (20<BF%<30), and High (BF%>30%) BF%.;); $APHR_{max}$%, Percentage of age predicted

maximal heart rate achieved; *significantly different than Group 3; ** significantly different than Group 1 †All groups are significantly different.

Table 4-4. Supine ANS Measurements by BF% Groups by Model

Model	Group	HR (bpm)	ln-RMSSD (msec)	ln-SDNN (msec)	PEP (msec)	ln-HF (msec2)	ln-LF ($msec^2$)
BF ANOVA	1	58(9)	4.57(0.46)	0.44(0.11)	100.5(15.5)	7.73(1.01)	7.63(0.91)
	2	57.(8)	4.62(0.50)	0.36(0.14)	96.1(24.7)	8.02(1.04)	8.20(0.71)
	3	59.(3)	4.72(0.80)	0.65(0.32)	87.3(23.9)	8.41(1.38)	7.89(0.80)
	p value	0.909	0.482	0.87	0.668	0.506	0.362
BF ANCOVA adjusting for VO_{2peak}	1	58.(9)	4.37(0.46)	4.37(0.44)	98.6(15.4)	7.73(1.01)	7.65(0.91)
	2	57(8)	4.48(0.50)	4.48(0.36)	96.1(24.7)	8.02(1.04)	8.20(0.71)
	3	57(3)	4.49(0.80)	4.49(0.65)	94.4(23.4)	8.39(1.38)	7.82(0.80)
	p value	0.945	0.936	0.964	0.865	0.659	0.41
BF ANCOVA adjusting for Lean VO_{2peak}	1	57(9)	4.57(0.46)	4.35(0.44)	101.0(15.4)	7.73(1.01)	7.63(0.91)
	2	58(8)	4.62(0.50)	4.49(0.36)	93.6(24.7)	8.0(1.0)	8.22(0.71)
	3	59.(3)	4.74(0.80)	4.55(0.65)	90.0(23.9)	8.4(1.4)	7.86(0.80)
	p value	0.973	0.506	0.87	0.679	0.512	0.367

Figure 4-3. Pre-Post ANS Metrics for BF% Groups



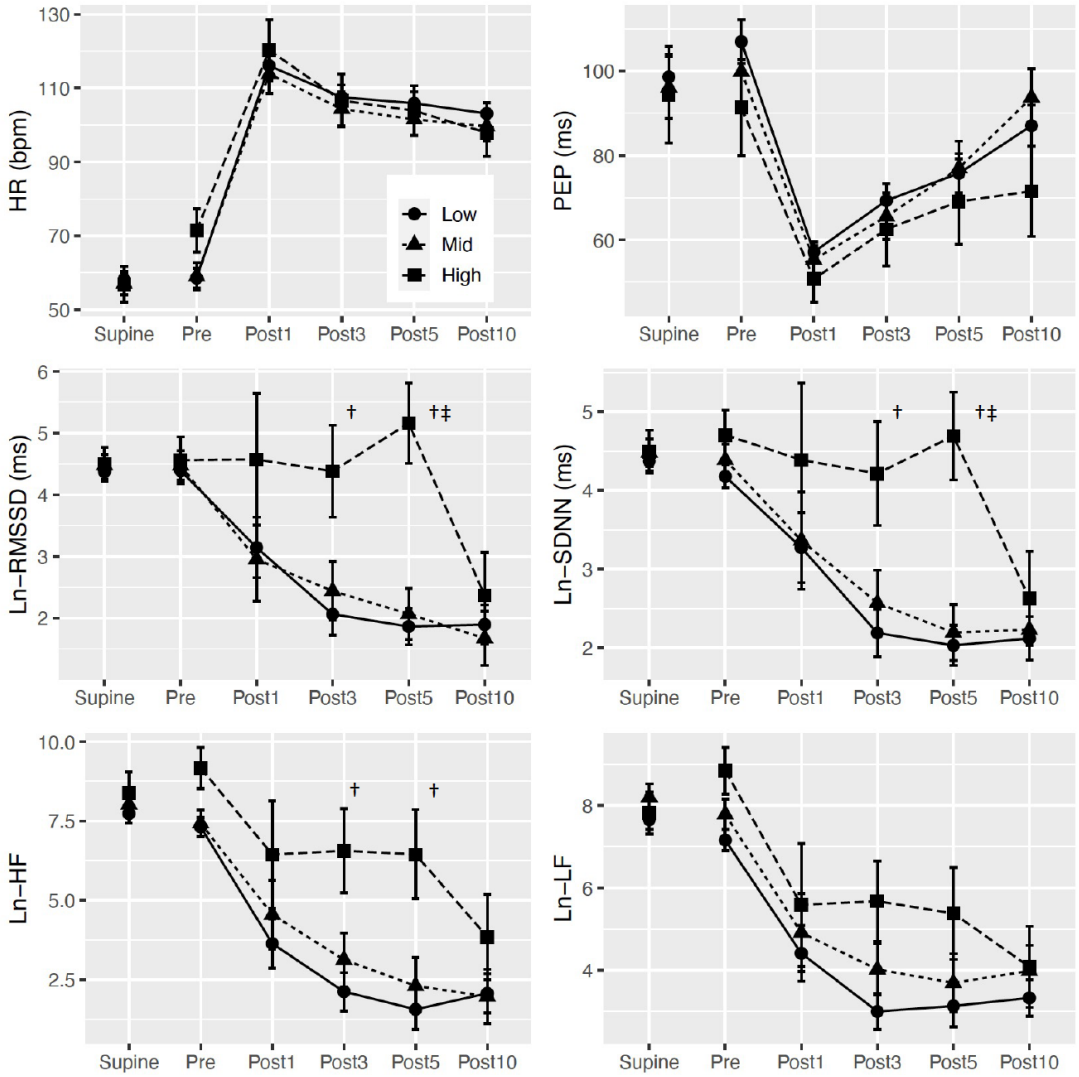
Note. Data are means ± standard error for each variable from the 10 minutes supine, semi-recumbent position pre- and post- 1,3,5, and 10 minutes maximal exercise. Supine values were not included in the repeated measures analyses and were compared with one-way ANOVAs. Groups represent Low (BF%<16%), Mid (20<BF%<30), and High (BF%>30%) body compositions respectively. Ln-HF, Natural logarithm of high frequency power; ln-LF, Natural logarithm of low frequency power; ln-RMSSD, Natural logarithm of the root mean

squared of successive differences; ln-SDNN, Natural logarithm of stand deviation of normal-to-normal beats.

Body Fat Percentage adjusted for VO_{2peak}

Controlling for VO_{2peak} resulted in no significant across group differences at baseline (Table 4.4). All variables, except PEP, had significant main effects across time (Figure 4.4). HR significantly increased immediately post exercise and remained elevated for the entire 10-minute recovery session. The Low and Mid groups had the same response in terms of ln-RMSSD and ln-SDNN across time in that they did not decrease until 3-minutes post exercise, remaining lower than pre-exercise levels for the remainder for the recovery session. The High group differed in its response and remained at pre-exercise levels until significantly decreasing at the post 10-minute time mark. The Low group displayed an immediate decrease in ln-LF power and ln-HF power following cessation of exercise, with levels remaining significantly lower for the entire 10-minute recovery period. The Mid group had a delayed decrease in HRV frequency metrics with ln-LF power and ln-HF power decreasing at post-3 minutes and remaining lower than pre-exercise levels. The High group had a similar trend in ln-LF power, significantly decreasing at post-3 minutes and remaining lower than pre-exercise levels. However, ln-HF power did not significantly decrease until the post-10 minute time point for the High group. There were significant between-group main effects for ln-RMSSD ($F = 4.33$, $p = .026$), ln-SDNN ($F = 4.26$, $p = .027$), and ln-HF power ($F = 3.93$, $p = .035$). Post-hoc pairwise comparisons revealed that the High group had significantly higher levels of all three variables compared to the Low group at 3- and 5-minutes post exercise recovery. Additionally, the High group had significantly higher time-domain HRV metrics compared to the Mid group at the post 5-minute time mark.

Figure 4-4. Pre-Post ANS metrics for BF% Groups Accounting for $VO2_{peak}$



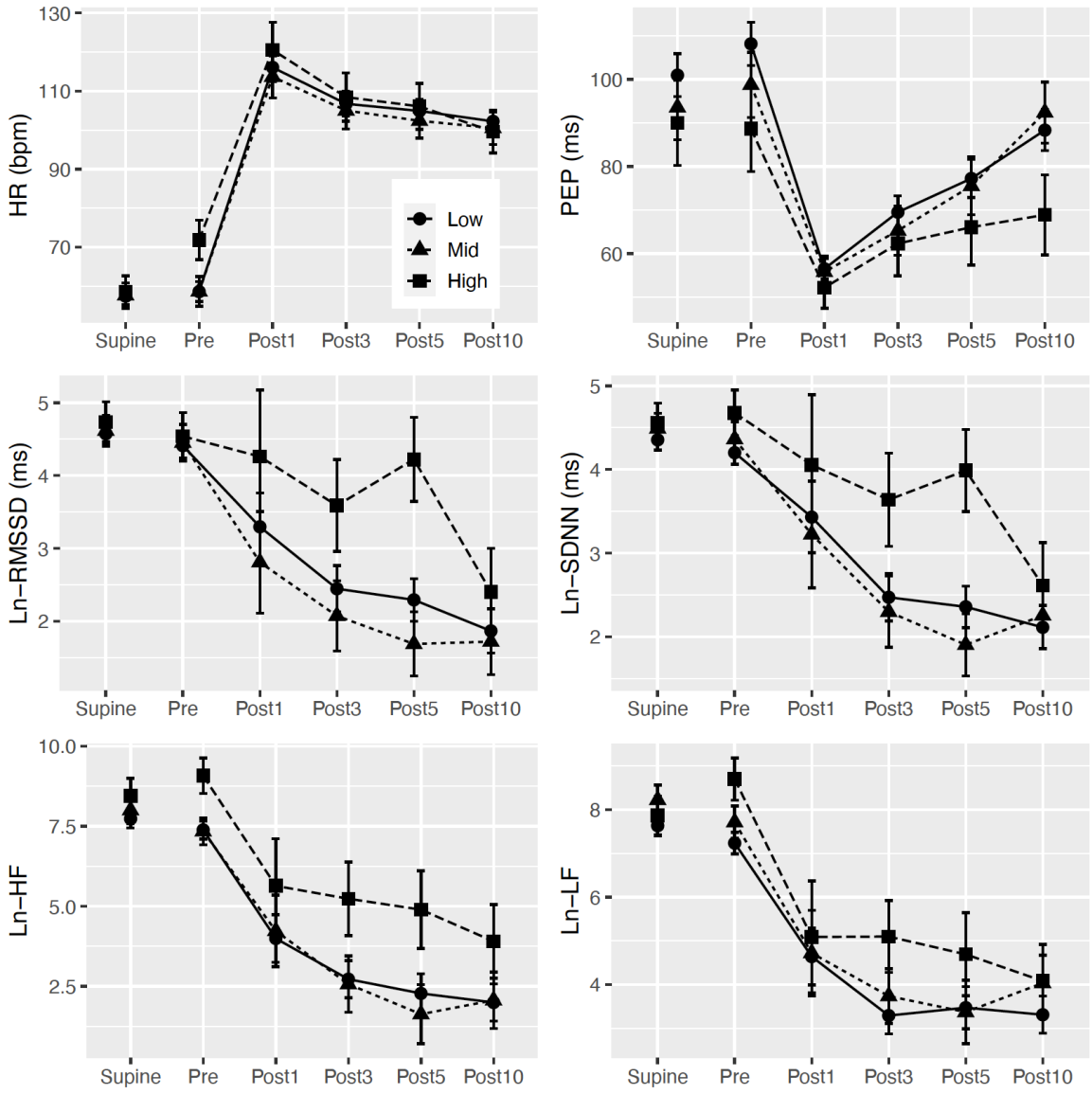
Note: Data are adjusted means ± standard error accounting for an average VO_{2peak} of 48.58 ml/kg/min for each variable from the 10 minutes supine, semi-recumbent position pre- and post- 1,3,5, and 10 minutes maximal exercise. Supine values were not included in the repeated measures analyses and were compared with one-way ANOVAs. Groups 1, 2, and 3 represent Low (BF%<16%), Mid (20<BF%<30), and High (BF%>30%) body compositions respectively. Ln-HF, Natural logarithm of high frequency power; ln-LF, Natural logarithm of low frequency power; ln-RMSSD, Natural logarithm of the root mean squared of successive differences; ln-

SDNN, Natural logarithm of stand deviation of normal-to-normal beats. † Denotes Low and High groups are significantly different; ‡ Denotes Mid and High groups are significantly different

Body Fat Percentage adjusted for Lean VO_{2peak}

As previously suggested, standardizing VO_2 values by division of overall body mass (mL·kg body mass^{-1}·min^{-1}) may not accurately reflect cardiorespiratory fitness in obese individuals. Thus, to further investigate the independent effects of adiposity on ANS performance, the BF% group results were adjusted for oxygen uptake expressed as a factor of lean mass. Correcting for Lean VO_{2peak} resulted in no significant between group differences at baseline (Table 4.4). Figure 4.5 depicts all HRV variables having significant main effects across time. Additionally, PEP did not display a significant main effect across time. All groups presented significantly increased HR immediately post-exercise and remained elevated compared to pre-exercise levels for the entire 10-minutes of recovery. The Low and Mid groups showed similar gradual decreases in ln-RMSSD and ln-SDNN at 3-minutes post exercise cessation while the High group remained at pre-exercise levels for both variables until 10-minutes post-exercise. While this difference in responses displayed meaningful trends, the between-group main effects for ln-RMSSD and ln-SDNN failed to reach statistical significances ($p = .065$ and .075 respectively). Furthermore, there were no significant between-group main effects for any variable amongst all three groups. Taken together, adjustment for Lean VO_{2peak} resulted in similar pre-post exercise trends observed with unadjusted BF% analyses and lacked the significant between-group differences observed with adjustments for VO_{2peak} (mL·kg body mass^{-1}·min^{-1}).

Figure 4-5. Pre-Post ANS metrics for BF% Groups Accounting for Lean $VO2_{peak}$



Note. Data are adjusted means ± standard error accounting for an average lean VO_{2peak} of 59.53 mL·kg lean body $mass^{-1}$·min^{-1} for each variable from the 10 minutes supine, semi-recumbent position pre- and post- 1,3,5, and 10 minutes maximal exercise. Supine values were not included in the repeated measures analyses and were compared with one-way ANOVAs. Groups 1, 2, and 3 represent Low (BF%<16%), Mid (20<BF%<30), and High (BF%>30%) body compositions respectively. Ln-HF, Natural logarithm of high frequency power; ln-LF, Natural

logarithm of low frequency power; ln-RMSSD, Natural logarithm of the root mean squared of successive differences; ln-SDNN, Natural logarithm of stand deviation of normal-to-normal beats.

Discussion

Results from the present study suggest that adiposity has limited influence on ANS activity at rest or following maximal exercise in apparently healthy, young males. No significant differences in HRV metrics or PEP existed across WC strata, with or without adjustment for cardiovascular fitness. Similarly, no differences were found at rest across BF% groups. However, most HRV markers (ln-HF, ln-RMSSD, and ln-SDNN) were significantly higher in the High BF% group compared to the Low BF% group during the third- and fifth-minutes post-exercise after adjusting for VO_{2peak}, but statistical significance was lost with adjustment for Lean VO_{2peak}. As Lean VO_{2peak} may more accurately reflect cardiovascular fitness in obese individuals (Krachler et al., 2015), a possible interpretation of these results is that ANS responses following maximal exercise do not differ substantially between lean individuals and those with higher adiposity.

Concerning resting ANS activity, our results suggest that in young apparently healthy males, there is no difference in ANS activity between groups of varying adiposity, regardless of the grouping method. Neither WC strata nor BF% clustering revealed significant differences in any of the various HRV metrics or PEP at rest in a supine or semi-recumbent position. These results differ from those of previous research employing similar recording epochs. In apparently healthy individuals with no known diseases, Yadav et al. (2017) reported that obese (BMI >30kg/m^2; mean WC=98.30 cm) participants had significantly lower RMSSD, SDNN, and HF power derived from 5-minute resting electrocardiogram recordings in a supine position than

aged-matched (mean age 30.48±8.01 years) non-obese (BMI< 30kg/m^2; mean WC=62.97 cm) individuals. Additionally, DBP differed between the obese and non-obese groups (84.97 versus 74.83 mmHg). As DBP did not differ among the three WC groups in the present study, HRV in the above study might have been more a reflection of vascular resistance than of WC. Further evaluation of the relations among adiposity, mild diastolic hypertension, and ANS indices is warranted.

Recent results from Osailan et al. (2020) showed no difference in heart rate recovery (HRR) following exercise to volitional fatigue between young men with BF% ≥30 (WC=109.18±15.12 cm) and <30 (WC=80.86±8.07 cm). Studies examining pre-post exercise comparisons between young (22.4±1.1 years) individuals with excessive body fat (≥30BF%) and individuals with normal body composition (≤30BF%) suggest no significant difference in ANS activity as determined by the use of heart rate recovery (HRR) (Osailan et al., 2020). The study also did not find a significant correlation between HRR and any measured metric of adiposity (BMI, WC, waist-to-hip ratio, or BF%). The authors suggested that the lack of differences in HRR following exercise tolerance testing is indicative of a similar parasympathetic response post-exercise regardless of differences in body composition. These results are in line with the findings of the current study in that between-group differences were not found in groups stratified by WC or BF% without adjustments for VO_{2peak}. Notably, Osailan et al. (2020) utilized an exercise protocol that ceased testing based on the participants reports of volitional exhaustion and did not collect expired gas during the exercise test, limiting the ability to determine if maximal exercise intensity was truly achieved. Furthermore, no methodological design or statistical adjustments for cardiorespiratory fitness were utilized beyond reporting the observed maximal heart rate of the excessive and normal fat groups (172±11.1 and 166.6±12.4 bpm,

respectively) which are less than the typically suggested minimum of ±10bpm of the age predicted maximal heart rate for maximal exercise.

Accounting for cardiorespiratory fitness may mitigate the relation of adiposity and post-exercise ANS responsiveness. Orri et al. (2018) evaluated postmenopausal women of differing training practices before and after maximal exercise. The study showed that women participating in moderate-intensity exercise training had HRV alterations during 2 minutes of active recovery following maximal exercise that were similar to those of women participating in vigorous-intensity exercise training. Despite stratification being based on self-reported exercise training status, the vigorous-intensity group had a higher WC (81.3 versus 74.1 cm) and similar VO_{2peak} (32.4 versus 34.5 $mL \cdot kg^{-1} \cdot min^{-1}$) compared to the moderate-intensity group, suggesting that WC does not cause HRV to differ across individuals when cardiovascular fitness is held constant. The Low and Mid groups of the present study possessed similar differences for WC with very little difference in VO_{2peak} and with no differences in HRV alterations following maximal exercise with or without adjustments for cardiorespiratory fitness. These findings collectively suggest that individuals with different WC but similar cardiorespiratory fitness display similar ANS activity post-maximal exercise.

The current study is strengthened by the employment of impedance cardiography as a non-invasive indicator of SNS activity. The PEP has been shown to be a valid indicator of SNS activity (Cacioppo et al., 1994; Michael, Graham, et al., 2017; Siedlecka et al., 2015) and can be used in conjunction with HRV metrics to more completely reflect ANS activity pre-post exercise. The relation between resting PEP and markers of adiposity is not well understood, with studies finding that lower PEP corresponds with higher WC (M. X. Hu et al., 2016; Licht et al., 2010), PEP is higher in severely obese individuals free of CVD (Romano et al., 1986), or that

PEP is not related to measures of adiposity (Vrijkotte et al., 2015). The present study found no significant between-group differences in resting PEP values regardless of adiposity stratification or adjustments for cardiorespiratory fitness.

Moreover, no across-group differences in PEP were found post-maximal exercise, regardless of adiposity stratification or adjustments for cardiorespiratory fitness. The maximal exercise used in the current study may have stimulated such a pronounced SNS response that capturing across-group differences in PEP might be limited by the recording methodology. It has been shown that maximal exercise delays parasympathetic reactivation and sympathetic withdrawal compared to submaximal and anaerobic threshold workloads (Imai et al., 1994). This delayed changed in ANS dominance may have occurred at a time point during recovery that was not accurately captured by the 1-minute time epochs used for the determination of PEP. The current time epochs were used to match those of the time-domain HRV metrics in attempts to reflect total ANS activity more accurately during the specified time segment. Nandi & Spodick (1977) observed differences in PEP values post-exercise at varying levels of exercise intensity up to 3 minutes post-exercise cessation utilizing recording epochs less than 10 seconds long. The true differences in PEP post-exercise could be lost in the current lengthier segments due to the rapid vagal reactivation and slower decay of SNS stimulation during the first few moments following exercise cessation. This time frame of heart rate recovery (HRR) is reported as the fast phase and is roughly defined as approximately the first 60 seconds post-exercise cessation (Peçanha et al., 2017). The fast phase is characterized as a steep decline in HR which is then followed by a gradual decay (slow phase) until HR reaches resting values. Given that the fast phase may be prolonged in those with decreased cardiorespiratory fitness and by exercise intensity (Imai et al., 1994), it is possible that the 1-minute time epochs may not be short enough

to distingue between the ANS activity of the fast phase and the slow phase of HRR. This may have inadvertently prohibited the observation of any across-group differences in sympathetic activity (PEP) where groups stratification resulted in differences in VO_{2peak} (e.g., BF% grouping).

The present study limited the total post-exercise recording period to 10-minutes. While this time frame would be more than adequate to evaluate the fast phase of HRR, it may not fully capture and evaluate the entire slow phase. Previous studies have shown that metrics of ANS activity remained significantly altered compared to resting values from 30-minutes (Du et al., 2005) up to 60 minutes (Cipryan & Vala, 2015; James et al., 2012) following exercise cessation in relatively fit and lean participants, suggesting that the gradual withdrawal of SNS activity may take considerably longer than 10 minutes. Accordingly, the trends in post-exercise PEP alterations for BF% groupings (Figures 4.3-4.5) appear to be separating with the passage of time. Taken together, it is worth investigating whether individuals with higher levels of adiposity present with a similar delayed return to resting ANS activity or display different ANS activity during the slow phase of HRR observable in prolonged post-maximal exercise recordings.

Adjusting to an average VO_{2peak} of 48.58 mL·kg body mass^{-1}·min^{-1} across groups stratified by BF% resulted in individuals with higher levels of adiposity having greater ln-HF, ln-RMSSD, and ln-SDNN at the same relative HR compared to leaner individuals at 3- and 5-minutes post exercise. However, these between-group differences lost statistical significance when values were adjusted for Lean VO_{2peak} instead of VO_{2peak}. Though the standard convention for expressing cardiorespiratory fitness in terms of maximal oxygen consumption is to report the volume of oxygen consumed per until of total body mass (mL·kg body mass^{-1}·min^{-1}, VO_{2peak}), using this metric may unintentionally underrepresent the cardiovascular fitness of those with higher levels of adiposity (Savonen et al., 2012). An alternative expression of oxygen

consumption per unit of lean body mass (mL·kg lean body mass^{-1}·min^{-1}, Lean VO_{2peak}) may more accurately represent an individual's true cardiorespiratory fitness regardless of total body mass (Krachler et al., 2015). As such, the results of the repeated measures ANOCVA accounting for Lean VO_{2peak} has an increased prognostic value (Osman et al., 2000) and most likely provides a more accurate reflection cardiorespiratory fitness across the adiposity strata (Krachler et al., 2015). Given the fact that 4 out of the 5 repeated measures analyses failed to find significant between-group differences pre- or post-maximal exercise, these data may more appropriately denote no significant differences among groups of varying adiposities irrespective of controls for cardiorespiratory fitness. While adjusting group means for VO_{2peak} (mL·kg body mass^{-1}·min^{-1}) resulted in significant differences between the Low and High BF% groups post-exercise, this may be due to the covariate imposing a "double penalty" on the High BF% group.

Possible complications may be present given the baseline characteristics of the BF% groups. While group stratification based on BF% was theoretically and statistically sound as deemed by risk stratification guidelines and post-hoc cluster analyses, it revealed differences across groups in cardiometabolic risk indices besides adiposity. The High BF% group had significantly higher values compared to the Low BF% group for resting diastolic blood pressure and serum insulin concentrations, and lower values for self-reported physical activity levels (MET-h/wk). Though none of the participants were clinically diagnosed for hypertension or diabetes mellitus, the concept that ANS activity might differ across preclinical ranges of blood pressure and glucose management should be considered. A compounding effect was demonstrated in a study by Verma et al. (2018) in that individuals with hypertension and type II diabetes displayed significantly lower HRV metrics compared to those with hypertension or diabetes alone, or those that had neither disease. This suggests that as health worsens, ANS

activity is significantly altered. Given that the mean diastolic blood pressure values for the High BF% group was above the hypertension threshold (85±11 mmHg), the rationale that those with worse disease metrics have altered ANS activity could be viable. Such differences were not present when group stratification was based on WC values, posing a possible explanation for the lack of any significant difference between the groups pre- and post-exercise. As our data set had only two individuals with a mean WC value signifying high risk for CVD (≥102m), the true influence of high WC values on ANS activity may have been blunted. Moreover, BF% stratification increased the observed between-group differences in baseline cardiometabolic characteristics which may be more indicative of "pre-diseased" vs "healthy" individuals.

While both time and frequency domain metrics of HRV were employed to evaluate ANS activity, non-linear metrics may be more adapt at evaluating the subtle differences across adiposity groupings. It is suggested that a loss of signal complexity or an increase in the predictability of a biological signal, such as HR, is a hallmark of a diseased state (Parker & Srivastava, 2013; Riganello et al., 2022). Recent studies investigating the change in metrics of HR complexity (sample entropy) following exercise were shown to have significant relations with body composition while traditional linear metrics of HRV did not (Berry et al., 2021). As such, more sophisticated metrics of ANS activity may provide unique insight into the alterations of ANS activity following exercise cessation in individuals of varying levels of adiposity.

This study is strengthened by the dual use of HRV and PEP to provide valid measurements of both the parasympathetic and sympathetic branches of the ANS pre- and post-maximal exercise. Typically, studies using non-invasive practices rely on measurements that reflect a single branch, most commonly the parasympathetic, to interpret ANS function. Our approach provides a more holistic representation of ANS activity at the cardiac level.

Additionally, we investigated the effect of more than one indicator of adiposity on resting ANS activity and ANS responsiveness post-maximal exercise, with and without adjusting for cardiorespiratory fitness. The intertwined nature of adiposity and fitness produces a challenging obstacle to consider when assessing their impacts on a third variable. Further investigation into the nuances of this relation is warranted. Our interpretations are limited in the current study by the low numbers of participants with excessively high WC or BF%. As noted, only two individuals had a WC signifying high risk for CVD (≥102cm) and only four had a BF% greater than 30%. Additionally, concerns with the post-exercise recording length, specifically during the fast phase of HRR, were addressed above and should be considered.

In summary, our results indicate that adiposity alone does not have a significant impact on parasympathetic or sympathetic nervous system activity, at rest or following exhaustive exercise, in young males with relatively healthy levels of adiposity and fitness. While accounting for the additive impact of cardiorespiratory fitness (VO_{2peak}) did produce a significant difference between BF% groups, controlling for Lean VO_{2peak} neutralized this effect. Given the rationale of expressing VO_{2peak} per lean body mass to provide an unbiased measurement of cardiorespiratory fitness in obese individuals compared to expressing VO_{2peak} per total body mass, a possible interpretation of our results is that there is no considerable difference in ANS across adiposity groups at rest or following maximal exercise.

CHAPTER V: OVERALL SUMMARY

The autonomic nervous system (ANS) plays fundamental roles in the development of cardiorespiratory diseases and their risk factors, namely hypertension, diabetes mellitus, obesity, and poor cardiorespiratory fitness (Alam, Lewis, Morgan, & Baxter, 2009; Erdogan et al., 2011; Soares-Miranda et al., 2014; Tarvainen, Laitinen, Lipponen, Cornforth, & Jelinek, 2014). While the human body regulates biological functions with a fine-tuned balance of activity from both the parasympathetic and sympathetic branches, any prolonged disturbance or asymmetry amongst the two can result in the development of several clinical and physiological conditions (Pagani et al., 1988; Zhou et al., 2012). Most often the case, reduced parasympathetic and elevated sympathetic activities are associated with increased CVD risk, including earlier mortality, myocardial infarction, and development of diabetes mellitus and hypertension (Gerritsen et al., 2001; Kleiger et al., 1987; Tsuji et al., 1996). In addition to its direct relation with altered ANS activity (Farah et al., 2013; Poirier et al., 2003; Windham et al., 2012), increased adiposity has a multifaceted relation with ANS activity meditated by its undesirable influence on the development of hypertension and diabetes mellitus as well as a strong association with poor cardiorespiratory fitness. Thus, the purpose of this dissertation was to provide a holistic evaluation of the influence of increased adiposity on ANS activity using two different methodological approaches. First, utilization of the Atherosclerosis Risk in Communities (ARIC) dataset allotted for the use of structural equation modeling to evaluate the effects of adiposity on markers of ANS activity while accounting for indices of glycemic impairment (GI) and cardiac stress (CS). This approach provided the statistical means to simultaneously evaluate the direct effects of adiposity on resting levels of both branches of the ANS while accounting for its indirect effects via GI and CS in a large, population-based study of older adults. Second, non-

invasive measurements of ANS activity were collected in apparently healthy, young adult males before and after maximal exercise. This approach allowed for assessment of physiological variables in a homogeneous sample and provided multiple methods to account for the influence of cardiorespiratory fitness on the observed data, namely through the use of adjusted means for VO_{2peak} (mL·kg body mass^{-1}·min^{-1}) and Lean VO_{2peak}(mL·kg lean body mass^{-1}·min^{-1}).

Given the interconnected nature of increased adiposity with the development of hypertension and diabetes mellitus and their independent links to altered ANS activity, the model depicted in Figure 3.1 was proposed to test the multifaceted influence of adiposity on ANS activity. Structural equation modeling suggested that adiposity had both significant direct (β = 0.208, p = 0.018) and indirect (β = -.217, p=.041) effects on PEP (marker of sympathetic activity) through GI while displaying no significant effect on RMSSD (marker of parasympathetic activity). Even though CS displayed a significant pathway (β = -0.524, p = 0.035) on RMSSD, the indirect effect of WC on RMSSD through CS was not significant. Interestingly, increased central adiposity had opposing direct and indirect effects on PEP. The conceptual model (Figure 3.2) suggests that increases in central adiposity (WC) might have a blunting effect on sympathetic activity (direct effect) while simultaneously increasing sympathetic drive by increasing (worsening) indices of glycemic impairment (indirect effect). These opposing actions mitigate any observable physiological changes in PEP. In a mixed population of older adults, it appears that adiposity's multifaceted influence on resting ANS activity results in a less than obvious overall changes in markers of ANS activity. This sentiment is echoed by those of the smaller cohort study discussed in Chapter 4. Group stratification by WC or body composition (BF%) across 26 young, adult males resulted in no meaningful between-group differences at rest regardless of adjustments for cardiorespiratory fitness.

Furthermore, ANS responsiveness to maximal exercised revealed mixed results following varying adjustments for cardiorespiratory fitness. Although no significant between-group differences were found with PEP, adjusting for VO_{2peak} (mL·kg body mass^{-1}·min^{-1}) revealed significant differences in ln-RMSSD, ln-SDNN, and ln-HF between the High and Low BF% groups at 3- and 5- minutes post maximal exercise, with the High group displaying the higher amount of heart rate variability (HRV). However, statistical significance was no longer present when adjusted for Lean VO_{2peak} (mL·kg lean body mass^{-1}·min^{-1}). These differing results shed light on the impact of cardiorespiratory fitness on the relation between adiposity and ANS activity given the propensity of for VO_{2peak} (mL·kg body mass^{-1}·min^{-1}) to be biased against obese individuals while Lean VO_{2peak} (mL·kg lean body mass^{-1}·min^{-1}) does not. Therefore, a plausible interpretation of these data is that adiposity does not have a substantial independent impact ANS activity at rest or during the first 10 minutes following maximal exercise and that accounting for cardiorespiratory fitness may have limited influence on ANS responsiveness post-maximal exercise.

There were notable differences between the two experiments, including the sample demographics, methods used in the ANS analyses. The cohorts differed greatly in terms of age with the larger cohort's participants having a mean age of 74.6±4.8 years compared to the smaller cohort being 25±5 years. Multiple studies have shown a strong association between age and ANS dynamics (Antelmi et al., 2004; Geovanini et al., 2020; Voss et al., 2015); most often increased sympathetic output, but decreased in sympathetic receptor responsiveness (Hogikyan & Supiano, 1994). Indeed, the larger cohort study resulted in WC having opposing direct and indirect relations with PEP (denoting sympathetic activity), with limited impact on RMSSD (parasympathetic activity). Yet, the total effect of WC on PEP was not significant, which is in

agreeance with the lack of a significant difference between groups regardless of group strata and adjustment for cardiorespiratory fitness used in the smaller cohort study. Additionally, the larger cohort included both male and female participants while the smaller cohort was limited to males. However, the age of the larger cohort could mitigate the influence of sex on ANS activity as differences in HRV metrics between the sexes appear to be diminished after approximately 60 years of age (Voss et al., 2015). Finally, both studies excluded those with known history or clinical diagnosis of any cardiometabolic complications. Despite this, the data suggest that individuals with worsening glycemic management capabilities and/or cardiorespiratory parameters may have influenced the results in the larger cohort study. Roughly 9% had DBP values between 80 and 90 mmHg and the sample means for fasting glucose suggest that a large section of the sample was in the prediabetes range. Both factors could have meaningful impact on the observed relations between adiposity and PEP, as both have been shown to be related to sympathetic activity (Erdogan et al., 2011; Williams et al., 2019). Similarly, the smaller cohort study only found significant between-group differences when BF% strata resulted in group comparisons suggestive of apparently healthy vs pre-diseased individuals. As those with multiple risk factors for CVD have altered ANS activity compared to those with singular or no risk factors (Verma et al., 2018), the inclusion of these individuals may have altered the independent effects of adiposity on ANS activity.

Both studies in this dissertation utilized HRV and PEP to evaluate ANS activity, though they differed in methodology. The ARIC study only determined time-domain metrics of HRV derived from a single 2-minute ECG rhythm strip, while the smaller cohort study calculated time-domain as well as frequency domain metrics over several epochs throughout 10-minute sampling periods. Additionally, PEP determination also varied with the smaller cohort study

utilizing impedance cardiography whereas the larger cohort derived PEP from brachial pulse wave velocity data. While these two methods differ in location and the physiological signal used to determine cardiac cycle timing, specifically the opening of the aortic valve, both have been shown to be reliable methods in the determination of systolic time intervals (Lorne et al., 2014; Su et al., 2013). Furthermore, the post-maximal exercise time epochs for PEP determination may have hindered the appearance of significant group differences, as discussed in Chapter 4.

Multiple indices can be used to describe the amount of adipose tissue an individual possesses. WC provides some indication of visceral fat, which is more metabolically deleterious than subcutaneous fat (Ibrahim, 2010; Ross et al., 2020), although it does not provide separate quantification of visceral versus subcutaneous abdominal fat. However, this metric only evaluates truncal fat content and may not accurately portray whole body adiposity. The choice of WC as our single marker of adiposity in the large population study could have influenced the observed effects and limited our ability to assess the potential impact of overall adiposity in the conceptual model. The smaller cohort study utilized both WC and BF%, as determined by air displacement plethysmography, in attempts to compare results to the larger cohort study while adding a more holistic marker of adiposity. The application of diverse metrics of adiposity such as height-adjusted body mass index, computed tomography scan, or dual energy x-ray absorptiometry, the gold standard of body composition, could further contribute to the current understanding of the relation between adiposity and ANS activity.

Collectively, the combined results from the above two studies shed light on the multifaceted influence of adiposity on ANS activity. Adiposity displayed nuanced associations with a marker of sympathetic nervous system activity, while having little influence on parasympathetic nervous system activity at rest in the larger cohort study. These findings were

in agreement with the lack of significant differences in ANS activity across adiposity groups at rest in this smaller cohort study. There is limited evidence that the influence of adiposity on ANS responsiveness following maximal exercise may be modified by adjustments for cardiorespiratory fitness as represented by VO_{2peak} (mL·kg body mass^{-1}·min^{-1}). However, there is contestation concerning the best practices on quantifying fitness across body composition strata. Although the data from these two studies highlight the nuanced involvement of adiposity on physiological parameters that influence ANS activity, they do not support the concept that adiposity has a strong, independent influence on ANS activity. Future research is needed to further unravel the complex entanglement between adiposity and autonomic health.

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APPENDIX A: UNADJUSTED WAIST CIRCUMFERENCE ANOVA TABLE

Variable	Source	SS	df	F	p-value
HR	Time	47486.693	2.474	355.744	<.001
	Group	2024.36	2	2.312	0.122
	Group X Time	944.397	4.949	3.537	0.008
	Within Error	3070.168	56.909		
	Total	53525.618	66.332		
PEP	Time	0.033	2.891	69.91	<.001
	Group	0	2	0.112	0.894
	Group X Time	0.001	5.783	1.104	0.369
	Within Error	0.011	66.499		
	Total	0.045	77.173		
ln-RMSSD	Time	100.427	3.457	19.115	<0.001
	Group	1.731	2	0.212	0.81
	Group X Time	3.996	6.914	0.38	0.91
	Within Error	120.836	79.509		
	Total	226.99	91.88		
ln-SDNN	Time	76.592	3.115	19.433	<0.001
	Group	2.121	2	0.354	0.706
	Group X Time	4.064	6.231	0.516	0.801
	Within Error	90.65	71.651		
	Total	173.427	82.997		
ln-HF	Time	498.913	4	38.352	<.001
	Group	17.558	2	0.532	0.594
	Group X Time	8.833	8	0.339	0.948
	Within Error	299.206	92		
	Total	824.51	106		
ln-LF	Time	303.463	4	38.79	<0.001
	Group	19.937	2	1.255	0.304
	Group X Time	16.262	8	1.039	0.413
	Within Error	179.933	92		
	Total	519.595	106		

APPENDIX B: WAIST CIRCUMFERENCE ADJUSTED FOR $VO2_{PEAK}$ ANCOVA TABLE

Variable	Source	SS	df	F	p-value
HR	Time	1480.297	2.548	12.136	<.001
	Group	2614.23	2	3.14	0.065
	Group X Time	1044.162	5.095	3.451	0.008
	Within Error	2860.351	53.574		
	Total	7999.04	63.217		
PEP	Time	508.026	2.975	1.184	0.323
	Group	811.424	2	0.476	0.628
	Group X Time	1367.837	5.95	1.376	0.238
	Within Error	8991.422	61.772		
	Total	11678.709	72.697		
ln-RMSSD	Time	14.063	3.314	2.762	0.043
	Group	3.898	2	0.48	0.625
	Group X Time	6.664	6.628	0.654	0.701
	Within Error	112.031	72.911		
	Total	136.656	84.853		
ln-SDNN	Time	8.647	3.061	2.196	0.095
	Group	3.794	2	0.63	0.542
	Group X Time	5.265	6.123	0.669	0.678
	Within Error	86.622	67.351		
	Total	104.328	78.535		
ln-HF	Time	62.842	4	5.198	<.001
	Group	27.199	2	0.817	0.455
	Group X Time	14.877	8	0.615	0.763
	Within Error	265.964	88		
	Total	370.882	102		
ln-LF	Time	22.554	4	2.85	0.028
	Group	25.945	2	1.622	0.22
	Group X Time	15.678	8	0.99	0.449
	Within Error	174.13	88		
	Total	238.307	102		

APPENDIX C: UNADJUSTED PRECENTAGE BODY FAT ANOVA TABLE

Variable	Source	SS	df	F	p-value
HR	Time	33332.483	2.327	211.522	<0.001
	Group	463.029	2	0.458	0.638
	Group X Time	390.14	4.654	1.238	0.305
	Within Error	3624.425	53.526		
	Total	37810.077	62.507		
PEP	Time	21785.37	2.67	46.631	<.001
	Group	2699.958	2	1.735	0.199
	Group X Time	1330.989	5.341	1.424	0.225
	Within Error	10745.272	61.418		
	Total	36561.589	71.429		
ln-RMSSD	Time	69.006	3.323	13.125	<.001
	Group	11.533	2	1.58	0.228
	Group X Time	4.735	6.645	0.453	0.857
	Within Error	120.097	76.42		
	Total	205.371	88.388		
ln-SDNN	Time	53.799	2.987	13.439	<.001
	Group	9.091	2	1.687	0.207
	Group X Time	2.644	5.975	0.33	0.918
	Within Error	92.071	68.709		
	Total	157.605	79.671		
ln-HF	Time	364.656	4	27.519	<.001
	Group	51.256	2	1.706	0.204
	Group X Time	3.263	8	0.123	0.998
	Within Error	304.776	92		
	Total	723.951	106		
ln-LF	Time	235.06	4	28.1	<.001
	Group	17.998	2	1.121	0.343
	Group X Time	3.799	8	0.227	0.985
	Within Error	192.395	92		
	Total	449.252	106		

APPENDIX D: PRECENTAGE BODY FAT ADJUSTED FOR $VO2_{PEAK}$ ANCOVA TABLE

Variable	Source	SS	df	F	p-value
HR	Time	1532.384	2.421	9.528	<.001
	Group	251.515	2	0.241	0.788
	Group X Time	421.742	4.842	1.311	0.274
	Within Error	3538.435	53.259		
	Total	5744.076	62.522		
PEP	Time	322.014	2.755	0.679	0.556
	Group	1111.12	2	0.692	0.511
	Group X Time	804.917	5.51	0.849	0.53
	Within Error	10431.741	60.615		
	Total	12669.792	70.88		
ln-RMSSD	Time	21.448	2.801	4.553	0.007
	Group	26.324	2	4.333	0.026
	Group X Time	15.054	5.602	1.598	0.167
	Within Error	103.641	61.623		
	Total	166.467	72.026		
ln-SDNN	Time	12.601	2.671	3.313	0.031
	Group	19.559	2	4.258	0.027
	Group X Time	8.198	5.341	1.078	0.384
	Within Error	83.69	58.753		
	Total	124.048	68.765		
ln-HF	Time	72.986	4	6.141	<.001
	Group	103.51	2	3.928	0.035
	Group X Time	19.386	8	0.816	0.591
	Within Error	261.456	88		
	Total	457.338	102		
ln-LF	Time	22.245	4	2.656	0.038
	Group	32.693	2	2.125	0.143
	Group X Time	5.569	8	0.332	0.951
	Within Error	184.24	88		
	Total	244.747	102		

APPENDIX E: PERCENTAGE BODY FAT ADJUSTED FOR LEAN $VO2_{PEAK}$ ANCOVA

TABLE

Variable	Source	SS	df	F	p-value
HR	Time	1294.313	2.424	8.193	<.001
	Group	322.681	2	0.307	0.739
	Group X Time	447.601	4.848	1.417	0.234
	Within Error	3475.452	53.33		
	Total	5540.047	62.602		
PEP	Time	241.286	2.763	0.508	0.663
	Group	2348.096	2	1.466	0.252
	Group X Time	1071.393	5.526	1.128	0.356
	Within Error	10449.681	60.789		
	Total	14110.456	71.078		
ln-RMSSD	Time	19.96	2.8	4.246	0.01
	Group	19.025	2	3.109	0.065
	Group X Time	8.32	5.6	0.885	0.427
	Within Error	103.422	61.595		
	Total	150.727	71.995		
ln-SDNN	Time	11.693	2.642	3.091	0.04
	Group	13.506	2	2.92	0.075
	Group X Time	5.296	5.296	0.7	0.633
	Within Error	83.234	58.134		
	Total	113.729	68.072		
ln-HF	Time	64.01	4	5.408	<.001
	Group	71.507	2	2.67	0.092
	Group X Time	5.663	8	0.239	0.982
	Within Error	260.375	88		
	Total	401.555	102		
ln-LF	Time	20.422	4	2.468	0.051
	Group	20.484	2	1.33	0.285
	Group X Time	5.64	8	0.341	0.948
	Within Error	182.048	88		
	Total	228.594	102		