# Associations of Sedentary Time with Heart Rate Variability

by

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Heart rate variability (HRV) is the gold standard non-invasive measure of cardiac-parasympathetic activity that is modulated by respiration. Lower resting HRV or cardiac-parasympathetic activity was proposed as a major linking mechanism between sedentary time (ST) and cardiovascular diseases (CVD). Several studies have examined associations of ST with HRV and reported favorable, unfavorable, or no associations. As such, it was challenging to draw a conclusion about the relationship of ST with HRV and cardiac-parasympathetic regulation. Thus, this dissertation sought to advance scientific knowledge by systematically reviewing and adding new investigations of ST with HRV.

First, we undertook a systematic review and meta-analysis (manuscript 1) which summarized the available observational literature and identified current research limitations and gaps. We found an unfavorable, but not clinically meaningful, association of ST with heart rate in males only; no correlations were observed with other HRV indices. Then, we conducted two original analyses (manuscripts 2 and 3) in a cohort of women and addressed many, but not all, of the observed limitations and gaps. We hypothesized that higher ST would be associated with unfavorable HRV. Our new analyses of self-reported activity and ST (manuscript 2) revealed that higher leisure moderate-to-vigorous intensity physical activity (MVPA) was associated with favorable HRV in women, while leisure ST was associated with unfavorable HRV only among inactive women. Occupational MVPA and ST were not associated with HRV. Our second original

analysis (manuscript 3) found that statistically replacing accelerometer-measured ST with long-bouts of MVPA resulted in favorable effects on HRV in women. Further, statistically replacing short- with long-bouts of MVPA was associated with more favorable HRV among women without preexisting conditions that may affect HRV.

Overall, the current literature as well as our new analyses found largely null or small associations between ST and HRV. Hence, lower resting HRV or cardiac-parasympathetic dysregulation does not appear to be a major linking mechanism between ST and CVD. To strengthen this conclusion, future research should address major limitations of the available literature including exclusive use of cross-sectional designs, rare implementation of gold standard approaches to evaluate ST and HRV, and lack of cardiac-sympathetic activity measurement.

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## Preface

*In the Name of Allah, the Most Gracious, the Most Merciful*

I dedicate this dissertation to:

My caring parents, Bandar and Roqayia,

My lovely and loyal wife, SAFFANAH,

My handsome son, Albaraa.

My sincere thanks go to the best mentor ever and my role model, Dr. BETHANY BARONE GIBBS, for her constant support and being a wonderful advisor during my entire PhD journey.

I would like to extend my thanks to my committee members for all their time, support, and effort they have put into this dissertation.

I also would like to thank all my family members and friends for their support.

Special thanks go to King Saud University for awarding me the full scholarship to pursue my graduate degrees in the United States.

# 1.0 Introduction

Reduced heart rate variability (HRV), i.e. reduced variation in the time intervals between consecutive heartbeats, is a manifestation of cardio-parasympathetic dysfunction and/or dysregulation. Reduced HRV has been found to be associated with many diseases, including cardiovascular disease (CVD). Reduced HRV can powerfully predict CVD incidence and mortality [1-3]. Physical inactivity and, more recently, sedentary behavior are modifiable risk factors that could impact HRV, where engaging in physical activity (PA) appears to increase HRV [4] and accumulating sedentary time (ST) may decrease HRV [5, 6].

The first chapter of this dissertation describes the anatomy and physiology of the autonomic nervous system (ANS), discusses the role of autonomic function/regulation in cardiovascular health, summarizes the relationship between autonomic dysfunction/dysregulation and CVD, and explains the most commonly utilized approaches to measure cardiac-autonomic dysfunction/dysfunction. Following that, the mechanisms, current measurement recommendations, best practices and limitations of HRV measurement are discussed. Also, definitions, types or domains, current guidelines, and measurement approaches for PA and sedentary behavior are described. Finally, the chapter concludes by summarizing what is known about the influence of the PA and sedentary behavior on HRV and identification of research gaps and future directions on this topic.

## 1.1 Autonomic Nervous System

### 1.1.1 Anatomy and Physiology of the Autonomic Nervous System

The ANS is a major, complex system that directly controls critical systems such as the cardiovascular and respiratory systems. Though it is a part of the peripheral nervous system, the ANS is under the control of the central nervous system and specifically the medulla oblongata in the brainstem, cerebral cortex, hypothalamus, and limbic system. The ANS is generally subdivided into: 1) the sympathetic nervous system (SNS), which is known for the 'fight or flight' response, and 2) the parasympathetic nervous system (PNS), which is known for the 'rest-or-digest' response [7]. Both the SNS and PNS dually innervate vital internal organs such as the heart and lungs (Figure 1-1) and usually exert opposite effects. Importantly, the SNS and PNS can be activated reciprocally, independently, and nonreciprocally [8].

The major function of the ANS is to maintain and regulate the body's internal physiology within narrow variations, a phenomenon was named 'homeostasis' (i.e., an ongoing maintenance) and 'allostasis' (i.e., adaptive changes to homeostasis) [9]. Specifically, the ANS regulates and maintains cardiovascular and respiratory functions, sweat rate, and insulin secretion. The ANS can also boost glycogenolysis, gluconeogenesis, and lipolysis, all of which are sources of energy production [7]. Working together, the SNS and PNS play a crucial role in ensuring well-balanced internal functions and appropriate responses to daily life situations and activities. For example, when transitioning between different bodily postures, the ANS is responsible for adjusting heart rate and blood pressure in order to maintain proper blood circulation. Another instance is when the

core body temperature is increased. In response, the ANS plays an important role in heat regulation and elimination by activating sweat glands and increasing skin perfusion via SNS activation. A final example is that both SNS and PNS interact to adjust the diameter of the pupil as ambient light changes. Thus, the ANS exerts profound regulatory effects on many internal physiological systems, including the cardiovascular system. This proper autonomic function and maintenance of homeostasis and allostasis is crucial for survival.

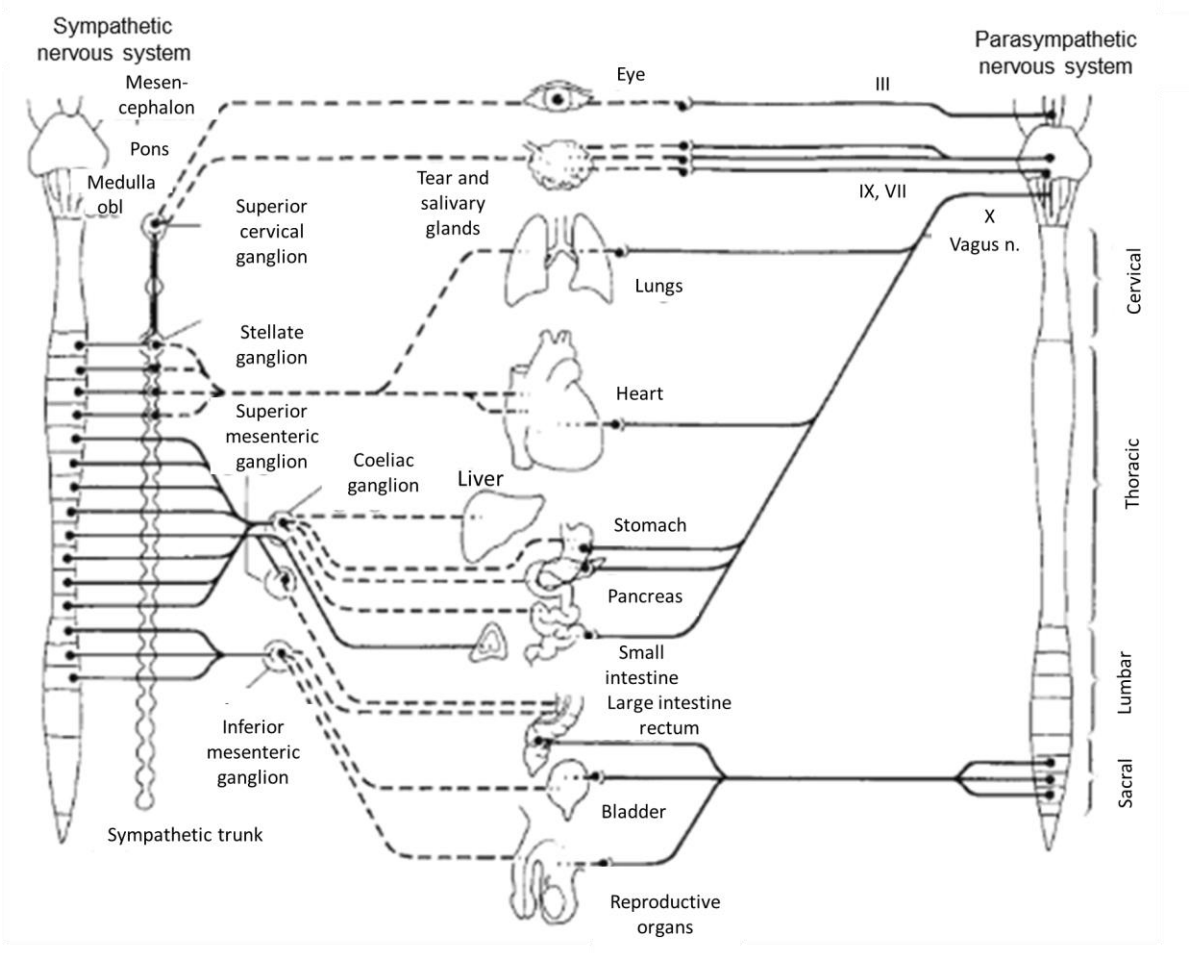



**Figure 1-1 Autonomic Innervation to Different Bodily Organs**
Figure from Mathias & Bannister [10]

### 1.1.2 Autonomic Function and Cardiovascular Health

The ANS plays a substantial role in cardiovascular health. Both the SNS and PNS innervate the heart and work together to regulate cardiovascular responses to internal and external stimuli. Specifically, the PNS innervates the sinoatrial (SA) node to adjust cardiac chronotropy (rate), the atrioventricular (AV) node to regulate cardiac dromotropy (conduction speed), and to a lesser extent the ventricles to reduce cardiac inotropy (contractility) [11]. On the other hand, the SNS innervates the SA node to increase cardiac chronotropy and the atria and ventricles to increase cardiac inotropy. Despite these complex effects, when resting, the primary role of the PNS is to influence chronotropy whereas the primary role of the SNS is to control inotropy [11]. Furthermore, the vasculature is primarily innervated by the SNS, which maintains sufficient blood pressure to provide adequate blood flow [11]. These physiological roles of the SNS and PNS allow them to interact and maintain healthy circulation and cardiovascular function.

The complex interaction of the SNS and PNS on cardiovascular function can be illustrated with the example of these systems' responses to a postural change from sitting to standing. Upon standing, gravitational force would result in blood pooling in the lower extremities, leading to decreased venous return, cardiac output, and blood pressure. Without compensatory regulation, this could then cause a series of undesirable cardiovascular effects such as reduced coronary and cerebral perfusion and vasovagal syncope [12]. To prevent such events, a healthy ANS enacts a cascade of responses. The PNS reduces its outflow to the heart, leading to immediate heart rate and cardiac output increases. At the same time, the SNS increases its outflow to the heart and vasculature, leading to increased cardiac contractility and vascular resistance and, eventually, increased venous return, stroke volume, cardiac output, and blood pressure. [12, 13]. Such interactions between the SNS and PNS occur constantly within the heart and blood vessels to

maintain cardiovascular homeostasis and allostasis by responding to various daily activities. However, as it is described in the next section, improper interaction between the SNS and PNS in the heart and/or vessels can lead to autonomic dysregulation/dysfunction, compromised cardiovascular health, and eventually to the development of CVD.

### 1.1.3 Autonomic Dysfunction and Cardiovascular Disease

Autonomic dysfunction, characterized by improper sympathetic and/or parasympathetic outflow to different bodily systems, can lead to various CVD outcomes. For instance, the failure of the SNS to increase its outflow upon standing can result in orthostatic hypotension, which is a fall in systolic blood pressure of $\geq$ 20 mmHg or diastolic blood pressure of $\geq$ 10 mmHg upon standing. This SNS dysfunction can have deleterious effects such as stroke due to reduced cerebral perfusion and heart failure due to reduced coronary perfusion [12, 13]. On the other hand, autonomic dysfunction that is characterized by sympathetic overactivation can affect ion regulation in pacemaker cells, especially calcium ions, leading to spontaneous depolarization and the initiation and persistence of atrial fibrillation and arrhythmia [14]. Additionally, sustained increases in sympathetic outflow to the blood vessels and ventricles has been proposed as a critical mechanism for the development of hypertension, and especially the development of essential hypertension [15]. Taken together, autonomic dysfunction that is characterized by impaired sympathetic control (i.e., failure to increase or overactivation) on the heart and/or vasculature can lead to serious CVD sequalae and even death.

Autonomic dysfunction can also occur due to impaired parasympathetic outflow to the heart. This type of the autonomic dysfunction is also associated with the development of common forms of CVD. For instance, depressed cardiac-parasympathetic activity, assessed by HRV, has

been associated with stroke [16], hypertension [2], heart failure [17] myocardial infarction [18], and coronary artery disease [19] in adults. Furthermore, impaired cardiac-parasympathetic activity predicts future CVD incidence and mortality, establishing temporality and strengthening causal inference. For example, an early study identified that post-myocardial infraction patients with reduced cardiac-parasympathetic activity, also evaluated by the measurement of HRV, had a 5.3-fold increase in the risk of mortality [1]. More recently, a meta-analysis of cohort studies among individuals with CVD (n = 3,094) revealed that reduced cardiac-parasympathetic activity, also assessed by HRV, was associated with an increased risk of all-cause mortality (HR = 2.12, 95% confidence interval [CI] = 1.64, 2.75) and cardiovascular events (HR = 1.46, 95% CI = 1.19, 1.77) [18]. Thus, like impaired SNS, impaired cardiac-parasympathetic activity also has documented pathological effects on the cardiovascular system that can lead to serious CVD and mortality.

### 1.1.4 Measurement of Autonomic Function

Autonomic dysfunction is typically a subclinical disease for many physiological systems including the cardiovascular system, and early detection can improve outcomes. As such, early identification of autonomic dysfunction is desirable to prevent its progression and consequences. Several invasive and non-invasive approaches to measure autonomic function have been developed over the past few decades. One of the earliest approaches was microneurography, which involves inserting a needle/insulated tungsten microelectrode directly into a peripheral nerve to record neural firings [20]. Microneurography is considered the gold standard invasive technique to examine the sympathetic impairment. Only recently and with help of ultrasound, microneurography has been adapted to additionally and directly evaluate parasympathetic impairment [21]. While promising in that a single method may be able to detect both sympathetic

and parasympathetic function, this method remains preliminary since its reliability and safety are yet to be demonstrated. Another commonly used technique to evaluate autonomic dysfunction is the measurement of plasma catecholamines levels, specifically norepinephrine and epinephrine [22]. These two catecholamines are released by the SNS into circulation. Thus, by measuring their levels, the evaluation of the sympathetic activity is possible through simple blood sampling. However, because the chemical substances released by the PNS do not reach circulation, it is not currently possible to assess parasympathetic impairment from the blood plasma. Therefore, currently available techniques can assess autonomic dysfunction that is characterized by sympathetic impairment; however, these approaches are not established methods for evaluating parasympathetic impairment.

Alternative, non-invasive approaches are able to measure parasympathetic impairment. One method is baroreflex sensitivity, which evaluates cardiac-parasympathetic activity by measuring heart rate alteration in response to blood pressure changes [22]. Yet, this technique is challenging because it usually requires direct manipulations of baroreceptors and involves more than one cardiovascular measurement (i.e., heart rate and blood pressure). The technical complexity can increase the risk of measurement errors and participant burden, limiting the usefulness of this measurement method. A second method to assess cardiac-parasympathetic activity is with different types of maneuvers such as a Valsalva or an orthostatic maneuver. For instance, when transitioning from supine to standing (i.e., an orthostatic maneuver) as previously mentioned, blood pools in the lower extremities causing reduced venous return and arterial blood pressure. Baroreceptors detect such changes and decrease afferent signals to brainstem, causing an immediate increase in heart rate that is mainly due to vagal withdraw. Changes in heart rate especially in the first 30 seconds after an orthostatic maneuver can be used as an assessment of

cardiac-parasympathetic activity [23]. Yet, similar to the measurement of baroreflex sensitivity, these maneuvers also require some manipulation to bring about heart rate alteration and are technically difficult. Further, both baroreflex sensitivity and maneuvers are only able to test parasympathetic reactivity and are unable to measure resting parasympathetic function. Lastly, the measurement of HRV (i.e., the variation in time intervals between consecutive heartbeats; Figure 1-2) is a non-invasive method that estimates cardiac-parasympathetic activity [24]. This variation in time intervals is the product of complex interaction between neural, hormonal, and mechanical factors (as explained below in the mechanism of HRV), and can be used to particularly estimate cardiac-parasympathetic activity. As such, HRV has an advantage over the other non-invasive techniques in that it can measure both reactivity and resting cardiac-parasympathetic activity and requires less complex measurement procedures. HRV is the most commonly used technique in research settings for non-invasive measurement of autonomic dysfunction. Thus, HRV is the primary measurement methodology to be used for the assessment of cardiac-autonomic dysfunction in this dissertation and is discussed in greater detail in the following section.

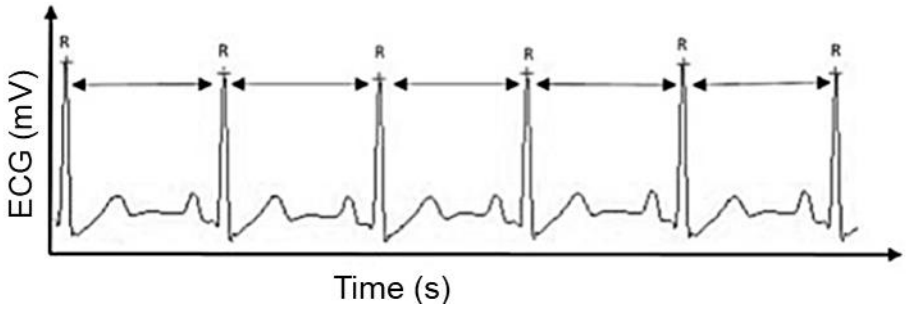



**Figure 1-2 HRV Calculation from Time Interval between Consecutive Heartbeats**
Figure from Laborde et al. [25]

Overall, several invasive and noninvasive methods are available to measure subclinical autonomic dysfunction. Though current established techniques can only evaluate either

sympathetic or parasympathetic impairment, HRV is perhaps the most commonly used as it is noninvasive and less technically demanding.

### 1.1.5 Summary

The ANS is a component of the peripheral nervous system that is responsible for 'homeostasis' and 'allostasis'. Two major divisions of the ANS, the sympathetic and parasympathetic, interact to regulate internal physiology, including cardiovascular physiology. Because the ANS innervates the heart and vessels and directly control their functions, autonomic dysfunction of the SNS and/or PNS can disrupt healthy cardiovascular functions and lead to CVD incidence and mortality. Fortunately, subclinical autonomic dysfunction can be detected before it progresses to overt disease. Various measurement methodologies are available for assessing autonomic dysfunction. Among these, HRV is most commonly used in clinical research because of advantages over the other approaches, including that it is noninvasive, can evaluate resting cardiac-parasympathetic activity and reactivity of cardiac-parasympathetic activity, and is technically simpler than other methods.

## 1.2 Heart Rate Variability

In line with the 'homeostatic' and allostatic' paradigms, a healthy heart rate is not monotonously regular [26]. In other words, the healthy heart has noticeable time variations between consecutive heartbeats (i.e., HRV). For several decades, blunted or reduced HRV has been used as an indication of impaired autonomic balance, regulation, and/or flexibility, and suggests vulnerability to CVD and even death [26]. The first clinical appreciation of HRV is credited to Hon and Lee in the 1960s, who demonstrated changes in the time interval between heartbeats in a fetus with reduced oxygen levels [27]. In the following decades, HRV was found to predict autonomic dysfunction in patients with diabetes, even prior to the onset of symptoms [28]. Further research identified that reduced HRV could accurately predict mortality [1]. Thereafter, the measurement of HRV has evolved and become a common, reliable method to evaluate cardio-autonomic dysfunction and predict CVD incidence and mortality [26].

Further, many high quality, epidemiological studies have reported that reduced HRV is associated with modifiable CVD risk factors. For instance, a cross-sectional analysis within the Framingham Cohort Study found that hypertensive individuals had lower (worse) HRV compared to normotensive individuals. The natural log transformed standard deviation of normal R-R intervals [lnSDNN] was 4.42 vs. 4.52 ($p < .0001$) for men and 4.36 vs. 4.46 ($p < .0001$) for women, respectively [2]. Similar findings have been reported among individual with diabetes, who have lower HRV compared to individuals without diabetes (i.e., standard deviation of normal R-R intervals [SDNN] = 70.2 vs. 79.4, $p = .003$) [29]. Among individuals without preexisting CVD, higher low density lipoprotein (LDL) cholesterol is related to lower HRV [30]. These studies are examples among many of the established inverse relationship between HRV and most CVD risk

factors. As such, this evidence reinforces the importance of HRV measurement to detect early CVD before its progression to overt disease.

In addition to the robust relationship between HRV and CVD risk factors, several large prospective studies have demonstrated deleterious associations between lower HRV and incidence of CVD morbidity and mortality. A meta-analysis of 28 cohort studies among individuals with CVD (n = 3,094) demonstrated that patients with lower HRV have 12% and 46% higher risk of all-cause mortality and cardiovascular events, respectively, compared to individuals with higher HRV [18]. Moreover, in another prospective study that enrolled myocardial infraction patients (n = 675), lower HRV was able to predict acute coronary events (hazard ratio [HR] =2.0, 95% CI = 1.2, 3.2) [31]. A final example is that, in patients with stable coronary artery disease (n = 588), HRV was found to have a significant, negative correlation with Gensini scores (a tool of assessing atherosclerosis based on coronary lesion severity where higher values indicate progressive atherosclerosis) [32].

Altogether, cardiac-autonomic dysregulation, measured by reduced HRV, has a robust association with CVD outcomes. As such, HRV may be a useful tool to detect and monitor progression of early CVD. In addition, because there are several cardiovascular factors that contribute to HRV, the measurement of HRV may be valuable to understand the mechanisms by which various CVD outcomes develop. In the following section, cardiovascular, neurological, and non-neurological contributing factors to HRV are discussed.

### 1.2.1 Mechanisms of Heart Rate Variability

Several mechanisms have been proposed to explain why a healthy heart rate is variable and to elucidate the link between HRV and CVD. The most well understood include direct cardio-

neurological (parasympathetic and sympathetic) interaction at the SA node, respiratory sinus arrhythmia (RSA), and baroreceptor responses to blood pressure fluctuations. First, the activation of PNS and SNS is a continuous dynamic process. Once the cardiac-parasympathetic (vagal) nerve at the SA node is activated, the post-ganglionic neuron releases the acetylcholine neurotransmitter, which binds to cholinergic (muscarinic) receptors. This binding causes a fast potassium ion efflux leading to hyperpolarization of the membrane potential and, thus, increased time intervals. On the other hand, when the cardiac-sympathetic nerve at the SA node is activated, the post-ganglionic neuron releases the norepinephrine neurotransmitter, which binds to adrenergic (β) receptors. This binding initiates a slower cascade of reactions (a second-messenger activation) that leads to slow ionic current changing, depolarizes the membrane potential, and, thus, attenuates time intervals [8, 33]. Accordingly, the PNS and SNS interact to regulate cardiac function at rest and the variation in processing rates creates variation in timing between consecutive heartbeats [26]. Thus, reduced HRV may be a manifestation of arrhythmic cardiac disease due to impaired cardio-neurological function at the SA node.

RSA is a second, well-understood mechanism that contributes to HRV. RSA is defined as the rhythmical changes in time interval between heartbeats during a breathing cycle (inspiration and expiration) [8]. When breathing in, heart rate increases due, in part, to vagal suppression. When breathing out, heart rate decreases due to vagal reactivation. Such fluctuations produce some variability in time intervals between heartbeats in individuals with healthy cardiac-autonomic regulation. In fact, several HRV indices are specifically utilized to index this cardiac-vagal fluctuation (explained below). Lastly, when blood pressure increases above the homeostatic level, baroreceptors detect this change and transduce the signal to cardiovascular centers in the brainstem via vagal afferent neurons. As a result, the cardiovascular centers react to regulate blood pressure

by increasing parasympathetic outflow to the heart with concurrent reduction in sympathetic outflow to the heart and vessels [26]. Together, both RSA and baroreflex create fluctuation in heart rate and significantly contribute to HRV. Hence, compromised respiratory, baroreceptor, and perhaps vascular functions are each likely to be reflected on HRV.

These established mechanisms, cardio-neurological interaction, RSA, and baroreflex reactivity, contribute to short-term HRV (i.e., over 5 minutes); however, several less established sources including chemoreceptors in response to arterial oxygen or carbon dioxide changes, circadian rhythm, core body temperature, metabolism, and cardiac intrinsic rhythm may contribute to long-term HRV (i.e., over 24 hours or longer) [26]. Because a number of short- and long-term factors influence HRV, caution should be taken in the measurement and interpretation of HRV in light of this complexity. To address this complexity, detailed recommendations are established to standardize the measurement and interpretation of HRV and to facilitate comparisons across studies. In the following section, best practices and current HRV measurement recommendations are described.

### 1.2.2 Heart Rate Variability Assessment Recommendations and Best Practices

Though a few techniques to measure HRV exist, electrocardiogram (ECG) is considered the gold standard method for HRV measurement [24, 34]. Via electrodes placed on the surface of the body, ECG measures voltage changes in the membrane of the cardiac cells that correspond to depolarization and repolarization [35]. The collected ECG signals can then be processed to derive multiple HRV domains. Among them, time and frequency domains are the most frequently used and are described in detail below.

For the time domain, simple indices such as mean heart rate and heart period (i.e., inter-beat intervals) are calculated. Using advanced statistical approaches, more time domain indices can be estimated, including SDNN, root mean square of successive differences (RMSSD), the number of interval differences of normal R-R greater than 50 milliseconds (NN50), and the proportion of NN50 divided by the total number of normal R-R intervals (pNN50). RMSSD, NN50, and pNN50 are correlated to each other. Nonetheless, RMSSD appears to have better statistical properties and is more stable; thus, RMSSD is the most preferable time domain index [34]. To provide the most accurate interpretation of time domain HRV, it is recommended to utilize SDNN to represent the overall variability and RMSSD to represent cardiac-vagal fluctuation that is modulated by respiration [24, 34].

The total variability of consecutive heartbeats can also be distributed over different frequency bands, yielding various frequency domain indices. The frequency domain describes how often the cardiac rhythm changes within a specific band of frequency. The frequency bands for HRV in adults include the high frequency (HF; 0.15 – 0.4 Hz), low frequency (LF; 0.04 – 0.15 Hz), very low frequency (VLF; 0.003 – 0.04 Hz), and ultra-low frequency (ULF; $\leq$ 0.003 Hz; for long-term measurement only). The ratio of LF and HF (LF/HF) is also commonly calculated. Among these frequency domain indices, HF power appears to be the only index that has a well-understood physiological representation. Specifically, when resting and breathing within a fixed range of rate and depth, validation and experimental studies found that HF accurately measures cardiac-vagal fluctuation that is modulated by respiration [24, 25, 34].

Though LF has been used to represent resting cardiac-sympathetic activity, recent evidence indicates that LF is not a measure of resting cardiac-sympathetic activity [36, 37] for the following reasons. First, studies have shown that LF fails to correlate to the “gold standard” measure of

cardiac-sympathetic activity (i.e., cardiac norepinephrine spillover) at rest. Additionally, cardiac-sympathetic blockade by segmental spinal anesthesia has no impact on LF. Lastly, the administration of a sympathetic agonist which increases both heart rate and norepinephrine level appears to have no influence on LF [36]. Thus, though LF (and therefore HRV) was formerly thought to measure both resting cardia-sympathetic and cardiac-parasympathetic function, current understanding is that HRV is limited to the measurement of total variability and cardiac-parasympathetic activity. Therefore, wherever HRV is mentioned in this dissertation, it refers to total variability and parasympathetic-related indices.

An importance methodological consideration that can affect the interpretation of HRV is the duration of HRV measurement. Although these time and frequency domain indices can be calculated from either short-term (commonly 2 – 5 minutes) or long-term (24 hours) measurement, they cannot be directly compared or used interchangeably [24, 34]. This mainly results from different contributing factors to HRV that influence short-term HRV vs. long-term HRV [38]. As such, the comparison of HRV across studies should be limited to studies that have similar measurement durations. Because of this, it is important to report the duration of HRV measurement (short- or long-term) and to not compare HRV indices across studies if estimated from different durations. To help reduce this discrepancy, the recommended measurement duration of HRV was standardized to 5 minutes for short-term and 24 hours for long-term HRV measurement [34].

There are several ECG-related guidelines that are also recommended for obtaining high quality HRV measurements. To begin, both heart rate and heart period (i.e., time intervals between heartbeats) can be calculated from the ECG signal and then used to calculate HRV indices. However, utilization of the heart period is favored as it is thought to provide more precise HRV estimates. This reflects that most HRV indices have linear correlations with heart period but not

with heart rate [24]. Furthermore, to obtain accurate ECG signals (and thus HRV), it is highly recommended that the ECG sampling rate be between 250 – 1000 Hz [24, 34]. Lower sampling rates can lead to signal deviation from actual points, causing error in R wave timing and, therefore, inaccurate estimation of HRV. Further, because ectopic beats, arrhythmia, signal noise, and missing values can significantly affect the power estimates of HRV, proper interpolation should be performed according to the guidelines [24]. Moreover, the utilization of a distribution-based artifact-detection algorithm concurrently with visual inspection is recommended to detect existing artifact, if any, which can considerably influence HRV [24]. Fortunately, many of these processing recommendations and algorithms are incorporated into advanced computer software such as Kubios HRV Premium, which enables precise ECG signal processing, cleaning, and power estimating [39]. Consistent with these best practices, Kubios HRV Premium was used in this dissertation to assess high quality HRV measurements derived from ECG signals.

Alongside the crucial ECG considerations listed above, there are several additional factors that can introduce error into the measurement of HRV and should ideally be controlled or considered during high quality measurement. First, both respiration rate and depth can dramatically affect HRV indices, especially HF, and can lead to misinterpretation of results [8]. Accordingly, it is highly recommended that both respiration rate and depth are to be monitored and controlled during HRV measurement and statistical analysis [24, 25]. HRV indices are generally considered valid and reliable measures of autonomic function, and in particular total variability and parasympathetic-related indices [40-43]. However, several studies suggest that the reliability of HRV could be compromised [44-46], especially if the respiration rate and depth are not considered [46]. This means that the respiration rate and depth are not only affecting HF power, but also the reliability of other HRV indices. Given that most commercial, non-ECG devices that

measures HRV (e.g., heart rate monitors) are not capable of measuring the respiration parameters [47, 48], poor reliability of HRV indices may result with these measurement methodologies and lead to misinterpretation of the findings.

Other personal and lifestyle factors are important measurement considerations for HRV. A participants' characteristics such as age, sex, and adiposity as well as cardioactive and contraceptive drugs are known to influence HRV [25]. Thus, these factors should be considered, and especially in observational designs that are susceptible to confounding. Lifestyle behaviors such as smoking, dietary intake, alcohol consumption, and physical exercise have also been found to impact HRV; therefore, it is recommended that participants abstain from smoking and eating at least 2 hours prior to HRV measurements, and alcohol consumption and physical exercise should be avoided 24 hours prior to HRV measurement. Thus, participant characteristics and lifestyle behaviors, including pre-visit instructions, are a final consideration in the measurement and statistical analysis of HRV.

### 1.2.3 Summary

HRV, the variation in time intervals between consecutive heartbeats, is a measure of cardiac-vagal fluctuation, has robust associations with CVD risk factors, and can powerfully predict CVD incidence and mortality. HRV reflects complex cardiovascular, neurological, and non-neurological mechanisms and can be susceptible to measurement error if influencing factors are not carefully controlled. As such, the measurement and interpretation of HRV should be carefully performed according to the available guidelines, including the use of ECG and respiration devices to accurately calculate HRV indices, proper interpretation of HRV indices, consideration of differences across populations, and standardization of pre-test lifestyle behaviors. Though

following standardized measurement recommendations can provide valid, reliable estimates, limitations in the interpretation of HRV as a measure of cardiac autonomic function remain. The standardized duration of HRV measurement (5 minutes for short-term HRV measurement and 24 hours for long-term HRV measurement) cannot to be interchangeably interpreted or compared. Further, HRV measures total variability and cardiac-parasympathetic activity but not cardiac sympathetic activity. Thus, HRV indices are useful but must be interpreted with these caveats.

## 1.3 Physical Activity and Sedentary Behavior

### 1.3.1 Physical Activity: Definition and Domains

PA, defined as any bodily movement that requires the use of skeletal muscles and results in increased energy expenditure (i.e., > 1.5 Metabolic Equivalents [MET]) [49], is a lifestyle behavior that associates with enormous health benefits. PA is accumulated across various intensities including light (LPA) [ 1.6 – 2.9 MET], moderate (MPA) [ 3 – 5.9 MET], and vigorous (VPA) [ > 6 MET] intensity PA. There are consistent data that demonstrate beneficial health gains with the accumulation of PA. Importantly, studies have reported dose-response effects where higher intensity PA is associated with greater benefits on aerobic capacity, body composition, diastolic blood pressure (DBP), and diabetes [50, 51]. Further, coherent evidence displays that regular aerobic PA at an intensity of 3 METs or higher (i.e., moderate-to-vigorous intensity physical activity [MVPA]) can reduce the risk of hypertension (HTN) [52], heart failure [53], ischemic heart disease [54], coronary artery disease [55], and breast and colon cancers [56]. In addition, regular lifetime PA, especially MVPA, is associated with a lower mortality rate [57, 58]. Thus, engaging in regular PA is an effective strategy to maintain health and reduce the risk of developing morbidity and mortality.

The regular accumulation of PA is achieved by incorporating PA into daily living activities. These daily living activities are generally performed within the following four domains: occupation, transportation, household, and leisure [57]. Occupational PA is performed while working; transportation physical activity is performed when moving from one place to another; household PA is performed while completing tasks in or around the home; and leisure PA is performed at one's will when not working, transporting, or performing household PA [57]. Though

most research has studied the health benefits of leisure PA, current literature indicates that total PA (across all domains) is associated with favorable effects on health [57, 59, 60].

Yet, some recent evidence suggests a harmful impact of specifically occupational PA on mortality risk and cardiovascular health [61]. This phenomenon, where occupational activity seems to have an opposite effect on health outcomes as compared to leisure activity, has been named the "PA Health Paradox" [61]. In a 2018 meta-analysis that pooled data from 17 studies, men with high occupational activity had an 18% (95% CI = 5%, 34%) increase in mortality risk [62]. Yet in the same meta-analysis, occupational activity was not associated with mortality among women. One specific example from a prospective analysis in the Copenhagen City Heart Study found that higher occupational PA was associated with a 31% and 56% increase in the risk of all-cause mortality in males with low or moderate levels of leisure PA, respectively [63]. These data suggest that considering domain-specific PA may be important to understand the best strategies to maintain health and prevent diseases.

The current PA Guidelines for Americans, which will be discussed in detail later, are mainly based on scientific evidence evaluating the health effects of leisure MVPA data [57]. Yet, the Guidelines recommend and allow for adults to accumulate adequate levels of PA across all domains, including during occupational time. The lack of occupational-specific PA guidelines likely reflects that the evidence associating occupational health with health outcomes is preliminary and with major limitations. Hence, future research investigating the role of domain-specific PA on a variety of health outcomes is needed to develop proper PA guidelines. This dissertation contributes to that research gap by considering domain-specific PA as it is related to HRV.

### 1.3.2 Sedentary Behavior: Definitions and Domains

Sedentary behavior, defined as "any waking behavior characterized by an energy expenditure of ≤1.5 METs while in a sitting, lying, or reclining posture" [64], has recently been associated with an increased risk of cardio-metabolic biomarkers [65], DM and HTN [66], CVD [67], and mortality [68]. Importantly, the term 'sedentary' has been historically used to describe physical inactivity (i.e., not meeting MVPA recommendations), potentially because of the difficulty in measuring LPA and that both share a portion of the same energy expenditure spectrum. However, the definition above and contemporary paradigm differentiate between inactivity and sedentary behavior because the relationships between ST and physical inactivity with various health outcomes and mortality are independent from one another. Specifically, more ST in exchange for less participation in standing and other LPA is independently associated with adverse health outcomes. For example, a meta-analysis of six cohort studies revealed that the relative risk (RR = 1.04; 95% CI = 1.03 to 1.04) of CVD mortality significantly increased for each additional hour of ST above 6 hours/day (Figure 1-3), independent of PA level [69]. Further, higher ST has been associated with higher risk of CVD, DM, and cancer incidence, also independent of PA level [70, 71]. Though sedentary behavior is a distinct risk factor, strong evidence indicates that the deleterious impacts of ST are more pronounced among individuals with low activity levels[72]. Therefore, accumulating high ST during the day is now considered a risk factor for adverse health outcomes that is separate from the failure to achieve adequate MVPA levels (i.e., inactivity).

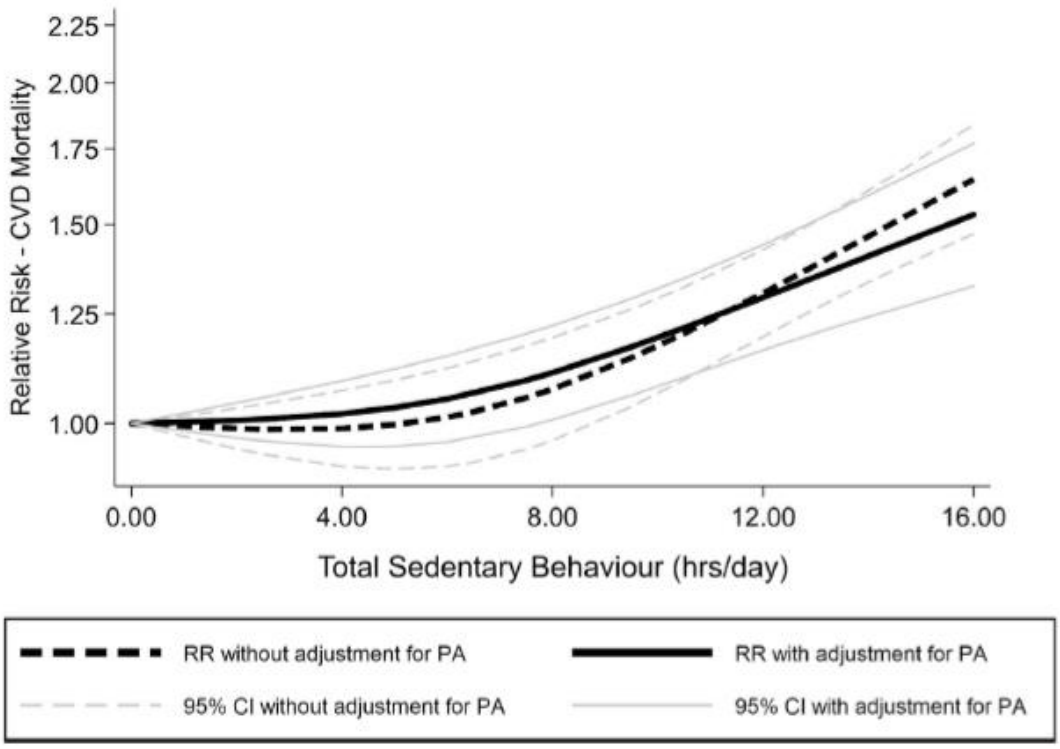



**Figure 1-3 Association between ST and CVD Mortality with and without Adjustment of PA.**

Figure from Patterson et al. [71]

Similar to PA, ST is also accumulated in various domains including occupational, transportation, household, and leisure [64]. Occupational ST takes place while at work; transportation ST occurs while transporting from one place to another; household ST occurs when completing tasks in or around home; and leisure ST occurs at one's will when not working, transporting, or completing household tasks. Consideration of domain-specific ST is emerging as important for building effective interventions across a socioecological framework. In addition, growing evidence suggests that the health effects of accumulating prolonged ST in different domains may be distinct.

Though many sedentary behavior studies have detected adverse associations between total ST and various diseases and mortality [73-75], much of this data also suggests that domain-specific

ST may be more important to consider for disease prevention and mortality reduction. For instance, a cross-sectional isotemporal substitution analysis from the Coronary Artery Risk Development in Young Adults (CARDIA) Study found that the statistically exchanging 2 hours of TV viewing for equivalent amounts of other sedentary behaviors (i.e., sitting in a car, using the computer, talking on the phone, completing paperwork, and reading) was associated with lower cardiometabolic risk scores (.06 - .09 standard deviation) [76]. Another example is that a cross-sectional study found that accumulating ≥ 2 hours/day of TV viewing, but not total ST, was associated with higher odds of DM [77]. Together, these findings suggest that considering domain-specific ST may be important to define more precise associations with health and guide interventions and recommendations.

### 1.3.3 Physical Activity and Sedentary Time: Current Guidelines

Research into the effects of physical activity on health outcomes and mortality rate has exponentially expanded over the past several decades. As a result, robust evidence on the health benefits gained by performing PA has accumulated to the level at which quantitative PA guidelines are justified. In the United States, the PA Guidelines for Americans were first released in 2008 and then updated in 2018 (Figure 1-4) [57]. These recommend that adults should accumulate at least 150 – 300 minutes/week of aerobic MPA, 75 – 150 minutes/week of aerobic VPA, or an equivalent amount of aerobic MVPA accumulated in any bout length to maintain overall and cardiovascular health. Additional health benefits are also achieved by exceeding these recommendations. In addition, the U.S. guidelines recommend performing muscle-strengthening PA at least twice per week [59, 78]. These quantitative PA guidelines were also recommended for individuals in the United Kingdom, Canada, and Australia. Of particular interest, these quantitative PA

recommendations can be accumulated during the day in forms of occupational, transporting, household, and leisure PA [59, 79, 80].

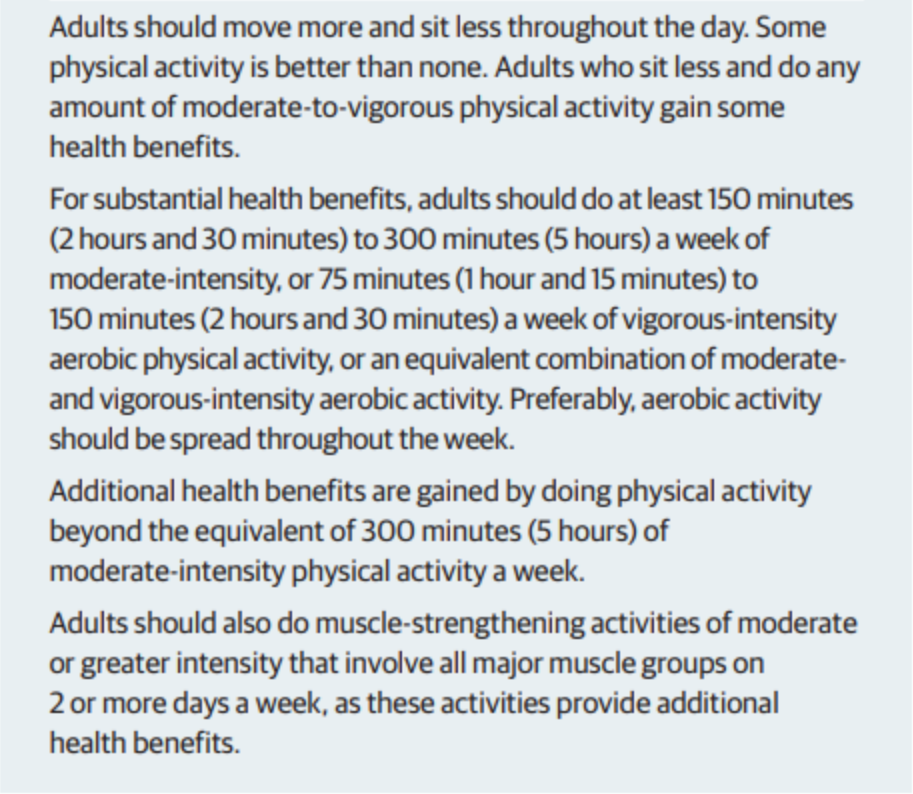



**Figure 1-4 PA Recommendations for Adults.**
Figure from Piercy et al. [81]

The guidelines also reflect that acute and chronic health gains that are achieved by performing any amount of PA. For example, a single bout of physical activity results in immediate health benefits including improved blood pressure [82, 83], insulin sensitivity [84, 85], and sleep [86, 87]. If performed regularly, coherent evidence demonstrates that PA chronically improves blood pressure [88, 89], blood sugar and lipids levels [90, 91], sleep [92, 93], and reduces body weight [94, 95], CVD incidence and mortality [96-98], and all-cause mortality [99, 100]. These benefits and others can be achieved by meeting the PA guidelines [57, 101].

Importantly, the majority of Americans do not meet these PA guidelines [57]. This is a public health problem because not meeting the PA guidelines is a major modifiable risk factor for

noncommunicable disease and mortality [102]. One international study of attributable risks estimated that 9% of worldwide mortality would be avoided if all individuals achieved recommended levels of PA [102]. Further, reducing daily PA can have immediate deleterious effects in active individuals, such as significantly attenuating flow-mediated dilation [103] and glycemic control [104]. These physiological responses to reduced daily PA shed light on how physical inactivity may lead to many diseases and death. Taken together, long-term adherence to PA recommendations can improve and maintain health. Further, developing strategies to increase participation in PA among low active individuals is an important public health goal for improving cardiovascular health and life expectancy.

In contrast to PA, sedentary behavior research is less robust and associations between sedentary behavior and health have only been studied extensively over the past decade. Consequently, sedentary behavior recommendations are more sparse and less consistent across guidelines. For example, the 2018 PA Guidelines for Americans emphasize the importance of reducing ST by "sitting less and moving more" across the day (see Figure 1-4) [57]. In contrast to MPVA guidelines, these recommendations are vague because they do not provide a specific quantitative behavioral target. Only recently, quantitative sedentary behavior guidelines were released as part of comprehensive, 24-hour guidelines by the Canadian Society of Exercise Physiology (Figure 1-5) [59]. In these, adults are recommended to limit their ST to no more than 8 hours/day of total ST and with no more than 3 hours/day of recreational screen time to gain health benefits [105]. Of particular note, these guidelines consider some aspects of domain (both total and leisure ST), but do not clarify the role of occupational or travel ST. A final recommendation released in 2015 specifically addresses occupational ST among desk workers. This statement was written by an international group of experts and recommends that desk workers

aim to break up occupational ST frequently, should accumulate no more than 4 – 6 hours/day of occupational ST, and should perform 2 – 4 hours/day of occupational standing and LPA [106]. Hence, accumulating evidence indicates that sedentary behavior may be harmful, and, in turn, guidelines are developing to inform general and even some quantitative targets for sedentary behavior reduction to improve health.

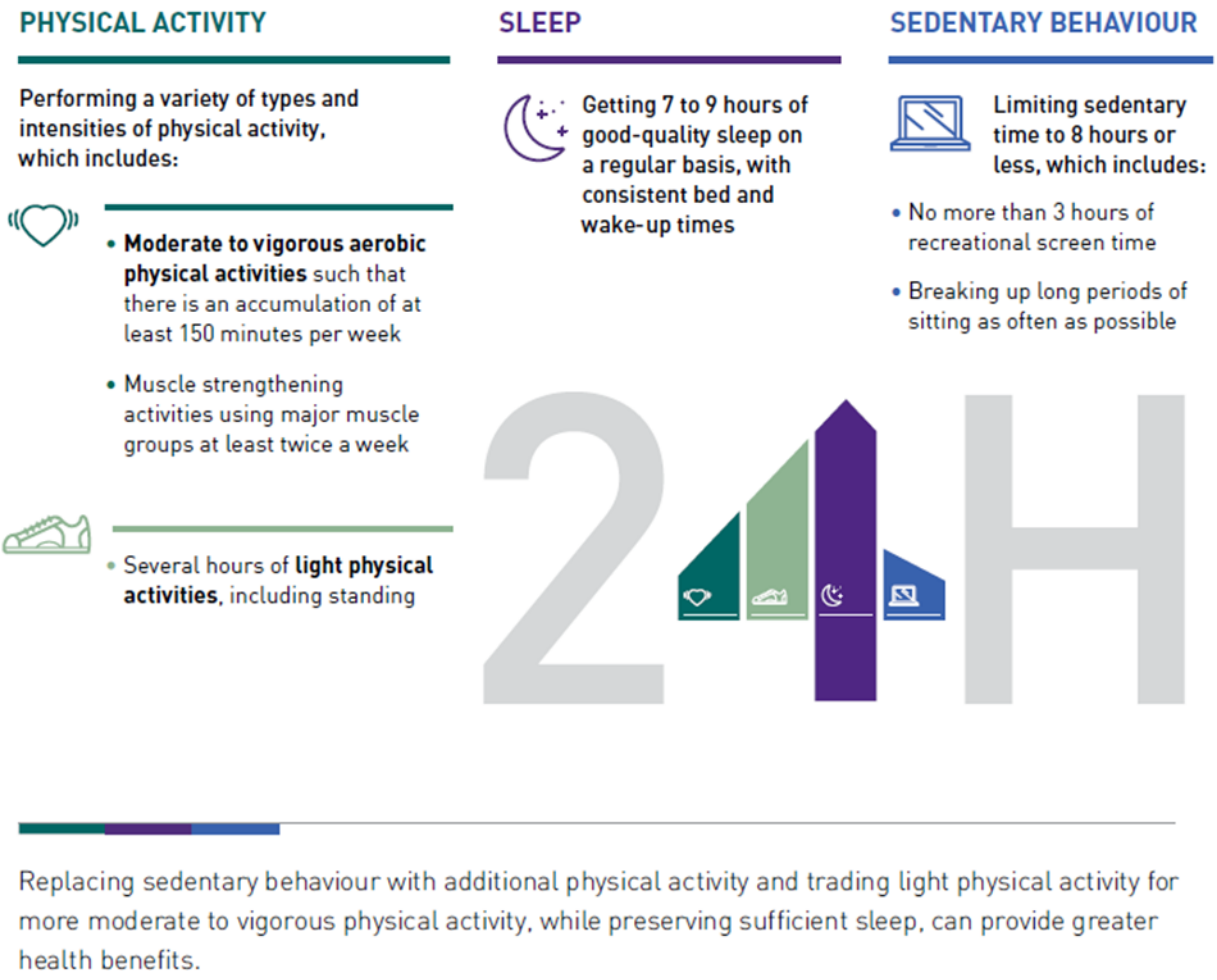



**Figure 1-5 Canadian 24-Hours Quantitative Behavior Guidelines.**
Figure from Ross et al. [59]

Most of the available evidence suggests negative health consequences of high or increasing daily ST. For instance, studies that measure the physiological effects of acute prolonged sitting, a

common sedentary behavior especially in industrialized communities, have reported several adverse responses including impaired glycemic control [107, 108], reduced cognitive functions [109, 110], increased blood pressure and vascular stiffness [111, 112], and reduced flow-mediated dilation [113, 114]. These immediate adverse health responses to acute ST are thought to evolve into diseases with long-term, frequent accumulation of ST. This assumption is supported by a number of observational studies that report associations between higher daily ST and an increased risk of DM [68, 70, 115], various CVD outcomes [67, 68, 70], and mortality [68, 70]. However, limitations to the current research including few longitudinal studies, poor measurement of sedentary behavior, limited experimental studies, and poorly clarified mechanisms make the strength of the evidence that sedentary behavior is associated with health outcomes less robust [116]. Therefore, more high-quality studies addressing these limitations are required prior to drawing a robust conclusion about the long-term effects of sedentary behavior on health. Nonetheless, the promotion of healthy lifestyle clearly includes increasing daily PA and preliminarily suggests that reducing daily ST are each important for health maintenance and disease prevention.

### 1.3.4 Physical Activity and Sedentary Time: Measurement

The accuracy of PA and ST measurement has evolved and improved over the past several decades. When it began, the major approach to estimate physical activity for research purposes was through the measurement of occupation. This approach was limited since it estimated overall activity levels based on occupational PA and perhaps ST according to an individual's job-related physical demands [57]. Of note, this method did not include estimation of leisure PA and ST and could not be used to measure activity levels in non-working individuals. Such limitations promoted

the establishment of better tools that can capture PA and ST across domains, regardless of occupational status.

An early advancement in PA measurement was the self-reported questionnaire that first evaluated leisure PA, and then later expanded to assess multiple domains of PA and sedentary behavior [117, 118]. Various questionnaires such as International PA Questionnaire (IPAQ) and Modifiable Activity Questionnaire (MAQ) are now commonly used to assess habitual levels of PA and sedentary behavior across domains [57, 119, 120]. Of particular interest, the MAQ, which was used in the research study that provided data for this dissertation, was developed initially to measure activity patterns among Native American adults and yielded reliable test-retest correlations (*r* ranged from 0.62 to 0.96 for occupational and leisure activity) [121]. Since then, the MAQ has been utilized in PA research worldwide [122-125] and is known for its ability to capture specific details of activity in each domain. For example, leisure ST is estimated by summing daily hours spent sitting, watching television, or using a computer for non-work-related tasks. This level of detail not only encompasses many potential activities in each domain to calculate a total, but also allows for investigation of types of activity within the activity domain. Such features may facilitate the establishment of total and domain-specific PA and sedentary behavior guidelines that are most strongly associated with health benefits.

Though self-report instruments (e.g., MAQ) provide unique data about domain-specific activities, estimation of habitual activity by self-report is known to have substantial measurement error including over- or underestimation as a result of recall or social desirability bias [126, 127]. Such errors are critical when evaluating the effects of interventions or when aiming to determine precise associations between activity levels and health outcomes [128, 129]. As a result, objective PA and ST methodologies such as pedometers and accelerometers have been developed. For

example, Actigraph accelerometers (i.e., GT3X, GT1M), also used in the third manuscript of this dissertation, are a reliable and valid device that senses bodily movement [130-133]. Using standardized algorithms that translate acceleration into 'counts', and then by applying validated cut points, time spent wearing the monitors can be partitioned into ST, LPA, and MVPA [130, 131, 134]. However, compared to self-report questionnaires, objective monitors do have disadvantages. These include that objective monitors increase the cost and complexity of measuring PA, increase participant burden, lack data on domain of activity, and can have technical or adherence issues that can affect the measurement [135, 136]. Thus, it is recommended to combine both subjective and objective activity measurements to accurately estimate PA and ST across various activity domains [132, 137].

### 1.3.5 Summary

PA and ST are distinct lifestyle factors that have opposite effects on many health outcomes. Overwhelming evidence suggests that increasing total and leisure PA improves cardiovascular health and, worldwide, current physical activity guidelines are largely in agreement that adults should achieve at least 150 minutes per week of MPA (or equivalent) and twice weekly strength training. However, preliminary evidence suggests occupational PA may result in opposing, harmful effects on these outcomes and current guidelines lack domain-specific PA recommendations. On the other hand, a smaller but growing research base suggests that greater total sedentary behavior is directly linked to many diseases such as CVD and mortality. Emerging data also suggests domain-specific ST, specifically leisure and television ST, may be more strongly associated with adverse health outcomes compared to total or occupational ST. Reflecting the preliminary state of science, sedentary behavior guidelines are less consistent but are beginning to

emerge. Future research should incorporate both subjective and objective measurement tools to accurately capture overall and domain-specific PA and ST and evaluate the relationship of these modifiable behaviors with health outcomes.

## 1.4 Physical Activity and Heart Rate Variability

### 1.4.1 Physical Activity and Heart Rate Variability: Observational Studies

Many robust observational studies have reported that higher PA, especially MVPA, is associated with higher (favorable) HRV in healthy or asymptomatic populations. For example, using a subjective measurement instrument (the Minnesota Leisure Activity Questionnaire), a cross-sectional analysis in the British Study of Civil Servants Study found that male workers (n = 2,334) in the highest PA quartile had higher SDNN (34.8 vs. 32.9 millisecond; p = .09) and HF (125.2 vs. 104.8 millisecond$^2$; p < .05) compared to those in the lowest PA quartile [138]. Of particular relevance to this dissertation which studied adult women, a cross-sectional study that utilized the MAQ found that physically active pregnant women had higher SDNN (53.9 vs. 38.7 millisecond in the first trimester; p = .01, 53.0 vs. 38.1 millisecond in the second trimester; p = .002, 55.8 vs. 44.6 millisecond in the third trimester; p = .03) and RMSSD (40.2 vs. 25.9 millisecond in the first trimester; p = .05, 32.7 vs. 22.9 millisecond in the second trimester; p = .07, 32.4 vs. 21.3 millisecond in the third trimester; p = .05) compared to physically inactive pregnant women [139]. These findings suggest that greater self-reported PA is associated with greater HRV in healthy male and female populations.

Comparable findings are also reported with objective PA measurement. For instance, a cross-sectional analysis of middle-aged adults (n = 1,668) in the CARDIA Study reported that isotemporal substitution of Actigraph-measured ST with VPA was associated with favorable SDNN (standardized $\beta = 0.06$; 95% CI = 0.03, 0.10) and RMSSD (standardized $\beta = 0.08$, 95% CI

= 0.05, 0.12) while substitution with LPA was associated only with more favorable RMSSD (standardized β = 0.05; 95% CI = 0.01, 0.08) [140]. Moreover, the Epidemiology and Human Movement (EPIMOV) Study examined the association between very VPA (≥ 8 MET) and HRV in healthy adults (n = 1,040) and found incremental positive effects of very VPA on SDNN and HF ($p < .001$ for both) [141]. These results suggest a favorable association between higher objectively-measured PA, especially VPA, with HRV benefits in healthy populations. Further, the preliminary data suggesting objectively-measured LPA is associated with higher HRV highlight the need for more research across the activity intensity spectrum.

Similarly, the positive associations between PA and HRV are also observed in CVD patients. For example, a cross-sectional study in patients with ischemic heart disease found that patients with high self-reported PA had higher SDNN and HF compared to moderate- or low-active patients ($p < .05$ for all) [142]. Moreover, significant associations between greater objectively-measured LPA and MPA with higher HRV were detected in stroke patients ($p < .05$ for all) [143]. Therefore, these studies support the hypothesis that PA has beneficial effects on HR in patients with CVD in additional to healthy adults.

Of particular interest and relevance to this dissertation, emerging evidence indicates that the association between leisure and occupational PA with HRV could be contrary. As mentioned above, this PA Health Paradox [144] hypothesizes that, while leisure PA is associated with better health outcomes (e.g., higher HRV), occupational PA is associated with more negative health outcomes (e.g., lower HRV). For instance, a cross-sectional study in Danish blue-collar workers (n = 514, 40% female) reported an interaction effect between the association of leisure vs. occupational PA, measured via Actigraph and a time-use diary, with nocturnal HRV. Specifically, leisure PA was associated with higher nocturnal HRV and occupational activity was associated

with lower nocturnal HRV for SDNN (p-for-interaction = .019), RMSSD (p-for-interaction = .004) and natural log transformed HF (p-for-interaction = .022) [144]. Similarly, another cross-sectional analysis from a Korean blue-collar cohort found that middle-aged and older men (n = 8; 45 ≥ years old) with higher occupational PA, also estimated from an accelerometer and self-reported diary, had lower SDNN, RMSSD, and HF ($p < .05$ for all) compared to older men with lower occupational PA (n = 16; 45 ≥ years old) [145]. These data support that the "PA Health Paradox" may manifest in cardiac autonomic function as measured by HRV; however, the limited data highlight the need for further investigation in future studies.

### 1.4.2 Physical Activity and Heart Rate Variability: Experimental Studies

Extensive experimental studies have demonstrated that different modalities of PA can improve HRV in various populations. These studies can be classified according to the modality of PA as follows: 1) resistance PA, 2) aerobic PA, and 3) mind-body PA. The effects of these three modalities of PA on HRV are discussed below.

Resistance PA has a demonstrated beneficial effect on HRV in diseased populations, but less so in healthy populations. A recent meta-analysis has synthesized the findings of 21 randomized controlled trials that evaluated the effect of different types (i.e., muscle- or bone-strengthening PA), intensities (i.e., ≥ 30% of one repetition maximum [1RM]), and frequency x durations (i.e., 30 – 50 minutes/week, 2 – 5 times/week for 6 weeks – 8 months) of resistance PA on HRV in healthy and diseased individuals [146]. Though there were no significant effects of resistance training on HRV in healthy individuals, resistance PA, and even with intensities as low as 30% 1RM, significantly improved RMSSD (standardized mean difference [SMD] = .95, 95% CI = .20, 1.73) and HF (SMD = .62, 95% CI = .03, 1.20) in patients with CVD, CVD-related, or

other diseases [146]. Similarly, a systematic review of randomized control trials and quasi-experimental studies qualitatively summarized the effects of inspiratory muscle training (IMT) with resistance (i.e., increasing maximal inspiratory pressure [MIP] which can enhance the strength of the respiratory muscles) on HRV in individuals with CVD, diabetic, and pulmonary disease [147]. It was concluded that IMT, even at low intensity (30% MIP), increased HF in patients with CVD and diabetes. In summary, bone- and muscle-strengthening PA and IMT appear to be an effective stimulus to bring about cardiac autonomic benefits, but only if the cardiac autonomic function is already compromised such as in populations with CVD and diabetes.

Many studies have reported that different intensities (e.g., MPA, VPA) of aerobic PA have a significant impact on HRV in healthy individuals. For example, a meta-analysis of 13 randomized control trials assessed the influence of aerobic PA (i.e., 40 – 60 minutes/session at 60 – 80 % of maximum intensity, for at least 4 weeks) on HF and R-R intervals in healthy adults [148]. Aerobic PA was found to significantly improve HF (effect size [*d*] = .48, 95% CI = .26, .70) and R-R intervals (*d* = .75, 95% CI = .51, .96). Other studies found positive impacts on other HRV indices. For example, a study compared the effects of MPA (i.e., walking and jogging at 62% of heart rate reserve, for 33 minutes x  3 times/week for 14 weeks) versus no exercise (control condition) on HRV in healthy adults [149]. The MPA intervention resulted in a 15% increase in SDNN ($p = .01$), while no changes were detected in the control group. Another higher intensity aerobic PA intervention (i.e., 80% of heart rate reserve, for 30 minutes x 3 days/week for 16 weeks) also increased SDNN, RMSSD, and pNN50 ($p < .01$ for all) in physically inactive healthy men [150]. These consistent findings suggest that aerobic PA may be an effective strategy to improve HRV in healthy populations.

A number of studies have reported comparable benefits of aerobic PA on HRV in clinical populations with CVD [151]. For instance, a study compared HRV responses to aerobic PA (i.e., 70% of heart rate max, 1.5 hours/session x 8 session/week for 8 weeks) in individuals with myocardial infraction, coronary artery bypass grafting, and angioplasty who underwent cardiac rehabilitation program versus a control group [152]. R-R intervals ($F = 12.41$; $p = .001$), SDNN ($F = 6.25$; $p = .015$), and natural log transformed HF ($F = 5.39$; $p = .024$) significantly increased in the intervention compared to the control group. Similarly, a randomized control trial found that aerobic PA (i.e., 60 – 85% of heart rate reserve, 40 minutes/session x 1 – 3 sessions/week, for 8 weeks) increased R-R intervals ($\Delta = 33.7$ vs. $– 42.6$; $p = .06$), total frequency power ($\Delta = .3$ vs. $-.4$; $p = .02$), and HF ($\Delta = .3$ vs. $.1$; $p = .06$) in patients after percutaneous transluminal coronary angioplasty. Together, the existing evidence suggests that aerobic PA can increase HRV in several CVD populations.

As an alternative to resistance and aerobic PA, several studies have evaluated mind-body PA (e.g., yoga or Tai Chi) as a strategy to improve HRV. The results of these studies were recently synthesized in a meta-analysis that included 12 randomized controlled trials [153]. Studies evaluating the effects of Tai Chi and yoga (i.e., 30 – 120 minutes x 2 – 7 times/week, for 6 – 16 weeks) on HRV in healthy and diseased (e.g., CVD, depression) populations were included. The pooled effect revealed that mind-body physical activity significantly improves HF (Hedge's $g = .37$, 95% CI = .22, .52). When subgroup analyses were performed, there was no significant difference in the effect on HF between healthy vs. diseased populations or when comparing Tai Chi vs. yoga interventions. These finding indicate that mind-body PA appears to be an effective for improving HRV in a variety of populations.

### 1.4.3 Physical Activity and Heart Rate Variability: Potential Mechanisms

Many studies have examined mechanisms by which PA improves HRV. The consensus is that PA improves HRV primarily through its effect to enhance cardiac vagal activity [151]. A few mechanisms have been proposed to explain pathways through which physical activity results in vagal activity enhancement, including increased nitric oxide (NO) bioavailability and suppressed angiotensin II [151]. NO and angiotensin II are potent cardiovascular regulators and usually exert opposite influences (e.g., NO dilates vessels and enhances vagal activity, while angiotensin II constricts vessels and inhibits vagal activity) [154]. Regular PA is known to increase NO bioavailability [155], which acts on the sympathetic center in the nuclei of brainstem and leads to decreased cardiac sympathetic outflow [156]. Concurrently, NO acts directly at the presynaptic channels of the vagus nerve, resulting in an increase in acetylcholine release and, thus, a decrease in heart rate [157]. It has been speculated that these NO-induced vagal changes are reflected on HRV. Furthermore, regular physical activity was found to decrease resting angiotensin II [158]. Because angiotensin II is known to inhibit vagal discharge [159], it has been hypothesized that reducing angiotensin II via PA would improve vagal activity and thus, HRV [151]. Yet, these mechanisms remain speculative and further research into these mechanisms and others is warranted to understand how PA improves HRV.

### 1.4.4 Summary

Overall, the current evidence indicates that MVPA is associated with better HRV across observational and experimental studies, various modalities of physical activity, and in healthy and clinical populations. From meta-analyses of experimental trials, resistance exercise appears to only

improve HRV in clinical populations (e.g., CVD, diabetes), yet aerobic and mind-body PA appear to have benefits across healthy and clinical populations. Mechanisms by which PA improves HRV have also been proposed and include increased NO bioavailability and suppressed angiotensin II; both are common physiological adaptations to PA that would boost cardiac vagal activity and lead to increased HRV. However, more studies examining these speculative pathways and other mechanisms are warranted to understand how PA improves HRV.

Important research questions remain regarding the effects of PA on HRV. First, preliminary observational data support HRV as a relevant outcome for the "PA Health Paradox," where leisure vs. occupational PA appear to have opposing beneficial/harmful effects on HRV. Thus, the effects of domain-specific PA on HRV are in need of further research. Of note, this research gap is addressed in the second manuscript of this dissertation. Further, the current literature has limited investigation of the influence of LPA on HRV. This dissertation also addresses this limitation in the final manuscript that investigated associations of objectively-measured PA across the activity spectrum (including LPA) with HRV. Lastly, though not addressed by this dissertation, direct comparison between the effects of different modalities of PA (i.e., resistance vs. aerobic vs. mind-body) on HRV is an area in need of future research to provide comprehensive exercise prescriptions for optimizing benefits.

## 1.5 ST and HRV

### 1.5.1 Sedentary Time and Heart Rate Variability: Observational Studies

In contrast to the available research investigating the effects of PA on HRV, research associating ST with HRV is relatively new and limited with no systematic review or meta-analysis currently available. When evaluating the handful of currently existing observational studies, associations between ST and HRV are inconsistent. For example, one cross-sectional analysis from the Northern Finland Birth Cohort found that greater total accelerometry-measured ST was not associated with HRV; however, bouted ST accumulated in bouts of at least 30 and 60 minutes was associated with higher (better) RMSSD [160]. Similarly, several studies with objectively-measured ST (e.g., Actigraph, Polar monitor) found no association between total ST and total power, SDNN, RMSSD, and HF ($p > .05$ for all) in healthy, obese, chronic fatigue syndrome, or hypotensive patients [160-164]. Findings from studies using self-reported ST also report highly variable results, including positive, null, and negative associations with HRV [165-167].

Together, these discrepant results across studies may be attributed to methodological differences and limitations, including small samples, differences in HRV assessment (i.e., devices and durations), or analytic shortcomings where analyses did not consider the interrelatedness of sedentary behavior and physical activity. Therefore, more systematic synthesis of the available literature as well as future studies are needed to address these limitations prior to drawing strong conclusions about the association between ST and HRV. The first manuscript of this dissertation

addresses the first research priority, by synthesizing the available studies, while the second and third manuscripts of this dissertation contribute new, higher quality investigations of ST and HRV.

Of relevance to this dissertation, two cross-sectional studies have examined the association between domain-specific ST (i.e., occupational and leisure) and HRV. In the first study, a cross-sectional analysis from a cohort of blue-collar workers revealed that higher occupational ST was associated with lower SDNN (β = - 5.07, 95% CI = - 8.48, - 1.67) and RMSSD (β = - 4.96, 95% CI = - 8.88, - 1.05) whereas higher leisure ST did not associate with any HRV indices [168]. However, the second cross-sectional study, also from a cohort of blue-collar workers, found no associations between domain-specific ST and HRV indices ($p > .05$ for all) [5]. Of note, both studies used high quality measures of nocturnal HRV, objective domain-specific ST, and concurrently adjusted for MVPA. Given the limited published studies, the inference of the association between domain-specific ST and HRV cannot be made, and further investigations are needed.

### 1.5.2 Sedentary Time and Heart Rate Variability: Experimental Studies

Similar to observational studies, limited experimental studies have assessed the impacts of ST on HRV and these report inconsistent results [6, 169-173]. To summarize these findings and identify gaps in the current literature, we have recently conducted a meta-analysis that has synthesized the acute effects of ST (i.e., prolonged sitting) on HRV [174]. Following the systematic search of the literature, only seven articles were identified and included in the analysis. Our pooled estimates suggested no statistically significant effects of acute prolonged sitting (i.e., ≤ 3 hours) on HRV. Though we intended to summarize the counteracting effects of frequent

interruptions of prolonged sitting on HRV, there were insufficient articles to perform such analyses. Moreover, most populations in these studies were apparently healthy young adults. Therefore, the acute effects of uninterrupted and interrupted prolonged sitting on HRV in individuals with preexisting CVD and those who are at higher CVD risk (e.g., older adults) remain unknown. Accordingly, more research is needed to draw stronger and more comprehensive conclusions regarding the acute effects of ST on HRV.

### 1.5.3 Sedentary Time and Heart Rate Variability: Potential Mechanisms

Though reduced cardiac-autonomic function is commonly hypothesized as a mechanism through which sedentary behavior increases cardiovascular risk, studies that investigate mechanisms by which sedentary behavior may influence HRV are scarce. Reduced peripheral blood flow in the lower extremities is hypothesized to explain reduced HRV in response to acute increases in sedentary behavior (e.g., sitting). To illustrate, when assuming a seated posture, blood pools in the lower limbs, leading to decreased venous return, cardiac output, and blood pressure [175-177]. These changes would then be detected by baroreceptors, which would send afferent signals to the cardiovascular centers in the brainstem [22] to reduce cardiac-parasympathetic activity (i.e., recued HRV) and increase cardiac sympathetic outflow to provide adequate blood pressure and flow. If the sitting lasts for a prolonged time, it is hypothesized that cardiac-parasympathetic activity will be depressed, and cardiac sympathetic activity will be overactivated. Repeated exposure to prolonged ST and the resulting interruption of blood flow is hypothesized to cause chronic HRV impairment. This hypothesis is supported by several studies that reported significant increases in blood pressure and vascular resistance (i.e., sympathetic overactivation) [172, 176] and decreases in RMSSD and HF [6, 169]. However, several other studies do not

support this hypothesis; they reported either positive or no effects of prolonged sitting on HRV [170, 171, 173]. Another speculative mechanism is that frequent exposure to sedentary behavior leads to reduced blood flow, shear stress, and, eventually, NO bioavailability. As a consequence, vagal discharge would decrease, resulting in a reduction in acetylcholine release [157] and, thus, reduced HRV. Yet, this mechanism remains speculative and has not been tested. Overall, further research into potential mechanisms is needed to understand how sedentary behavior may affect HRV.

### 1.5.4 Summary

The literature suggests a harmful association between high ST and cardiovascular health, and reduced cardiac-autonomic function has been hypothesized as a mechanism through which this association occurs. Yet, the current literature considering ST and HRV, the most commonly used noninvasive research measure of cardiac-autonomic function, is limited. Observational studies are all cross-sectional, often small, use highly variable methods, and reveal inconsistent findings. Experimental research has only examined the effects of short-term ST (i.e., $\leq$ 3 hours) on HRV and has typically studied healthy young adults. Lastly, limited studies have examined mechanisms by which sedentary behavior impacts HRV, with reduced cardiac vagal tone to compensate for interrupted blood flow as the leading (though still highly speculative) hypothesis.

These limited data inform remaining research questions and future directions. Recent observational evidence indicates that ST accumulated in prolonged bouts and domain-specific ST may have an important role in explaining some of the variation in findings. Future observational studies that consider these factors and address other methodological limitations of the existing literature (i.e., have larger samples, consider interrelationships with PA, use gold standard

methodology for measuring sedentary behavior and HRV) are needed. We addressed many of these limitations in the current dissertation, as described in the following chapter outlining the Specific Aims. However, additional observational and experimental research is needed. In particular, the effects of long-term experimental manipulation of ST on HRV have not been evaluated. Also, the effects of acute sitting on HRV in middle-aged and older adults as well as clinical populations are in need of research. Lastly, these future research studies should extend the investigations into mechanisms and explore hypothesized and other pathways by which ST may affect HRV.

## 2.0 Specific Aims.

Though autonomic dysfunction (low heart rate variability [HRV]) is proposed as a linking mechanism between sedentary time (ST) and cardiovascular disease (CVD), observational studies report inconsistent associations between ST and HRV. To clarify associations and inform future research, including randomized controlled trials, this dissertation addressed the following three aims:

### 2.1 Specific Aim for Manuscript 1:

Qualitatively summarize and quantitatively synthesize the available evidence on the association between ST and heart rate (HR) and HRV in adults. This manuscript helps to formulate data-based directions for future research investigations of the mechanisms by which sedentary behavior leads to CVD and mortality in adults.

### 2.2 Specific Aim for Manuscript 2:

Examine the independent and joint associations between self-reported total and domain-specific ST and physical activity (PA) with HRV in young to middle-aged women enrolled in the POUCHmoms Study. This manuscript provides insight into the associations between ST and PA with autonomic function in women and clarifies the role of domain in these associations.

## 2.3 Specific Aim for Manuscript 3:

Assess the effects of statistically replacing accelerometer-measured ST with PA on HRV in women in the POUCHmoms Study using the isotemporal substitution analysis. This study provides insight into specific sedentary behavior reduction recommendations to improve HRV and cardiovascular health, especially among adult women.

# 3.0 Manuscript 1: Is Sedentary Time Associated with Unfavorable Heart Rate and Variability in Adults? A Systematic Review and Meta-Analysis of Observational Studies (Specific Aim I)

## 3.1 Abstract

**BACKGROUND:** Sedentary time (ST) is associated with cardiovascular disease (CVD), but whether ST relates to heart rate (HR) and variability (HRV) is unclear. **PURPOSE:** To evaluate if ST is associated with unfavorable HR and HRV in adults. **METHODS:** We systematically searched PubMed and Google Scholar through June 2020. Inclusion criteria were: observational design, humans, adults, English language, ST as the exposure, resting HR/HRV as the outcome, and (meta-analysis only) availability of the quantitative association with variability. After qualitative synthesis, meta-analysis used inverse variance heterogeneity models to estimate pooled associations. **RESULTS:** Thirteen and eight articles met criteria for the systematic review and meta-analysis, respectively. All studies were cross-sectional and few used gold standard ST or HRV assessment methodology. The qualitative synthesis suggested no associations between ST and HR/HRV. The meta-analysis found a significant association between ST and HR ($\beta$=0.24 bpm per hour ST; CI: 0.10, 0.37) that was stronger in males ($\beta$=0.36 bpm per hour ST; CI: 0.19, 0.53). Pooled associations between ST and HRV indices were non-significant ($p>0.05$). Substantial heterogeneity was detected. **CONCLUSION:** The limited available evidence suggests an unfavorable but not clinically meaningful association between ST and HR, but no association with HRV. Future longitudinal studies assessing ST with thigh-based monitoring and HRV with electrocardiogram are needed.

## 3.2 Introduction

Cardiovascular disease (CVD) remains the leading cause of death in adults worldwide [178, 179]. Recent evidence indicates that sedentary behavior (i.e., any waking behavior that has an energy expenditure of ≤ 1.5 metabolic equivalents and occurs in lying, reclining, or seated position [64]) is associated with CVD incidence and mortality [67, 180]. Importantly, this association is distinct from the harmful impacts of physical inactivity, which is defined as not engaging in sufficient levels of moderate-to-vigorous intensity physical activity (MVPA) [57]. This association was graded as 'strong' by the 2018 Physical Activity Guidelines Advisory Committee [72]. Yet, mechanisms by which greater ST leads to elevated CVD risk remain unclear.

Higher resting heart rate (HR) [181, 182] and lower resting heart rate variability (HRV) (i.e., the variation in time intervals between consecutive heartbeats) [1, 183] are manifestations of cardiac-autonomic dysfunction. This dysfunction is associated with increased CVD risk and is purported to partially explain the relationship between sedentary time (ST) and CVD [116, 166, 168]. However, observational studies evaluating the association between ST and HR or HRV in adults have reported inconsistent results, including negative, positive, and null associations [5, 160, 162, 163, 165-168]. To address this uncertainty, we systematically reviewed the current evidence relating ST to HR and HRV to shed light on whether this proposed mechanism is supported by the available research and to clarify the potential role of cardiac-autonomic dysfunction in the association of ST with CVD and mortality. Thus, the primary aim of this study was to qualitatively summarize and quantitatively synthesize the available evidence from observational studies examining the association of ST with HR and HRV in adults. It was hypothesized that higher time spent in sedentary behavior would be associated with higher resting HR and lower resting overall variability, indicating cardiac-autonomic dysregulation in adults.

## 3.3 Materials and Methods

This systematic review and meta-analysis was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database (registration ID: CRD42020196516) and was performed according to Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [184].

### 3.3.1 Data Sources and Search Strategy

PubMed and Google Scholar were searched systematically by two independent researchers (AA and LB) using the following terms: ("sitting" OR "sedentarism" OR "sedentary" OR "television time" OR "screen time") AND ("HRV" OR "heart rate variability" OR "heart rate"). The reference lists of all identified trials and relevant reviews or editorials were also manually examined. The search was limited to include only published studies between database inception and June 3rd, 2020.

### 3.3.2 Study Selection

Two independent reviewers (AA and LB) comprehensively screened the titles, abstracts, and entire manuscripts, when needed, of all identified studies using the inclusion criteria listed below. Any discrepancies for inclusion between reviewers were settled by consensus or, when necessary, a third reviewer (BG or LS). Inclusion criteria for the systematic review were: 1) human participants ≥ 18 years old, 2) English language, 3) observational research designs, including cross-sectional, longitudinal, or case-control studies, 4) objective or self-reported measures of ST,

including total, bouts, and domain-specific ST as the exposure variable, and 5) at least one reported outcome of interest including resting HR and/or HRV indices (e.g., SDNN, RMSSD, LF, HF, and LF/HF ratio). The same inclusion criteria were used for the meta-analysis with the addition of a reported estimate of the association between ST and HR or HRV. For HR, we required the difference in outcome per unit increase in exposure (β) or a correlation coefficient (*r)* along with some measure of variability that would allow β calculation (e.g., standard deviation). For HRV, we required a correlation coefficient (*r*) or a regression coefficient (β) along with some measure of variability that would allow for *r* calculation.

### 3.3.3 Data Extraction and Quality Assessment

Two independent reviewers (AA and BG) extracted data from each eligible article, including name of first author and year of publication, country, characteristics of sample, method of assessment for ST, method of assessment of HR and/or HRV, and number and description of estimates. To calculate the pooled effect, associations between ST and HR and/or HRV indices were extracted or calculated using reported β, r, standard deviations, and sample sizes (manual calculations are described below). An eligible article could have reported more than one estimate across subgroups [160, 166, 167] or within subjects (i.e., separately for occupational and leisure-time sedentary behavior, or separately for weekend and weekday ST)[5, 167, 168]; in such cases, data for each estimate were extracted separately. If articles had a missing estimate and lacked sufficient data for manual calculation, corresponding authors were contacted. If authors failed to provide necessary data, their study was retained in the systematic review but excluded from the meta-analysis.

Two authors (AA and BG) adapted the NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies quality score [185] to measure 13 specific quality elements (e.g., objective of the study, risk of selection and measurement biases, evaluation of temporality, validity and reliability of measurement methodology) important for rigor when evaluating associations between ST and HR or HRV. Each element was assigned one point if the answer was yes and zero points if the answer was no. Total points of all elements were aggregated to calculate the final quality score for each article which could range from 0 to 13 points.

### 3.3.4 Data Synthesis

All studies meeting inclusion criteria were qualitatively (descriptive) and semi-quantitatively synthesized. Semi-quantitative synthesis counted the number of articles with direct, null, or inverse associations and arrived at an overall interpretation using previously published methods [186]: 1) "no association" if > 50% of the studies reported null findings, 2) "inconclusive" if exactly 50% of the studies reported no associations and 50% of the studies demonstrated significant in one direction (positive or negative) association, 3) "some evidence for association" if >50% of the studies demonstrated a positive (or negative) association, and 4) "consistent evidence for association" if all the studies (100%) showed significant association in a positive (or negative) direction.

For the quantitative synthesis (meta-analysis), we applied a correction by adjusting the sample size (adjusted n = original n/number of estimates) for any estimate from an article reporting more than one estimate either across subgroups or within-subjects. This allowed for use of all estimates in the pooled effect size calculation by correcting (reducing) weights of multiples estimates from the same study [187]. For associations between ST and HR, the pooled effect size

(β) was calculated as the change in heart rate (bpm) per unit increase of ST (hour/day) with 95% confidence interval. Estimates not reported per hour of ST were scaled appropriately. Studies reporting Pearson's *r* rather than β were converted using the following formula: β = r * (standard deviation of the outcome/standard deviation of exposure). Standard error (SE) was calculated as $\sqrt{[(1-r^2)/(n-2)]}$ or estimated from 95% confidence intervals if not specifically reported [188]. HRV indices were reported using various units and some studies reported more than one measurement unit simultaneously. Thus, we systematically extracted HRV indices according to the following priority order regarding measurement unit: 1) natural log of milliseconds/milliseconds squared, 2) milliseconds/milliseconds squared, 3) percentage, and 4) normalized units. Due to this heterogeneity in the measurement units, associations between ST and HRV were extracted as or converted to unitless correlation coefficients (r) and then combined in a pooled estimate [189]. If not reported, Pearson's r was manually calculated as follows: r = standardized β + 0.05λ, where λ = 1 if the standardized β is positive and λ = 0 if the standardized β is negative [190].

### 3.3.5 Data Analysis

The Stata Metan package (Stata Statistical Software; StataCorp, LLC, College Station, Texas, USA) and MetaXL software (https://www.epigear.com/index_files/metaxl.html) were utilized to perform the meta-analyses. If there were three or more estimates with the same outcome measure, meta-analysis was conducted. Because the included studies had substantial between-article variability in the sample characteristics, measurement methodology for ST, HR, and HRV assessment, the inverse variance heterogeneity (IVHET) model was selected to account for the potential heterogeneity [191]. The association between ST and HR was pooled and reported as β (bpm per hour of ST). Further, Cohen's *d* was calculated as the pooled β (bpm per hour ST)/median

standard deviation of baseline HR across studies (bpm). The magnitude of association was evaluated using Cohen's *d* as follows: *d* <0.2 is trivial; *d*=0.2 is small; *d*=0.5 is moderate; and *d*=0.8 is large [192]. On the other hand, the association between ST and HRV was pooled and reported as *r*, which was also used to evaluate the magnitude of the association as follows: r=0.2 is small; r=0.5 is medium; r=0.8 is large [193].

Subsequent to running the IVHET models, we examined the robustness of the pooled results and the potential for publication bias. Sensitivity analyses removed one study at a time to test the robustness of the pooled results. If the pooled estimate was altered in statistical significance or in magnitude of effect grading by removing any one study, the pooled estimate was reported with and without that study. Though we were unable to visually evaluate publication bias by the Begg's funnel plot test due to less than 10 included studies [194], we statistically evaluated publication bias by Egger's regression test [195]. Lastly, statistical heterogeneity was assessed by the $I^2$ statistic, where <25% indicates low risk of heterogeneity, 25–75% indicates moderate risk of heterogeneity, and >75% indicates considerable risk of heterogeneity [196]. If sufficient data were available (at least three unique studies across at least two sub-groups), the following prespecified sub-group analyses were conducted to explore potential sources of heterogeneity: sex (i.e., male, female, or combined), sedentary behavior assessment (i.e., subjective vs. objective), sedentary behavior domain (i.e., total, television only, leisure, occupational), timing of HR or HRV measurement (i.e., nocturnal vs. diurnal), using ECG to measure HRV (yes vs. no), and covariate adjustment for MVPA (yes vs. no).

## 3.4 Results

### 3.4.1 Literature Search and Trial Selection

Figure 3-1 displays the results of the systematic literature search. Using the predetermined terms and filters, 2,283 articles were initially found through database searching. Two additional articles were manually identified. Following comprehensive examination of titles, abstracts, and full text when needed, 2,272 articles were excluded due to not meeting one or more inclusion criteria. Thirteen articles met the inclusion criteria for the systematic review. Though only eight articles met criteria for inclusion in the meta-analysis, these articles yielded a total of 19 estimates. The remaining five articles did not report estimates of associations and the corresponding authors did not provide the necessary quantitative data to calculate pooled effects.

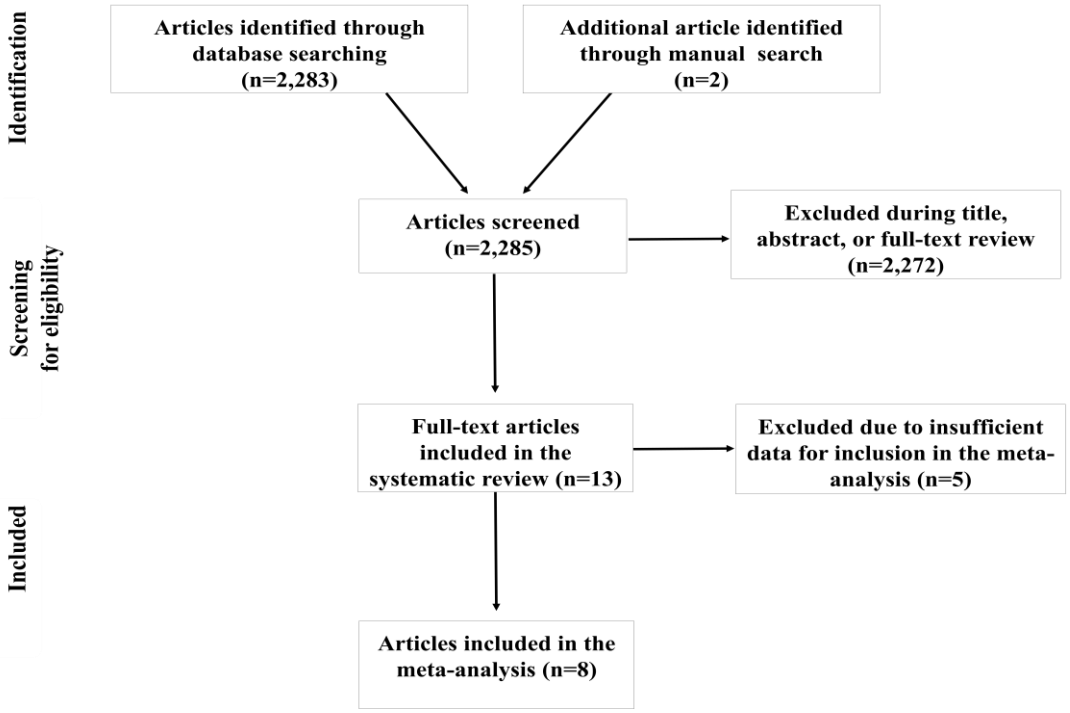



**Figure 3-1 Article Selection Flow Chart.**

### 3.4.2 Characteristics of Included Articles and Quality Assessment

The characteristics of the included articles in the systematic review and meta-analysis are presented in Table 3-1. The included articles were published between 2011 and 2020. The populations were from Australia [167], Brazil [161, 162, 164, 165, 197], Canada [198], Denmark [5, 168], Finland [160], Spain [199], the United Kingdom [163], and Sweden [166]. The sample sizes of the included articles ranged from 35 [165] to 46,832 [166]. One article included only male participants [165], and the remaining included both sexes and analyzed them either together [5, 161-164, 168, 197-199] or separately [160, 166, 167]. To measure ST, five articles utilized self-report instruments [165-167, 197, 199] and eight used objective devices [5, 160-164, 168, 198]. Further, the included studies in the meta-analysis reported either a single estimate [162, 163, 165], multiple within-subject estimates [5, 167, 168], or multiple subgroup estimates [160, 166, 167]. Lastly, out of the thirteen included articles in the systematic review, one had a quality score of nine [168], one had a quality score of eight [5], four had a quality score of seven [160, 164, 166, 198], three had a quality score of six [167, 199] [165], three had a quality score of five [161, 163, 164], and one had a quality score of four [162]. In general, most studies earned quality points for stating a research objective, evaluating ST as a continuous outcome, and for a low chance of selection bias. Studies typically had lower scores due to cross-sectional designs, failure to use a thigh-based accelerometer to measure ST, and failure to measure ST at more than one timepoint (Supplemental Table 3-1).

## Table 3-1 Population Characteristics, ST Measurement, Subgroups, and Quality of Included Articles.

| Reference | Country | Sample N (% male); mean age | ST measurement | Description of estimates | Quality |
|---|---|---|---|---|---|
| **Included in meta-analysis and systematic review** | | | | | |
| **Beijer et al., 2018 [166]** | Sweden | 46832 (41%); 47 yr | self-reported TV watching | age x sex subgroup estimates<br>a (M; 26 y)<br>b (M; 47 y)<br>c (M; 68 y)<br>d (F; 26 y)<br>e (F; 47 y)<br>f (F; 68 y) | 7 |
| **dos Santos et al., 2019 [165]** | Brazil | 35 (100%); NR | self-reported sitting time | single estimate | 6 |
| **Hallman et al., 2019 [5]** | Denmark | 490 (56%); 45 yr | thigh + trunk-mounted accelerometer | domain-specific estimates<br>a (occupational sitting time)<br>b (leisure sitting time) | 8 |
| **Hallman et al., 2015 [168]** | Denmark | 126 (55%); 46 yr | thigh + trunk-mounted accelerometer | domain-specific estimates<br>a (occupational sitting time)<br>b (leisure sitting time) | 9 |
| **Huynh et al., 2014 [167]** | Australia | 2328 (49%); 31 yr | self-reported sitting time | type of day x sex estimates<br>a (M; weekday sitting time)<br>b (M; weekend sitting time)<br>c (F; weekday sitting time)<br>d (F; weekend sitting time) | 6 |
| **Newton et al., 2011 [163]** | United Kingdom | 107 (NR); NR | multi-sensor armband | single estimate | 5 |
| **Niemelä et al., 2019 [160]** | Finland | 4150 (45%); 47 yr | wrist-worn accelerometer | sex subgroup estimates<br>a (M)<br>b (F) | 7 |
| **Spina et al., 2019 [162]** | Brazil | 485 (37%); 48 yr | waist-worn accelerometer | single estimate | 4 |
| **Included in systematic review only** | | | | | |
| **Delfino et al., 2020 [197]** | Brazil | 245 (24%); 45 yr | self-reported sitting time | | 7 |
| **Gerage et al., 2015 [164]** | Brazil | 87 (21%); 58 yr | hip-worn accelerometer | | 5 |
| **McGregor et al., 2018 [198]** | Canada | 7,776 (50%); 47 yr | hip-worn accelerometer | | 7 |
| **Oliveira et al., 2020 [161]** | Brazil | 64 (14%); 39 yr | wrist-worn accelerometer | | 5 |
| **Recio-Rodriguez et al., 2013 [199]** | Spain | 732 (41%); 57 yr | self-reported TV watching | | 6 |

N: sample size; N/A: not applicable; NR: not reported; ST: sedentary time; yr: years.

Regarding outcomes (Table 3-2), five articles measured only HR [166, 167, 197-199], six measured only HRV [161-165, 168], and two measured both HR and HRV [5, 160]. HR and/or HRV were measured using ECG [5, 163, 168], heart rate monitors [160-162, 164, 165], or oscillometers (HR only) [166, 197, 198]; two studies did not report the device used [167, 199]. When reported and not including oscillometer HR measurements, the duration of the HR and HRV measurement ranged from three minutes [160] to three x five-minute segments [5, 168] and were performed during the daytime [160-164, 166, 167, 197-199], in the afternoon [165], or at night [5, 168]. The measurements of HR and HRV were obtained in supine [5, 161, 162, 164, 165, 168] and seated postures [160, 166, 167, 197, 198], with posture not reported in two articles [163, 199].

**Table 3-2 Description of Outcomes from the Included Articles.**

| | Outcome(s) | HR or HRV measurement device | HR or HRV measurement duration, posture, and type |
|---|---|---|---|
| | **Included in meta-analysis and systematic review** | | |
| **Beijer et al., 2018 [166]** | HR | oscillometer | N/A; seated; resting daytime |
| **dos Santos et al., 2019 [165]** | HRV (HF, LF, LF/HF) | HR monitor | 5 minutes; supine; afternoon |
| **Hallman et al., 2019 [5]** | HR; HRV (SDNN, RMSSD, HF, LF, LF/HF) | ECG | 3 x 5-minutes; supine; nocturnal |
| **Hallman et al., 2015 [168]** | HRV (SDNN, RMSSD, HF, LF, LF/HF) | ECG | 3 x 5-minutes; supine; nocturnal |
| **Huynh et al., 2014 [167]** | HR | NR | NR; seated; resting daytime |
| **Newton et al., 2011 [163]** | HRV (LF/HF) | ECG | 10 minutes; NR; daytime |
| **Niemelä et al., 2019 [160]** | HR; HRV (RMSSD, LF/HF) | HR monitor | 5 minutes; seated and standing; daytime |
| **Spina et al., 2019 [162]** | HRV (SDNN, RMSSD, HF, LF, LF/HF) | HR monitor | 5 minutes; supine; resting daytime |
| | **Included in systematic review only** | | |
| **Delfino et al., 2020 [197]** | HR | oscillometer | N/A; seated; resting daytime |
| **Gerage et al., 2015 [164]** | HRV (HF, LF) | HR monitor | 5 minutes; supine; resting daytime |
| **McGregor et al., 2018 [198]** | HR | oscillometer | N/A; seated; resting daytime |
| **Oliveira et al., 2020 [161]** | HRV (LF, HF, LF/HF) | HR monitor | 10 minutes; supine; resting daytime |
| **Recio-Rodriguez et al., 2013 [199]** | HR | NR | NR; NR; daytime |

HR; heart rate, HRV; heart rate variability, N/A; not applicable, NR; not reported, SDNN; standard deviation of normal R-R intervals, RMSSD; root mean square of successive differences, LF; low frequency, HF; high frequency

### 3.4.3 Association between Sedentary Time and Heart Rate

For the qualitative assessment, 53% (n=17) of estimates detected no relationship between ST and HR. Because more than 50% of estimates reported null findings, the qualitative synthesis suggested no association between ST and HR.

For the quantitative evaluation, 14 estimates from the four included articles found trivial and statistically nonsignificant association between ST and HR, where each hour increase in ST

was associated with a 0.19 bpm (95% CI: -0.07, 0.45; *d*=0.03) increase in HR (Figure 3-2a). However, sensitivity analyses removing one article at a time suggested one article substantially altered the statistical inference of the pooled estimate [160]. The direction of the association in this article opposed the direction of the association in all other articles; when reanalyzed after excluding the influential article, the association became statistically significant but remained trivial, with each hour increase in ST associated with a 0.24 bpm (95% CI: 0.10, 0.37; *d*=0.04) increase in HR (Figure 3-2b). Egger's regression test indicated no asymmetry ($\beta_0$=-1.29; p=0.57). There was statistically significant and considerable heterogeneity ($I^2$=96.6%, p<0.001). Due to an insufficient number of estimates within subgroups, we were only able to perform a subgroup analysis by sex, which found a significant association between ST and HR in males but not in females or mixed-sex populations (Supplemental Figure 1).

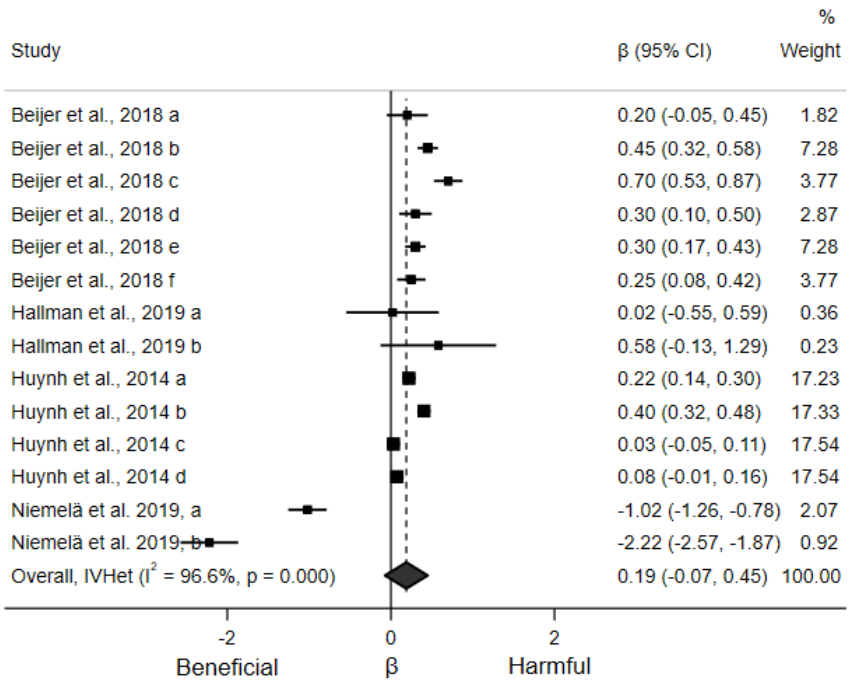
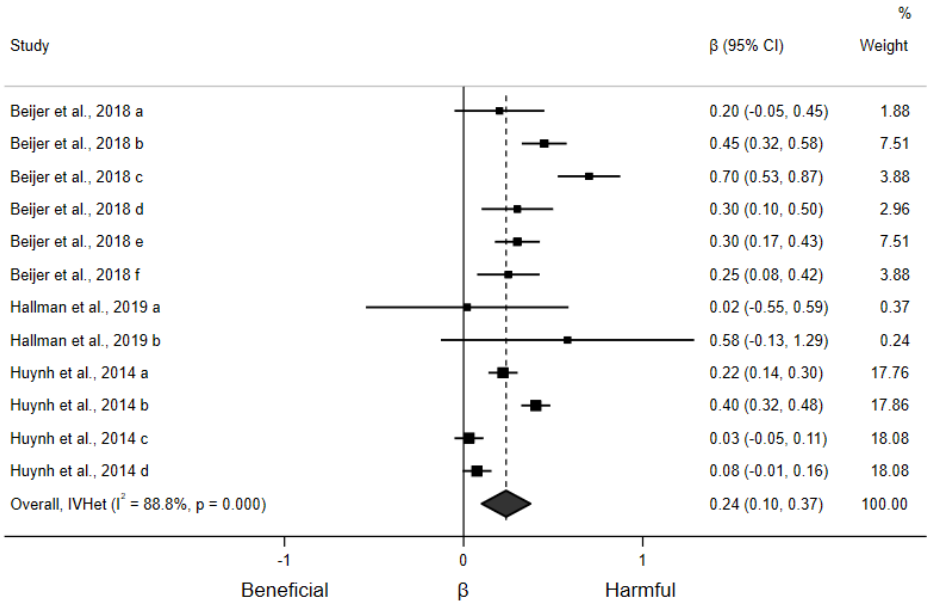



**Figure 3-2 Forest Plots of Articles Examining the Association between ST and Heart Rate.**
a.Association between ST (Hour/Day) and HR (Beat/Minutes) Including All Articles.




b. Association between ST (Hour/Day) and HR (Beat/Minutes) After Excluding the High Influence Article [160].

### 3.4.4 Association between Sedentary Time and Time Domain Indices of Heart Rate Variability

For the qualitative assessment, 80% (n=5) and 71% (n=7) of the estimates found no correlation between ST and SDNN and RMSSD, respectively. Because more than 50% of the estimates reported null findings, the qualitative synthesis suggested no association between ST and SDNN or RMSSD.

For the quantitative assessment, five estimates from the three included articles found a statistically nonsignificant, small, and inverse correlation (r=-0.02; 95% CI: -0.12, 0.08) between ST and SDNN (Figure 3-3a). Egger's regression test indicated no asymmetry ($\beta_0$=-1.34; p=0.27).

There was a moderate, but not statistically significant, risk of heterogeneity ($I^2$=50.5%, p=0.089). Similarly, seven estimates from the four included articles found a non-statistically significant, small, and inverse correlation (r=-0.03; 95% CI: -0.06, 0.01) between ST and RMSSD (Figure 3-3b). Egger's regression test ($\beta_0$=-0.15; p=0.83) indicated no asymmetry. There was also low and statistically nonsignificant heterogeneity ($I^2$=14.9%, p=0.32). Sensitivity analyses removing one article at a time suggested no article statistically and significantly influenced these pooled estimates. None of our prespecified subgroup analyses could be conducted due to an insufficient number of estimates within subgroups.

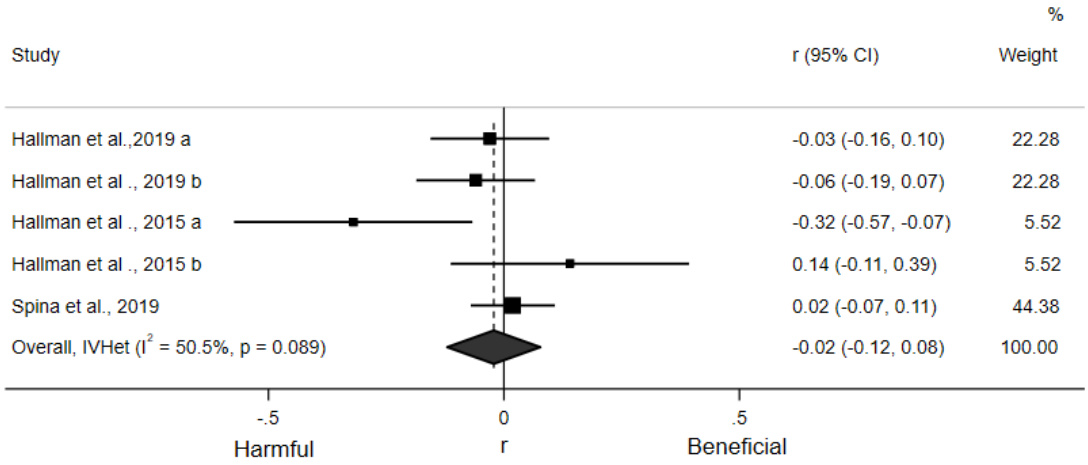
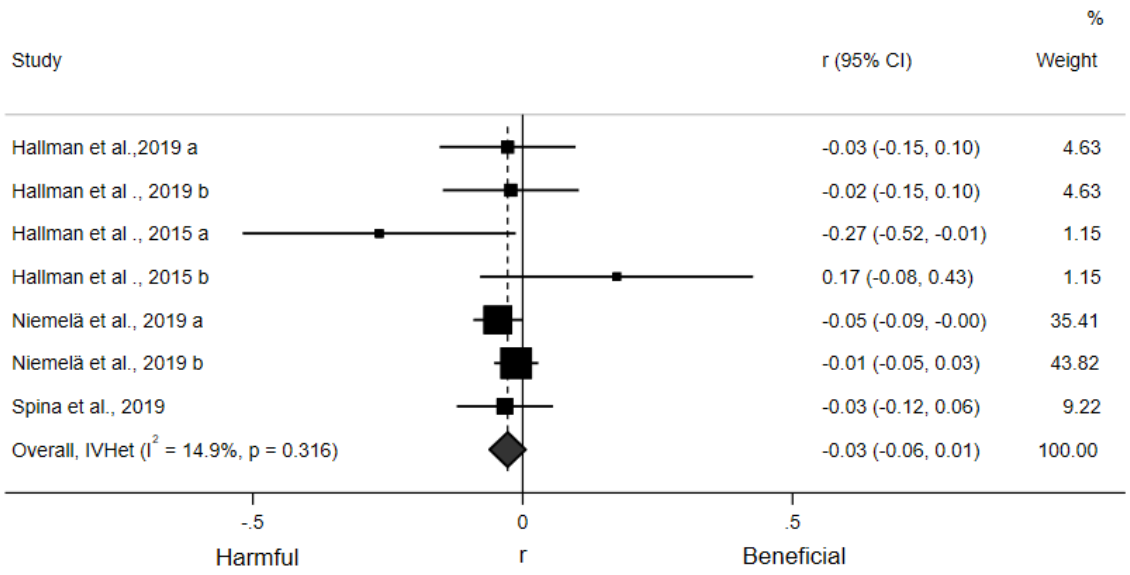



**Figure 3-3 Forest Plots of Articles Examining the Association between ST and Time Domain Indices of HRV.**

a. Association between ST and SDNN.




b. Association between ST and RMSSD.

### 3.4.5 Association between Sedentary Time and Frequency Domain Indices of Heart Rate Variability

For the qualitative assessment, 87% (n=8), 100% (n=6), and 90% (n=10) of the estimates detected no correlation between ST and LF, HF, and LF/HF ratio, respectively. Because more than 50% of the estimates reported null findings, the qualitative synthesis indicated no association between ST and LF, HF, or LF/HF ratio.

For the qualitative assessment, six estimates from the four included articles found a statistically nonsignificant, small, and inverse correlation (r=-0.02; 95% CI: -0.16, 0.13) between ST and LF (Figure 3-4a). Egger's regression test indicated no asymmetry ($\beta_0$=-2.60; p=0.12). There also was moderate and statistically significant heterogeneity ($I^2$=70.8%, p=0.004). Likewise, six estimates from the four included articles detected a statistically nonsignificant, small, and

inverse correlation (r=-0.03; 95% CI: -0.13, 0.08) between ST and HF (Figure 3-4b). Egger's regression test indicated no asymmetry ($\beta_0$=-1.46; p=0.13). There was moderate, but not statistically significant, heterogeneity ($I^2$=54.0%, p=0.054). Lastly, nine estimates from the six included articles revealed a statistically nonsignificant and negligible correlation (r=-0.00; 95% CI: -0.05, 0.04) between ST and the LF/HF ratio (Figure 3-4c). Egger's regression test ($\beta_0$=0.37; p=0.59) indicated no asymmetry. There was moderate, but not statistically significant, heterogeneity ($I^2$ = 31.0%, p=0.17). Sensitivity analyses removing one article at a time suggested no article statistically and significantly affected any of these pooled estimates. Due to an insufficient number of estimates within subgroups, we were only able to perform subgroup analyses for LF/HF ratio by adjustment for MVPA and using ECG which yielded small and statistically nonsignificant associations (Supplemental Figure 3-1, 3-2a and 3-2b).

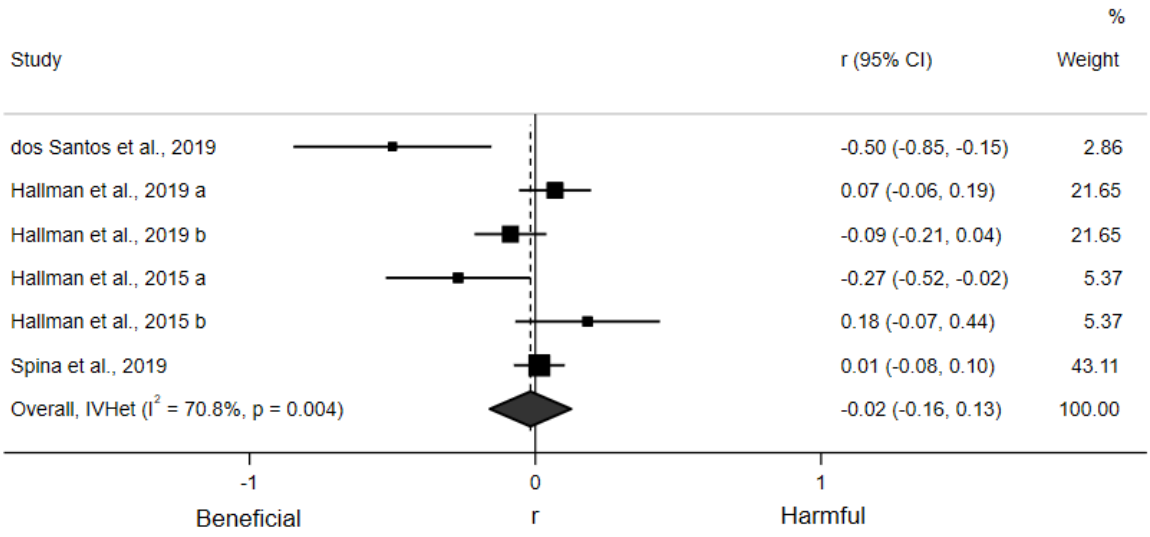
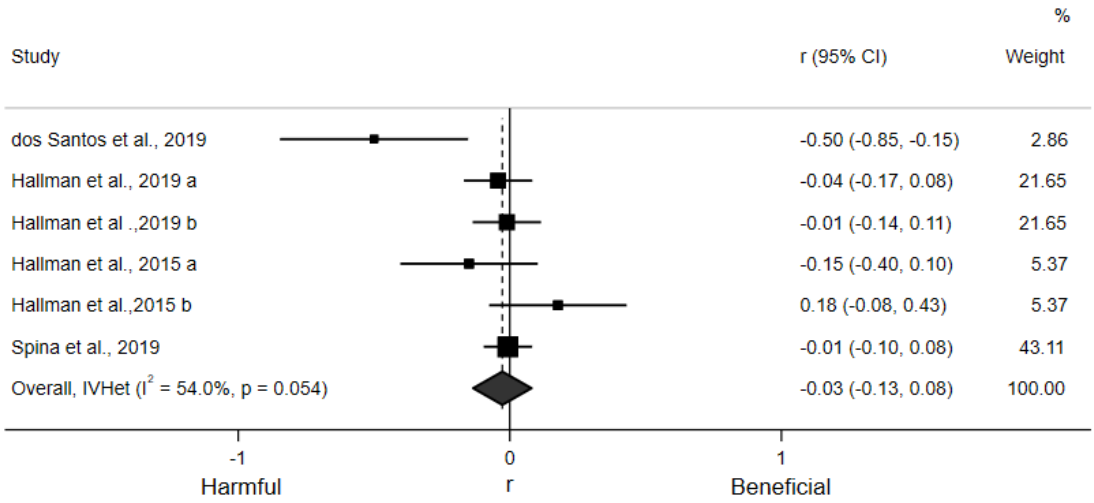
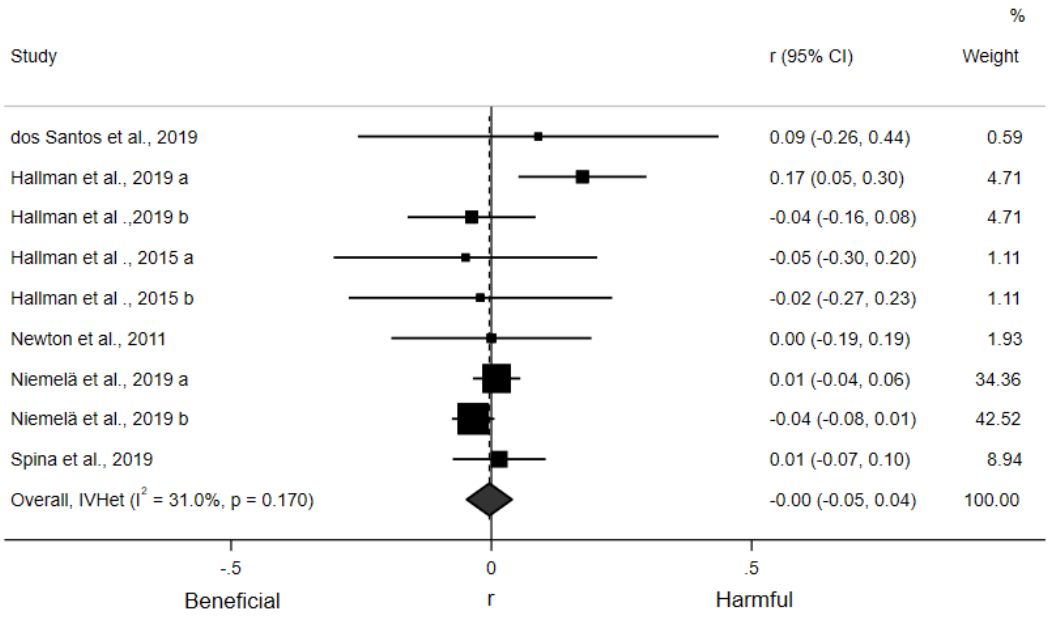



**Figure 3-4 Forest Plots of Articles Examining the Association between ST and Frequency Domain Indices of HRV.**

a. Association between ST and LF.




b. Association between ST and HF.




c.Association between ST and LF/HF Ratio.

## 3.5 Discussion

This study was the first systematic review and meta-analysis to synthesize the existing literature on the observational association of ST with HR and HRV in adults. Only thirteen studies have assessed the association of ST with HR and HRV. Among these studies, study quality was generally low with studies, on average, only meeting 6 of 13 quality criteria. Overall, we found a statistically significant and direct, yet trivial, association between ST and HR. Subgroup analysis found this association to be only apparent in males. However, there were no associations between ST and any of HRV indices.

### 3.5.1 Association between Sedentary Time and Heart Rate

Though our meta-analysis statistically supported our hypothesis that higher ST would be associated with greater HR, this association was very small. Each hour increase in ST was associated with 0.24 bpm increase in HR, corresponding to $d$=0.04, which is likely of minimal clinical significance. This small effect can be benchmarked against a previous meta-analysis reporting that a 10-bpm increase in resting HR was associated with 9% and 8% higher risk of all-cause and cardiovascular mortality, respectively [200]. Thus, greater HR is unlikely to explain much of the association between ST and CVD and mortality. Conceivably, other cardiovascular mechanisms such as impaired metabolic and vascular function may explain the association between higher ST and CVD and mortality [201].

Of note, we detected considerable risk of heterogeneity in the pooled HR estimate. Our sex subgroup analysis revealed that the association of ST with HR was stronger in males

(Supplemental Figure 2). This disparity may be partially explained by sex differences in the autonomic control of the heart, where females usually have higher vagal activity and lower sympathetic activity, granting them cardioprotective effects as compared to males [202]. As such, the observed impact of sedentary behavior on HR might be attenuated in females compared to males. Other potential factors (e.g., ST measurement instruments, domain of ST, posture of HR measurement) may explain more of this considerable heterogeneity in the association between ST and HR and warrant further investigation. For example, two of the four included HR studies in the meta-analysis used self-report instruments [166, 167] which are subject to measurement error that could have affected the association between ST and HR [203]. Additionally, two of the four studies examined the association between ST and HR using domain-specific components of ST [5, 166]. Though not fully understood yet, the association between ST and HR may differ by sedentary behavior domains as it does with other health outcomes [76, 204]. Lastly, methodological issues such as whether pre-visit abstention from PA and food/caffeine/nicotine intake were implemented could have contributed to the observed heterogeneity [205, 206]. These factors were not frequently mentioned or accounted for in the articles included in this review; future research should account for these factors when assessing HR.

### 3.5.2 Association between Sedentary Time and Heart Rate Variability

It was hypothesized that higher ST would be associated with lower overall (i.e., lower SDNN) and cardiac-parasympathetic HRV indices (i.e., lower RMSSD and HF). However, our meta-analyses revealed no associations between ST and HRV indices (i.e., SDNN, RMSSD, LF, HF, LF/HF ratio). These conclusions were limited due to the low-quality and small number of

published studies; this also prevented in-depth examinations using subgroup analyses. Still, these findings suggest that higher ST may not be associated with cardiac-autonomic impairment. Yet, an important consideration when interpreting these findings is that HRV is only able to measure overall and cardiac-parasympathetic activity when resting. Therefore, these findings do not provide data to evaluate whether higher ST is associated with cardiac-sympathetic overactivation, a mechanism that has been specifically hypothesized as the primary autonomic pathway linking ST with CVD [201]. Future studies evaluating ST with specifically cardiac-sympathetic overactivation, as well as addressing the noted limitations of the current research, are needed to better understand whether autonomic dysfunction is indeed a mechanism linking ST and CVD.

Similar to our pooled HR estimate, there were several potential sources of heterogeneity (e.g., participant characteristics, ST measurement instruments, domain of ST, duration and posture of HRV measurements, and HRV measurement devices) that could have impacted our pooled HRV estimates. Yet, we could only perform limited subgroup analyses due to the insufficient number of estimates within subgroups. The few subgroup analyses we were able to conduct did not identify factors likely to be responsible for the observed heterogeneity. Furthermore, our pooled null estimates were potentially affected by several important methodological aspects of the available studies including cross-sectional designs and the lack of control for important covariates such as respiration rate, which can significantly affect HRV [24]. Altogether, though our pooled HRV estimates found no relationship, the observed considerable heterogeneity indicates that there may be more than one true underlying association between sedentary behavior and HRV.

### 3.5.3 Hypothesized Physiological Mechanisms

It has been proposed that frequent exposure to adverse, acute cardiovascular responses to sedentary behavior could manifest as chronic associations between high levels of sedentary behavior with increased HR and decreased HRV. Acutely, sedentary behavior such as prolonged sitting causes blood pooling in the lower extremities leading to interrupted blood flow and reduced blood pressure [175]. To compensate, the sympathetic nervous system would increase its outflow and the vagus nerve would likely reduce its outflow to increase HR to adjust blood flow and pressure [175, 207, 208]. This would lead to reduced overall and cardiac-parasympathetic HRV indices [6, 169]. Concurrently, this decrease in blood flow causes a reduction in shear stress and, eventually, nitric oxide (NO) bioavailability [175, 209]; additionally to its vasodilatory effect, NO acts as vagal activity enhancer leading to augmented acetylcholine release [157]. Thus, reduction in NO bioavailability may attenuate acetylcholine release and, therefore, HRV. Repeated exposure to sedentary behavior and these resulting responses were hypothesized to manifest as chronic augmentation in HR and reduction in HRV. However, our findings do not lend support to a theory where repeated exposure to these acute physiological responses would lead to chronic, adverse effects.

### 3.5.4 Limitations

There are several limitations that should be considered when interpreting our results. First, the currently published observational studies relating ST to HR and HRV all had cross-sectional designs, which are susceptible to biases such as residual confounding and reverse causality. In

addition, significant heterogeneity was observed. This may be partly explained by differences across ST measurement instruments and domains. For example, only two studies used a thigh-worn accelerometer[5, 168], which is the gold standard measure of ST because it can accurately distinguish between seated and standing postures and can therefore provide a more precise ST estimation compared to wrist- or waist-worn monitors or self-reporting tools [203, 210, 211]. As such, our pooled estimates were likely impacted due to ST measurement error in the remaining studies [160, 162, 163, 165]. Furthermore, growing evidence indicates that various domains of ST relate differently to a variety of health outcomes [76, 204]. Our pooled estimates could not account for such potential disparities due to the insufficient number of studies within subgroups.

Equally important, there are other limitations related specifically to HRV that potentially influenced our pooled estimates. Because HRV is a time-dependent measure [34] and short- vs. long-term HRV is affected by different mechanisms [38], it may be inappropriate to compare and aggregate HRV indices that were obtained from different measurement durations [34]. Moreover, another notable limitation is that only one study [162] accounted for respiration rate, which could have significantly affected our pooled estimates [8, 24]. Furthermore, HRV measurement posture (i.e., seated, supine, standing) and timing (i.e., morning, afternoon, nocturnal) varied substantially. Both of these measurement-related factors could have introduced variability and influenced our pooled estimates [212]. Lastly, only half of the studies [160, 162, 165] assessed HRV with the gold standard technique of ECG [34]; this represents another important limitation that might have influenced our pooled estimates. This was suggested when we compared LF/HF ratio estimates based upon whether ECG was used; the pooled associations were in opposing direction, though both were statically nonsignificant (Supplemental Figure 3B). Unfortunately, due to the limited

number of available studies, we were unable to perform this subgroup analysis for other HRV indices.

### 3.5.5 Implications and Future Directions

The findings of this systematic review and meta-analysis do not support the hypothesis that HR and HRV are mechanisms linking sedentary behavior and CVD. As the association between sedentary behavior and CVD is more established [201], our systematic review and meta-analysis suggest that there might be other more important mechanisms (e.g., vascular dysfunction, metabolic disturbances, or sympathetic overactivation) that might explain such associations. Yet, given the above-mentioned limitations to the available data, future research should consider and address existing methodological limitations to confirm our null findings with greater rigor. Specifically, there is a need for studies that have longitudinal designs to establish temporality in the association between ST and HR and HRV. In addition, intervention studies that examine the effects of reducing ST on HR and HRV may be the best design to assess causality between ST and cardiac autonomic regulation. Studies should also utilize the gold standard techniques to measure ST (i.e., a thigh-worn monitor) and HRV (i.e., ECG with guideline-based processing and accounting for respiration rate). Other sources of potential heterogeneity that we were unable to disentangle may also be important future research directions, such as preexisting CVD, domains and patterns of ST, and diurnal vs. nocturnal HRV measurement [145, 213-215].

## 3.6 Conclusion

Overall, the available, low-quality evidence suggests an unfavorable but not clinically meaningful association between ST and HR, but no association between ST and HRV. These results do not support the hypothesis that increases in HR and decreases in HRV are mechanisms linking increased ST and CVD. Future research that is longitudinal, uses optimal and standardized measurement methodology for sedentary behavior and HRV, and evaluates potential sources of heterogeneity is needed to draw more comprehensive conclusions.

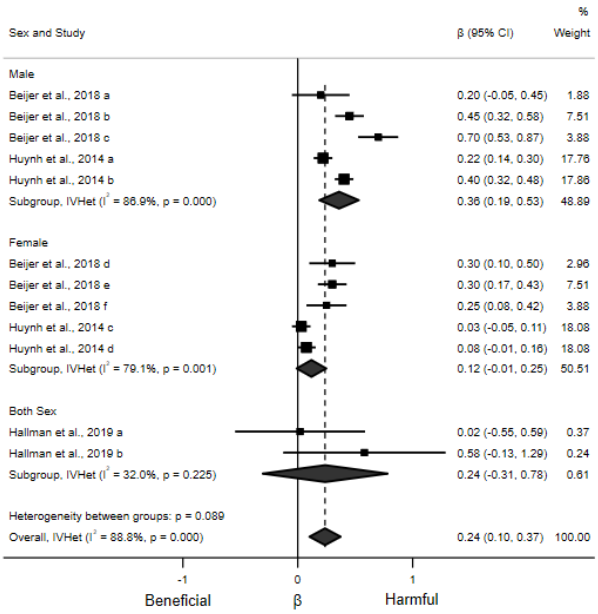
## 3.7 Supplemental Tables and Figures

### Supplemental Table 3-1 Quality Assessment of the Included Studies

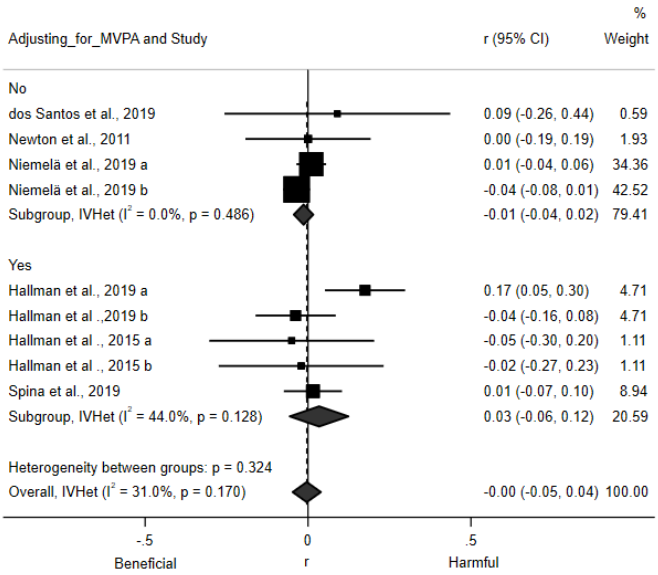
| Name | Was the research question or objective in this paper clearly stated? | Was the study population clearly specified (i.e., demographics, period, location)? | Was the sample population-based? | Were all the subjects selected or recruited from the same or similar populations (including time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants? | Was a sample size justification, power description, or variance and effect estimates provided? | For the analyses in this paper, Was temporality established? | Was the timeframe sufficient so that one could reasonably expect to see an association between ST and HR/HRV if it existed? | For ST, did the study examine different levels? (e.g., categories or continuous)? | Was ST assessed using validated, objective, thigh-worn device? | Was ST assessed more than once over time? | Was HR/HRV measured with valid and reliable methods, including pre-visit abstention from physical activity and intake (dietary, caffeine, nicotine) if appropriate? | Was HR objectively measured and HRV was measured by ECG (gold standard), if applicable? | Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between ST and HR/HRV (specifically, age, sex, MVPA)? | Quality score (out of 13) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Beijer et al., 2018 [166] | ■ | ■ | ■ | ■ | | | | ■ | | | | ■ | ■ | 7 |
| dos Santos et al., 2019 [165] | ■ | | | ■ | ■ | ■ | | ■ | | | ■ | | | 6 |
| Hallman et al., 2019 [5] | ■ | ■ | | ■ | | | | ■ | ■ | | ■ | ■ | ■ | 8 |
| Hallman et al., 2015 [168] | ■ | ■ | | ■ | ■ | | | ■ | ■ | | ■ | ■ | ■ | 9 |
| Huynh et al., | ■ | ■ | ■ | ■ | | | | ■ | | | | | ■ | 6 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2014 [167]** | | | | | | | | | | | | | | |
| **Newton et al., 2011 [163]** | ■ | ■ | | ■ | | | | ■ | | | | ■ | | **5** |
| **Niemelä et al., 2019 [160]** | ■ | ■ | ■ | ■ | | | | ■ | | | ■ | | ■ | **7** |
| **Spina et al., 2019 [162]** | ■ | | | ■ | | | | ■ | | | ■ | | | **4** |
| **Delfino et al., 2020 [197]** | ■ | ■ | ■ | ■ | ■ | | | ■ | | | | ■ | | **7** |
| **Gerage et al., 2015 [164]** | ■ | ■ | | ■ | ■ | | | ■ | | | | | | **5** |
| **McGregor et al., 2018 [198]** | ■ | ■ | ■ | ■ | | | | ■ | | | | ■ | ■ | **7** |
| **Oliveira et al., 2020 [161]** | ■ | ■ | | ■ | ■ | | | ■ | | | | | | **5** |
| **Recio-Rodriguez et al., 2013 [199]** | ■ | ■ | ■ | ■ | ■ | | | ■ | | | | | | **6** |

Each question earned one point if the answer was yes, and zero if the answer was no.



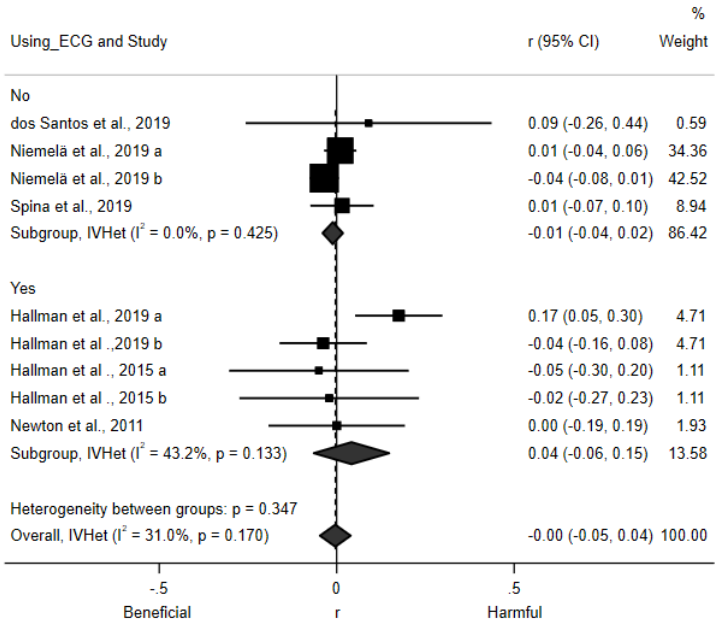

**Supplemental Figure 3-1 Forest Plots of Association between ST and HR in Subgroup Analyses by Sex.**




**Supplemental Figure 3-2 Forest Plots of the Association between ST and LF/HF Ratio in Subgroup Analyses.**

a. Subgroup analyses by MVPA adjustment.

No indicates no adjustment for MVPA; Yes indicates adjustment for MVPA.




b. Subgroup analysis by using electrocardiogram (ECG).
No indicates not using ECG to measure HRV; Yes indicates using ECG to measure HR.

# 4.0 Manuscript 2: Associations of Total, Leisure, and Occupational Moderate-to-Vigorous Intensity Physical Activity and Sedentary Time with Cardio Autonomic Regulation in Women (Specific Aim II)

## 4.1 Abstract

Lifestyle factors, including moderate-to-vigorous intensity physical activity (MVPA) and excessive sedentary time (ST), may contribute to cardiovascular risk in women, perhaps via cardiac-autonomic dysregulation. **AIMS:** To examine associations of total, leisure, and occupational MVPA and ST with cardiac-autonomic regulation in women. **METHODS:** This cross-sectional, secondary analysis included 522 women (age=37.7±5.7 yr; 59%white). MVPA and ST (hours/day) were self-reported using the Modifiable Activity Questionnaire. Cardio-autonomic regulation was assessed by calculating resting HRV indices (average HR, lnSDNN [total variability], lnRMSSD [cardiac-parasympathetic activity]) with Kubios software from a 5-minute, seated ECG. Progressive generalized linear models evaluated associations of total, leisure, and occupational MVPA and ST with HRV indices while adjusting for confounders (demographics, health-related factors) and then potential mediators (clinical variables). A final model evaluated the relationship between ST and HRV stratified by MVPA level. **RESULTS:** Adjusting for confounders, total and leisure MVPA were associated with favorable lnSDNN (B=0.027 [p=0.014] and B=0.074 [p=0.009], respectively) and lnRMSSD (B=0.036 [p=0.015] and B=0.075 [p=0.043], respectively). Adjustment for mediators tended to strengthen the observed significant associations. No associations were found between occupational MVPA or any ST

measure with HRV indices (p>0.05). Neither MVPA nor ST were associated with HR. When stratified by MVPA level, leisure ST was associated with unfavorable lnSDNN (B=-0.041, p=0.047) and lnRMSSD (B=-0.050, p=0.030) only among women who did not meet leisure MVPA recommendations. **CONCLUSION:** Cardiac-autonomic dysregulation may be a mechanism through which low leisure MVPA and, among low-active women, high leisure ST contribute to CVD risk among women.

## 4.2 Introduction

Recent evidence indicates that young to middle-aged women (i.e., ages 20-55 years old) have had blunted improvements in rates of cardiovascular disease (CVD) as compared to declining CVD rates in similarly aged men and older women [216, 217]. The ability to reduce accumulating CVD risk in these women requires an investigation of contributing factors and mechanisms through which they accumulate CVD risk. Lifestyle factors such as physical inactivity and excessive ST are suggested as contributing factors for CVD.

Physical inactivity is defined as not engaging in sufficient levels of moderate-to-vigorous intensity physical activity (MVPA) [57]. Physical inactivity is prevalent among adults worldwide and is higher among women compared to men [218]; this is unfortunate since physical inactivity is a major and modifiable CVD risk factor [219]. In addition, excessive time spent in sedentary behavior (i.e., any waking activity that occurs in a lying, reclining, or seated posture and has energy expenditure of ≤ 1.5 metabolic equivalents) [64] is emerging as a CVD risk factor, independent of physical inactivity [220]. Excessive sedentary behavior is also prevalent among women, who

spend > 55 % of their waking time in sedentary behavior [221]. Taken together, women tend to be physically inactive and accumulate excessive ST, both of which may contribute to CVD risk in this population. Yet, the physiological pathways that relate physical inactivity and excessive ST to CVD in women are not fully understood.

Cardiac-autonomic dysregulation is a physiological mechanism that links risk factors, such as hypertension (HTN) and diabetes (DM), to CVD outcomes [222]. Cardiac-autonomic regulation is commonly assessed by measuring heart rate variability (HRV), a non-invasive method that measures the variation in time intervals between consecutive heartbeats [24]. Lower variability in the time intervals indicates altered cardiac-autonomic balance, regulation, and/or flexibility and suggests vulnerability to CVD [26]. Many studies have consistently found associations between reduced HRV with CVD and mortality [18]. Evaluation of HRV in young to middle-aged women may be especially relevant as reduced HRV is subclinical and has been associated with sex-specific factors relevant to women such as adverse pregnancy outcomes [223]. Thus, reduced HRV is likely detectable before progression to overt CVD and may provide insight into the pathway between lifestyle risk factors (e.g., physical inactivity and excessive ST) and elevated CVD risk in these women.

Robust evidence indicates that MVPA is associated with greater HRV in adults, including young to middle-aged women [224]. However, limited research has examined the association between sedentary time (ST) and HRV in adults [5, 161, 162, 165, 168]. These studies have yielded inconsistent findings, and no studies have been conducted specifically in young to middle-aged women. Moreover, most studies had small samples, did not use gold standard HRV assessment procedures (i.e., ECG), and/or did not consider joint associations with MVPA. This final limitation is important as recent evidence indicates that the harmful effects of excessive ST on health

outcomes may be attenuated in the presence of high MVPA [220]. Additionally, accumulating data suggest that MVPA and ST across occupational and leisure domains may differently affect CVD risk. Therefore, associations of CVD risk across domains of these behaviors should be researched [5, 144, 168].

The primary aim of this study was to examine the independent and joint associations between total MVPA and ST with HRV in young to middle-aged women. We hypothesized that greater MVPA would be associated with higher (i.e., better) HRV, while greater ST would be associated with lower (i.e., worse) HRV. We further aimed to explore associations across leisure and occupational domains. Lastly, we hypothesized whether any adverse effects of sedentary behavior on HRV would be more apparent among inactive women versus women who meet leisure MVPA recommendations.

## 4.3 Materials and Methods

This study was a secondary, cross-sectional analysis of data from the follow-up study of the Pregnancy Outcomes and Community Health (POUCH) Study [225]. Briefly, the POUCH Study enrolled 3019 women during pregnancy to prospectively examine social and biological factors linked to preterm delivery. A subset of women (sub-cohort n=1371) was studied in greater detail. The selection strategy for the sub-cohort included all women who had a preterm delivery (<37 weeks gestation), women with specific risk factors for preterm delivery, and a random sample of the remaining women. Sub-cohort women who agreed to be recontacted were invited into the POUCHmoms Study for follow-up 7 to 15 years after delivery (between 2011-2014). To be

included in POUCHmoms, women could not have been currently pregnant or pregnant in the past six months.

### 4.3.1 Study Population

Among eligible POUCH Study sub-cohort women, 678 participated in the POUCHmoms follow-up assessment [225]. To be included in the current analysis, participants additionally had to have complete self-reported ST and MVPA data and HRV measurements of sufficient quality. All participants provided written informed consent. This follow-up study was approved by the Institutional Review Boards of the Michigan State University and the University of Pittsburgh.

### 4.3.2 Measurements

#### 4.3.2.1 ST and MVPA

The interview-based MAQ questionnaire was used to measure ST and MVPA [121]. This questionnaire assesses daily ST and MVPA during leisure and occupational time during the past 12 months. Leisure ST was evaluated by asking the participants about their typical daily hours spent sitting including activities such as TV watching, computer use unrelated to work, reading, crafts and helping children with homework. For occupational ST, the participants were asked about their daily hours spent sitting at work in a job that they had held for at least one month over the past year. Leisure and occupational ST were examined as individual domains and as total ST, calculated by summing the two domains. To assess leisure MVPA, the participants were asked to estimate the frequency and average minutes for each time they participated in planned physical

exercise that they had performed at least 10 times over the past year. These average minutes were converted into hours/day. Occupational MVPA was measured by asking the participants about their average daily hours spent in physically demanding activities at work in a job that they had held for at least one month over the past year. Leisure and occupational MVPA were examined as individual domains and as total MVPA, calculated by summing the two domains.

#### 4.3.2.2 HRV measurement

HRV was measured using ECG at the POUCHmoms follow-up visit. Participants were instructed to fast for at least 8 hours prior to the study visit. Upon arriving, several assessments including blood sample collection and self-reported questionnaires were completed followed by a 45–60-minute snack break. Thereafter, ECG measurements were obtained while participants were seated quietly in a chair with both feet flat on the floor. Two electrodes were placed on the participant's upper chest, and one electrode was placed on the participant's abdomen to record resting ECG signals using the Biopac MP36RWSW system (Goleta, CA). Sampling rate was set at 1000 Hz. Thereafter, 6 minutes of ECG signals were recorded and were later exported as AQC files (Biopac AcqKnowledge). AQC files were imported into Kubios Premium HRV analysis software (version 3.3.1, MATLAB, The MathWorks, Inc) for processing and deriving HRV indexes. ECG signals were successfully recorded from 604 participants.

Established guidelines were followed to calculate HRV from ECG signals [24]. Among participants with at least 5 minutes of data, the automatic correction was employed to detect artifacts. Any files that had > 5 % artifacts were immediately excluded. Thereafter, files that had ≤ 5 % artifacts underwent further visual evaluation for noise, distortion, missing or premature R waves, ectopic beats, arrhythmias, or irregular rhythms; abnormal samples were corrected if

possible according to the guidelines [24] or otherwise excluded. To account for the potential effects of respiration on HRV, Kubios Premium software estimated the respiration rate from ECG using the amplitude of R waves (ECG-derived respiration rate) [226] to use in sensitivity analyses (described below). We selected HRV indices that have a well-understood physiological and statistical basis and predict CVD outcomes. As such, HR, SDNN (representing the overall variability), and RMSSD (representing cardiac parasympathetic activity) were selected as outcomes of interest.

#### 4.3.2.3 Covariates

The POUCHmoms follow-up visit linked prospectively collected pregnancy data from the POUCH study and measured confounders and mediators of our hypothesized associations. Demographic, lifestyle, and health-related factors including age, race (i.e., non-Hispanic white, African American, or other), education (i.e., high school or less, some college, or college degree), working status (i.e., currently working or not working), type of health insurance (i.e., private, Medicaid, or none), and current smoking status (i.e., yes or no) were self-reported. In addition, waist and hip circumferences were measured in triplicate with a Gulick tape measure. The average of hip and waist measurements was used to calculate waist-to-hip ratio (WHR). Following five minutes of seated rest, systolic (SBP) and diastolic (DBP) blood pressures were measured three times using an Omron HEM-907 (Omron Healthcare, Inc.; Lake Forest, IL) with an appropriately sized cuff. The average of the second and third measurements was calculated as the resting blood pressure [227]. Women with SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg or who reported using anti-hypertensive medications were classified as hypertensive. Finally, the presence of DM and/or glucose-lowering medications were self-reported.

### 4.3.3 Analytical Method

Participant characteristics were summarized descriptively as means with standard deviations, medians with 25$^{th}$ and 75$^{th}$ percentiles, or numbers and percentages, as appropriate. Characteristics of included versus excluded women were compared using independent t-tests or Mann-Whitney *U* tests for normally and non-normally distributed continuous variables, respectively, and $\chi^2$ test for categorical variables. Outcome variables that were not normally distributed (i.e., SDNN, RMSSD) were natural log transformed. Confounders and/or mediators were defined *a priori* by constructing a direct acyclic graph (DAG) (Supplemental Figure 4-1). Pearson's correlations between R-R intervals with HR, lnSDNN and lnRMSSD were also checked [228] .

Generalized linear models examined the cross-sectional relationship between self-reported ST and MVPA with HRV. **Model 1** included simultaneous adjustment for ST, MVPA, and confounders including demographics, lifestyle, and health-related factors. Because women who had preterm delivery or were at higher risk of preterm delivery were oversampled in the POUCH Study sub-cohort and therefore in POUCHmoms as well, sampling weights were applied to all analyses. **Model 2** was further adjusted for clinical factors as potential mediators including HTN, DM, antihypertensive, and glucose-lowering medications. This progressive covariate adjustment was employed first for total ST and MVPA and then after separation of ST and MVPA into domains (leisure and occupational). Lastly, because prior research suggests that the relationship between ST and health outcomes may differ according to MVPA level [220], models evaluating relationships between ST and HRV were repeated separately among women who met (i.e., active) and did not meet (i.e., inactive) current leisure MVPA recommendations of 2.5 hours/week.

Sensitivity analyses excluded women with underlying medical conditions that could have affected cardiac-autonomic function and HRV (e.g., hypoglycemia, post-traumatic stress disorder, carpal tunnel syndrome, heart arrhythmias, neuropathy, cardiac problems), and then further excluded women who did not meet the ECG-derived respiration rate criteria (9-24 breaths/minute). Because most HRV indices have a positive correlation with heart period such that, as heart period increases, HRV indices also increase, researchers have suggested that HRV should be adjusted for heart period or rate [228]. Therefore, adjusted HRV indices were calculated according to the current recommendations using the coefficient of variation (CV) technique as following: $_{CV}$HRV index = 100 x HRV index / heart period [228]. Sensitivity analyses using the adjusted HRV indices were conducted to evaluate the potential influence of heart period.

Stata version 15.0 (StataCorp, College Station, TX) was used to conduct all statistical analyses. The significance level was set as $\alpha < 0.05$. Cohen's *d* was calculated as the $\beta$ divided by the standard deviation of the dependent variable to examine the magnitude of association as recommended for HRV: $d=0.25$ is small; $d=0.5$ is medium; and $d=0.9$ is large [229].

## 4.4 Results

A total of 678 women completed the POUCHmoms follow-up assessment visit. Of them, 604 women had sufficient ECG records for 5 minutes of HRV analysis. Of these women, 82 women had invalid HRV records due to the following reasons that prevented HRV calculation: ECG distortion (n=49), arrhythmia/irregular ECG (n=20), > 5% artifacts (n=10), excessive noise

(n=2), and file error (n=1). Thus, following exclusions, 522 women were included in the current analyses (Figure 4-1).

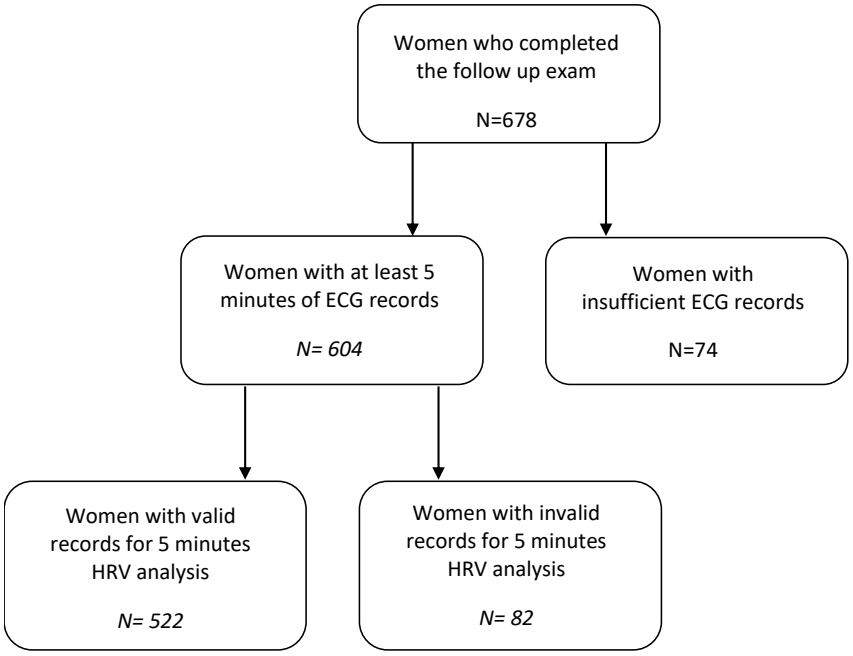



**Figure 4-1 Flowchart of Women who Completed ECG Measurement.**

Compared to included women, excluded women (n=156) had similar demographic characteristics and lifestyle behaviors, but tended to have higher SBP and DBP values, a higher prevalence of HTN, and more frequent use of anti-hypertensive and glucose-lowering medications (Supplemental Table 4-1).

Table 4-1 presents characteristics of the sample. The majority were non-Hispanic white (59.0%), non-smoking (71.5%), currently working (73.4%), and insured by Medicaid (54.8%). On average, systolic and diastolic blood pressures were in the normal range, though some participants had hypertension (18.8%) or diabetes (4.8%). Median self-reported total ST was 7 hours/day and total MVPA was 0.82 hours/day. Based on leisure-time activity, 48.9% of women met MVPA recommendations (i.e., $\geq$ 2.5 hours/week).

## Table 4-1 Characteristics of Participants (n=522).

| Characteristic | Mean (SD), Median ($25^{th}$– $75^{th}$), or n (%) |
|---|---|
| **Age (years)** | 37.7 (5.7) |
| **Race** | |
| White | 308 (59.0) |
| African American | 184 (35.2) |
| Other | 30 (4.7) |
| **Education** | |
| High School or Less | 128 (24.5) |
| Some College | 244 (46.7) |
| College Degree | 150 (28.7) |
| **Working Status** | |
| Currently Working | 383 (73.4) |
| Currently Not Working | 139 (26.6) |
| **Insurance** | |
| Private | 189 (36.2) |
| Medicaid | 286 (54.8) |
| None | 47 (9.0) |
| **Currently Smoking** | |
| No | 373 (71.5) |
| Yes | 149 (28.5) |
| **Waist-to-Hip Ratio** | 0.8 (0.1) |
| **Systolic Blood Pressure (mmHg)** | 114.0 (13.7) |
| **Diastolic Blood Pressure (mmHg)** | 75.5 (11.1) |
| **Hypertension** | |
| No | 424 (81.2) |
| Yes | 98 (18.8) |
| Using Medication | 62 (63.3) |
| Not Using Medication | 36 (37.7) |
| **Diabetes** | |
| No | 497 (95.2) |
| Yes | 25 (4.8) |
| Using Medication | 13 (52.0) |
| Not Using Medication | 12 (48.0) |
| **HR (beats/minute)** | 76.8 (10.1) |
| **lnSDNN** | 3.6 (0.5) |
| **lnRMSSD** | 3.4 (0.6) |
| **Total ST (hours/day)** | 7.0 (4.5 - 9.5) |
| Leisure ST (hours/day) | 3.0 (2.0 – 5.0) |
| Occupational ST (hours/day) | 3.0 (1.0 – 5.0) |
| **Total MVPA (hours/day)** | 0.8 (0.2 – 3.5) |
| Leisure MVPA (hours/day) | 0.3 (0.1 - 0.7) |
| Meeting Recommendations | 255 (48.6) |
| Not Meeting Recommendations | 267 (51.1) |
| Occupational MVPA (hours/day) | 0.0 (0.0 - 2.6) |

HR: heart rate; ln: natural logarithm; mmHg: millimeters of mercury; MVPA: moderate-to-vigorous physical activity; ms: millisecond; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time.

Table 4-2 displays the independent associations between self-reported total ST and MVPA with HR and HRV. Model 1 (i.e., adjusted for confounders) and 2 (i.e., Model 1 with further adjustment for mediators) found no associations between self-reported total ST with HR, lnSDNN, or lnRMSSD (each $p>0.05$; *d* range: 0.00 – 0.02). In contrast, both Model 1 and 2 detected small, favorable, and statistically significant relationships between self-reported total MVPA with lnSDNN ($p=0.014$ and 0.008; $d=0.05$ and 0.06, respectively) and lnRMSSD ($p=0.024$ and 0.015; $d=0.05$ and 0.06, respectively), but not with HR ($p>0.05$; $d=0.04$).

**Table 4-2 Associations between Total ST and MVPA with HR and HRV.**

| Variables | Model | Total ST | | Total MVPA | |
|---|---|---|---|---|---|
| | | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** |
| **HR (beats/minute)** | 1 | 0.105±0.154 (0.495) | 0.01 | -0.371±0.239 (0.122) | 0.04 |
| | 2 | 0.033±0.146 (0.823) | 0.00 | -0.447±0.249 (0.073) | 0.04 |
| **lnSDNN** | 1 | -0.012±0.009 (0.166) | 0.02 | **0.027±0.011 (0.014)** | 0.05 |
| | 2 | -0.009±0.008 (0.282) | 0.02 | **0.029±0.011 (0.008)** | 0.06 |
| **lnRMSSD** | 1 | -0.010±0.010 (0.292) | 0.02 | **0.032±0.014 (0.024)** | 0.05 |
| | 2 | -0.007±0.009 (0.456) | 0.01 | **0.036±0.015 (0.015)** | 0.06 |

*d*: Cohen's *d*; HR: heart rate; ln: natural log-transformed; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time. Bold indicates significant associations (p<0.05).
Model 1 is adjusted for total ST and total MVPA, age, race, education, working status, insurance, smoking.
Model 2 adds adjustment for hypertension, antihypertensive medications, diabetes, glucose-lowering medications, and waist-to-hip ratio.

Table 4-3 displays the independent associations after separation of ST and MVPA into domains (leisure and occupational) with HR and HRV. No associations were detected between leisure ST, occupational ST, or occupational MVPA with HR, lnSDNN, or lnRMSSD (each p>0.05; *d* range: 0.00 – 0.05). However, small, beneficial, and statistically significant relationships were observed between leisure MVPA and lnSDNN and lnRMSSD in fully adjusted models (p<0.001 [*d*=0.20] and p=0.003 [*d*=0.17], respectively). Leisure MVPA was not associated with HR (p>0.05).

**Table 4-3 Associations between Leisure and Occupational ST and MVPA with HR and HRV.**

| Variables | Model | Leisure ST | | Occupational ST | | Leisure MVPA | | Occupational MVPA | |
|---|---|---|---|---|---|---|---|---|---|
| | | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** |
| **HR (beats/ minute)** | 1 | 0.458±0.239 (0.056) | 0.05 | -0.130±0.238 (0.587) | 0.01 | -0.796±0.600 (0.185) | 0.08 | -0.350±0.306 (0.253) | 0.03 |
| | 2 | 0.339±0.221 (0.127) | 0.03 | -0.176±0.234 (0.454) | 0.02 | -1.228±0.631 (0.052) | 0.12 | -0.335±0.306 (0.273) | 0.03 |
| **lnSDNN** | 1 | -0.024±0.014 (0.091) | 0.05 | 0.001±0.010 (0.936) | 0.00 | **0.074±0.028 (0.009)** | 0.15 | 0.019±0.013 (0.139) | 0.04 |
| | 2 | -0.015±0.013 (0.238) | 0.03 | -0.001±0.010 (0.955) | 0.00 | **0.099±0.024 (<0.001)** | 0.20 | 0.016±0.013 (0.255) | 0.03 |
| **lnRMSSD** | 1 | -0.030±0.017 (0.073) | 0.05 | 0.005±0.013 (0.719) | 0.01 | **0.075±0.037 (0.043)** | 0.13 | 0.028±0.018 (0.120) | 0.05 |
| | 2 | -0.021±0.015 (0.174) | 0.04 | 0.005±0.013 (0.710) | 0.01 | **0.103±0.035 (0.003)** | 0.17 | 0.024±0.018 (0.169) | 0.04 |

*d*: Cohen's *d*; HR: heart rate; ln: natural log-transformed; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time. Bold indicates significant associations (p<0.05).
Model 1 is adjusted for leisure and occupational ST and MVPA, age, race, education, working status, insurance, smoking.
Model 2 adds adjustment hypertension, antihypertensive medications, diabetes, glucose-lowering medications, and waist-to-hip ratio.

Table 4-4 displays the associations of self-reported leisure and occupational ST with HR and HRV following stratification of women based on leisure-time MVPA recommendations, adjusted for confounders (Model 1). Small, unfavorable, and statistically significant associations were observed between leisure ST with lnSDNN (p=0.047; *d*=0.08) and lnRMSSD (p=0.030; *d*=0.08) in women who did not meet leisure MVPA recommendations; no associations were observed among women who did meet leisure MVPA recommendations. Occupational ST did not have statistically significant relationships with HR or HRV regardless of leisure MVPA group.

**Table 4-4 Associations of Leisure and Occupational ST with HR and HRV in Active (N = 255) vs. Inactive (N = 267) Women.**

| Variables | Leisure MVPA Status | Leisure ST | | Occupational ST | |
|---|---|---|---|---|---|
| | | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** |
| **HR (beats/minute)** | Active | 0.258±0.281 (0.358) | 0.03 | 0.350±0.302 (0.248) | 0.03 |
| | Inactive | 0.628±0.338 (0.064) | 0.06 | -0.063±0.257 (0.807) | 0.01 |
| **lnSDNN** | Active | 0.001±0.012 (0.918) | 0.00 | -0.015±0.012 (0.216) | 0.03 |
| | Inactive | **-0.041±0.020 (0.047)** | 0.08 | -0.007±0.013 (0.589) | 0.01 |
| **lnRMSSD** | Active | -0.001±0.016 (0.950) | 0.00 | -0.025±0.016 (0.127) | 0.04 |
| | Inactive | **-0.050±0.023 (0.030)** | 0.08 | 0.005±0.017 (0.782) | 0.01 |

*d*: Cohen's *d*; HR: heart rate; ln: natural log-transformed; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time. Bold indicates significant associations($p<0.05$). Active women were defined as those who self-reported accumulating leisure MVPA of ≥ 2.5 hours/week; inactive women were defined as those who self-reported accumulating leisure MVPA of < 2.5 hours/week.

All models adjusted for leisure and occupational ST, age, race, education, working status, insurance, and smoking.

Sensitivity analyses that excluded women with potential underlying medical conditions that could have affected HRV and women who did not meet ECG-derived respiration rate yielded similar results. Comparable relationships were also observed when adjusted cvHRV indices were utilized (Supplemental Table 4-2 and 4-3).

## 4.5 Discussion

This study examined the independent and joint associations between total and domain-specific MVPA and ST with HRV in young to middle-aged women. The main findings were that higher total MVPA was independently associated with higher HRV; this association appeared to

be primarily driven by leisure MVPA. Yet importantly, the magnitude of these associations was small. In contrast, neither total nor domain-specific ST were independently associated with HRV in the full sample analysis. However, small and adverse associations were observed where higher leisure ST was associated with lower (worse) HRV specifically among women who did not meet leisure MVPA recommendations.

Consistent evidence indicates that regular MVPA can reduce the risk of CVD [52]. These cardiovascular benefits gained by MVPA are hypothesized to be attributed, in part, to improved cardiac-autonomic regulation [230]. This hypothesis is supported by a meta-analysis of experimental clinical trials reporting beneficial associations between MVPA and HRV [224]. Building on this evidence, we also found that higher MVPA was associated with better cardiac-autonomic regulation (i.e., higher HRV) in women. These data indicate that cardiac-autonomic dysregulation may be a mechanism linking physical inactivity and CVD in these women.

Of note, the favorable relationships observed between MVPA and HRV in the current study were apparently due to leisure MVPA, whereas occupational MVPA was found not to be associated with HRV. This finding is of particular interest because emerging evidence indicates the existence of a "PA Health Paradox" effect on HRV [144], which suggests that leisure PA is usually MVPA, performed over short durations with sufficient recovery, and, thus, bring about cardiovascular benefits including enhanced HRV [144, 231]. On the other hand, occupational PA is usually performed over long durations with short recovery periods, and is hypothesized to cause constant cardiovascular overload and cardiac-autonomic dysregulation (reduced HRV) [144, 231]. This proposed phenomenon was supported by a cross-sectional study in Danish blue-collar workers (n = 514, 40% female) that reported an interaction effect where leisure vs. occupational PA was more favorably associated with nocturnal HRV [144]. Our findings are consistent in that

leisure MVPA was more favorably associated with HRV as compared to occupational MVPA, by both magnitude of effect and statistical significance. These similar findings were observed despite study design differences including population (mixed gender vs. all female), differing instruments used to estimate occupational MVPA (i.e., accelerometry vs. questionnaire), and nocturnal vs. waking HRV assessment. Further research addressing this phenomenon is warranted.

Strong evidence also suggests that excessive ST may increase the risk of CVD incidence and mortality, independent of physical inactivity [220]. Cardiac-autonomic dysregulation is hypothesized to partially explain this deleterious relationship [201]. Yet, the current limited literature displays ambiguous findings, with studies reporting no associations [5, 161, 162, 168] or correlations between higher total, leisure, or occupational ST with worse HRV [5, 165, 168]. When adjusting for MVPA, one small study including only young men found a negative association between total ST and HRV [165]. Two other studies including mixed sex samples reported no independent relationship between total ST and HRV [161, 162], similar to our findings. The source of the differing results is not entirely clear, and could be a result of age, sex, or assessment methodology. Future research that considers these differences is warranted.

Though both leisure and occupational ST were hypothesized to associate with unfavorable HRV, these associations were expected to be stronger for leisure ST. This is because adults tend to spend greater time in prolonged bouts (≥30 minutes) ST during leisure, which could have exaggerated the deleterious impacts on health outcomes [232-234]. Only two previous studies have evaluated the associations of leisure and occupational ST with HRV, both of which were in blue-collar workers. The first study reported no correlations [5], while the other observed a negative relationship between occupational ST and nocturnal HRV [168]. Herein, our stratified analysis detected an inverse association between leisure ST (but not occupational ST) with diurnal HRV

only among women who did not meet the leisure MVPA guidelines. These disparities may be explained by MVPA status (i.e., active vs. inactive), timing of HRV measurement (i.e., nocturnal vs. diurnal), and type of occupation (i.e., only blue-collar vs. blue- and white-collar). Regardless, our results are in agreement with a recent harmonized meta-analysis that revealed the strongest dose-response effect of leisure ST on CVD mortality was in adults who did not meet leisure MVPA guidelines [220]. Importantly, meeting leisure MVPA recommendations may eliminate the deleterious impacts of leisure ST on HRV in these women.

Several direct and indirect physiological mechanisms have been proposed to explain how MVPA and ST influence HRV. The consensus is that MVPA improves HRV primarily through increasing cardiac vagal activity [151]. This vagal enhancement may be attributed to increased nitric oxide bioavailability and/or suppressed angiotensin II, both of which can exert direct effects on the vagal nerve [151]. Furthermore, MVPA may indirectly improve HRV by boosting oxytocin concentration [235], which reacts at the cardiovascular centers in the brainstem, causing vagal enhancement [236]. Our findings are most consistent with the proposed mechanisms where higher MVPA was associated with higher resting vagal activity (i.e., higher lnRMSSD). On the other hand, ST may directly affect HRV by chronic reductions in shear stress and, eventually, decreased nitric oxide bioavailability [209], leading to attenuated vagal activity. This hypothesized mechanism was partially supported by our findings where higher leisure ST related to lower resting vagal activity (i.e., lower lnRMSSD) in the absence of sufficient MVPA. Yet, more research into potential mechanisms is needed to advance our understanding of how MVPA and ST may affect HRV.

A strength of our current study is that we separately evaluated the associations of total and domain-specific MVPA and ST with HRV specifically in young to middle-aged women. Previous

studies included either mixed sex samples [5, 144, 161, 162, 168] or young men only [165] and examined association of only total [161, 162, 165] or domain-specific [5, 144, 168] physical activity. Thus, our study provides more comprehensive data of the relationships between MVPA and ST with HRV. Furthermore, our unique sample was a cohort of multiracial women, which enhances the generalizability of our results. We also utilized the gold standard measure of HRV (i.e., ECG) and carefully implemented the robust guidelines to process the ECG data.

Yet, several limitations should be considered when interpreting our findings. Our study was observational and cross-sectional, making it susceptible to biases such as residual confounding and reverse causality. Studies with longitudinal designs that establish temporality or use experimental manipulation of ST are needed. In addition, the reported estimates may only be applied to relatively healthy women. Furthermore, although the MAQ is valid and provides useful estimates of total and domain-specific activity [121], it is susceptible to measurement error and self-report bias that might influence our estimates. Therefore, future studies should consider using gold standard measures of MVPA (i.e., accelerometers) and ST (i.e., thigh-worn monitors) in conjunction with self-report instruments. Though the respiration rate was estimated from ECG using the amplitude of R waves [226], this feature has not yet been validated in Kubios software. Thus, respiration rate could have significantly influenced our estimates [24] and should also be considered in future research. Lastly, HRV only reflects overall and cardiac-parasympathetic activity when resting. As such, the association of ST with cardiac-sympathetic overactivation, a mechanism that has been specifically hypothesized as the primary autonomic pathway linking ST with CVD [201], remains to be evaluated.

## 4.6 Conclusion

Altogether, our results suggest that leisure MVPA and, among inactive women, leisure ST may be determinants of cardiac-autonomic regulation. Importantly, these findings contribute mechanistic insight into the pathway between low MVPA, high ST, and CVD risk development among women. These data are especially important as MVPA and ST are major modifiable risk factors for CVD. Further, among women with significant barriers to achieving MVPA, lower ST is potentially an additional strategy to improve HRV and mitigate CVD risk in women. Future longitudinal studies and experimental trials with manipulation of ST are needed to confirm associations with cardiac-sympathetic activity.

## 4.7 Supplemental Tables and Figures

### Supplemental Table 4-1 Characteristics of Included vs. Excluded Women from the Analyses.

| **Total N = 678** | **Included Participants (N = 522) Mean (SD), Median ($25^{th}$– $75^{th}$), or N (%)** | **Excluded Participants (N = 156) Mean (SD), Median ($25^{th}$– $75^{th}$), or N (%)** | **p-value** |
|---|---|---|---|
| **Age (years)** | 37.73 (5.7) | 37.59 (5.8) | 0.792 |
| **Race** | | | |
| White | 308 (59.0) | 82 (52.6) | 0.277 |
| African American | 184 (35.2) | 66 (42.3) | |
| Others | 30 (4.7) | 8 (5.1) | |
| **Education** | | | |
| High School or Less | 128 (24.5) | 51 (32.7) | 0.125 |
| Some College | 244 (46.7) | 64 (41.0) | |
| College Degree | 150 (28.7) | 41 (26.3) | |
| **Insurance** | | | |
| Private | 286 (54.8) | 85 (54.5) | 0.148 |
| Medicaid | 189 (36.2) | 64 (41.0) | |
| None | 47 (9.0) | 7 (4.5) | |
| **Current Smoking** | | | |
| No | 373 (71.5) | 115 (73.7) | |
| Yes | 149 (28.5) | 41 (26.3) | 0.581 |
| **Waist-to-Hip Ratio** | 0.81 (0.1) | 0.81 (0.1) | 0.919 |
| **Systolic Blood Pressure (mmHg)** | **113.97 (13.7)** | **118.41 (15.0)** | **<0.001** |
| **Diastolic Blood Pressure (mmHg)** | **75.41 (11.1)** | **78.19 (10.7)** | **<0.001** |
| **Hypertension** | | | |
| No | **424 (81.2)** | **115 (73.7)** | **0.042** |
| Yes | **98 (18.8)** | **41 (26.3)** | |
| Using Medication | **62 (63.3)** | **33 (80.5)** | **0.019** |
| Not Using Medication | **36 (36.7)** | **8 (19.5)** | |
| **Diabetes** | | | |
| No | 497 (95.2) | 143 (91.7) | 0.091 |
| Yes | 25 (4.8) | 13 (8.3) | |
| Using Medication | **13 (52.0)** | **11 (84.6)** | **0.007** |
| Not Using Medication | **12 (48.0)** | **2 (15.4)** | |
| **Total ST (hours/day)§** | 7.00 (4.5 – 9.5) | 7.00 (5.0 – 10.0) | 0.705 |
| Leisure ST (hours/day)§ | 3.00 (2.0 – 5.0) | 4.00 (2.0 – 5.0) | 0.263 |
| Occupational ST (hours/day)§ | 3.00 (1.0 – 5.0) | 3.00 (1.5 – 4.3) | 0.747 |
| **Total MVPA (hours/day)§** | 0.82 (0.2 – 3.5) | 0.55 (0.2 – 3.2) | 0.092 |
| Leisure MVPA (hours/day) | 0.34 (0.1 – 0.7) | 0.33 (0.1 – 0.6) | 0.183 |
| Occupational MVPA (hours/day)§ | 0.00 (0.0 – 2.6) | 0.00 (0.0 – 2.4) | 0.112 |

§: non-normally distributed; mm/Hg: millimeters of mercury; ms: millisecond; MVPA: moderate-to-vigorous physical activity; ST: sedentary time. Bold indicates significant difference (p<0.05).

## Supplemental Table 4-2 Associations between Total ST and MVPA with Heart Rate and HRV.

| Variables | Model | Total ST | | Total MVPA | |
|---|---|---|---|---|---|
| | | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** |
| **HR (beat/minute)** | 1 | 0.105±0.154 (0.495) | 0.01 | -0.371±0.239 (0.122) | 0.04 |
| | +(cv) | N/A | | | |
| | 2 | 0.033±0.146 (0.823) | 0.00 | -0.447±0.249 (0.073) | 0.04 |
| | 3 | 0.059±0.151 (0.696) | 0.01 | -0.425±0.254 (0.094) | 0.04 |
| | 4 | 0.066±0.152 (0.667) | 0.01 | -0.347±0.255 (0.173) | 0.03 |
| **lnSDNN** | 1 | -0.012±0.009 (0.166) | 0.02 | **0.027±0.011 (0.014)** | 0.05 |
| | +(cv) | -0.010±0.008 (0.166) | 0.02 | **0.022±0.009 (0.016)** | 0.04 |
| | 2 | -0.009±0.008 (0.282) | 0.02 | **0.029±0.011 (0.008)** | 0.06 |
| | 3 | -0.008±0.008 (0.325) | 0.02 | **0.030±0.011 (0.006)** | 0.06 |
| | 4 | -0.010±0.009 (0.255) | 0.02 | **0.027±0.011 (0.014)** | 0.05 |
| **lnRMSSD** | 1 | -0.010±0.010 (0.292) | 0.02 | **0.032±0.014 (0.024)** | 0.05 |
| | +(cv) | -0.009±0.009 (0.301) | 0.02 | **0.028±0.012 (0.022)** | 0.05 |
| | 2 | -0.007±0.009 (0.456) | 0.01 | **0.036±0.015 (0.015)** | 0.06 |
| | 3 | -0.007±0.010 (0.481) | 0.01 | **0.037±0.015 (0.013)** | 0.06 |
| | 4 | -0.009±0.010 (0.368) | 0.02 | **0.033±0.015 (0.029)** | 0.06 |

cv: coefficient of variation; *d*: Cohen's *d*; HR: heart rate; ln: natural log-transformed; ms: milliseconds; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time. Bold indicates significant associations ($p<0.05$).

Model 1 adjusted for total ST and total MVPA, age, race, education, working status, insurance, and smoking.

Model +(cv) used adjusted HRV indices and adjusted for total ST and total MVPA, age, race, education, working status, insurance, and smoking.

Model 2 adjusted for total ST and total MVPA, age, race, education, working status, insurance, smoking, hypertension, hypertensive medications, diabetes, diabetic medications, and waist-to-hip ratio.

Model 3 adjusted for total ST and total MVPA, age, race, education, working status, insurance, smoking, hypertension, diabetes, smoking, hypertension, hypertensive medications, diabetes, diabetic medications, and waist-to-hip ratio and participants with diseases (n = 17) were excluded.

Model 4 further excluded participants who did not meet breathing frequency (n = 18) were excluded.

An adjusted HRV index = 100 ∗ HRV index/inter-beat interval; All these adjusted HRV parameters were natural log transformed.

**Supplemental Table 4-3 Associations between Leisure and Occupational ST and MVPA with Heart Rate and HRV.**

| Variables | Model | Leisure ST | | Occupational ST | | Leisure MVPA | | Occupational MVPA | |
|---|---|---|---|---|---|---|---|---|---|
| | | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** |
| **HR (beat/ minute)** | 1 | 0.458±0.239 (0.056) | 0.05 | -0.130±0.238 (0.587) | 0.01 | -0.796±0.600 (0.185) | 0.08 | -0.350±0.306 (0.253) | 0.03 |
| | [+](cv) | N/A | | | | | | | |
| | 2 | 0.339±0.221 (0.127) | 0.03 | -0.176±0.234 (0.454) | 0.02 | -1.228±0.631 (0.052) | 0.12 | -0.335±0.306 (0.273) | 0.03 |
| | 3 | 0.384±0.226 (0.091) | 0.04 | -0.169±0.238 (0.477) | 0.02 | -1.172±0.652 (0.073) | 0.12 | -0.333±0.313 (0.288) | 0.03 |
| | 4 | 0.361±0.228 (0.114) | 0.04 | -0.138±0.240 (0.567) | 0.01 | **-1.340±0.652 (0.040)** | 0.13 | -0.179±0.313 (0.566) | 0.2 |
| **lnSDNN** | 1 | -0.024±0.014 (0.091) | 0.05 | 0.001±0.010 (0.936) | 0.00 | **0.074±0.028 (0.009)** | 0.15 | 0.019±0.013 (0.139) | 0.04 |
| | [+](cv) | -0.019±0.013 (0.138) | 0.04 | -0.002±0.009 (0.836) | 0.00 | **0.062±0.024 (0.010)** | 0.12 | 0.015±0.011 (0.164) | 0.03 |
| | 2 | -0.015±0.013 (0.238) | 0.03 | -0.001±0.010 (0.955) | 0.00 | **0.099±0.024 (<0.001)** | 0.20 | 0.016±0.013 (0.255) | 0.03 |
| | 3 | -0.016±0.014 (0.234) | 0.03 | 0.001±0.010 (0.996) | 0.00 | **0.096±0.025 (<0.001)** | 0.19 | 0.018±0.013 (0.162) | 0.04 |
| | 4 | -0.017±0.014 (0.220) | 0.03 | -0.002±0.010 (0.881) | 0.00 | **0.100±0.025 (<0.001)** | 0.20 | 0.013±0.013 (0.323) | 0.03 |
| **lnRMSSD** | 1 | -0.030±0.017 (0.073) | 0.05 | 0.005±0.013 (0.719) | 0.01 | **0.075±0.037 (0.043)** | 0.13 | 0.028±0.018 (0.120) | 0.05 |
| | [+](cv) | -0.024±0.014 (0.094) | 0.04 | 0.004±0.011 (0.740) | 0.01 | **0.063±0.031 (0.041)** | 0.11 | 0.024±0.015 (0.117) | 0.04 |
| | 2 | -0.021±0.015 (0.174) | 0.04 | 0.005±0.013 (0.710) | 0.01 | **0.103±0.035 (0.003)** | 0.17 | 0.024±0.018 (0.169) | 0.04 |
| | 3 | -0.022±0.016 (0.178) | 0.04 | 0.005±0.013 (0.720) | 0.01 | **0.101±0.036 (0.005)** | 0.17 | 0.027±0.018 (0.139) | 0.05 |
| | 4 | -0.023±0.016 (0.158) | 0.04 | 0.002±0.013 (0.855) | 0.00 | **0.106±0.036 (0.004)** | 0.18 | 0.020±0.018 (0.276) | 0.03 |

cv: coefficient of variation; *d*: Cohen's *d*; HR: heart rate; ln: natural log-transformed; ms: milliseconds; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time. Bold indicates significant associations ($p<0.05$).
Model 1 adjusted for leisure and occupational ST and MVPA, age, race, education, working status, insurance, and smoking.
Model [+](cv) used adjusted HRV indices and adjusted for leisure and occupational ST and MVPA, age, race, education, working status, insurance, and smoking.
Model 2 adjusted for leisure and occupational ST and MVPA, age, race, education, working status, insurance, smoking, hypertension, hypertensive medications, diabetes, diabetic medications, and waist-to-hip ratio.
Model 3 adjusted for leisure and occupational ST and MVPA, age, race, education, working status, insurance, smoking, hypertension, diabetes, hypertensive medication, diabetic medication, and waist-to-hip ratio and participants with diseases (n = 17) were excluded.
Model 4 further excluded who did not meet breathing frequency (n = 18) were excluded.
An adjusted HRV index = 100 ∗ HRV index/inter-beat interval; All these adjusted HRV parameters were natural log transformed

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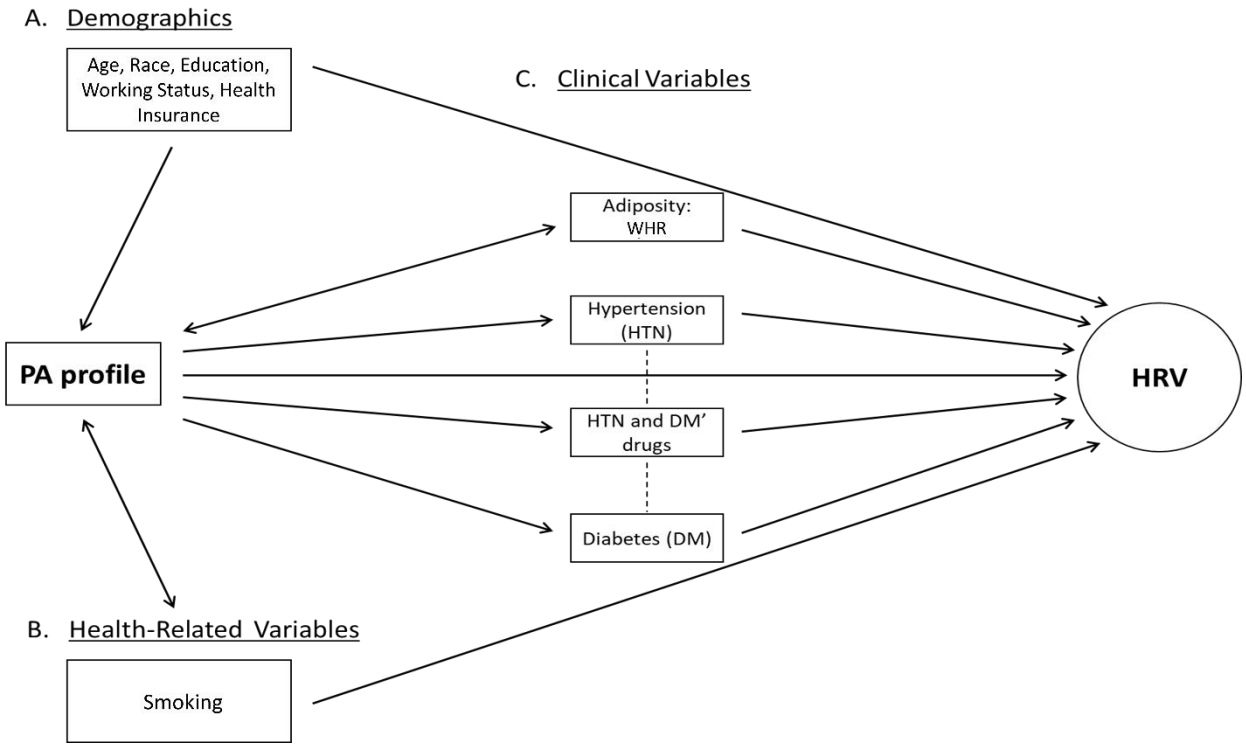



**Supplemental Figure 4-1 Directed Acyclic Graph (DAG) for Potential Confounders and Mediators between Physical Activity (PA) Profile (i.e., Sedentary Behavior and Physical Activity) and Heart Rate Variability (HRV).**

In addition to the previous sensitivity analyses, further analyses were performed, but not reported in the submitted manuscript, to examine 1) the role of intensity in the relationship between PA with HR and HRV, and 2) compare the associations of ST and PA with HR and HRV among currently working vs. currently not working women, women with preterm vs. term pregnancy, and African American vs. non-African American women.

Supplemental Table 4-4 displays the associations between total ST, MPA, and VPA with HR and HRV, adjusting for confounders. No associations between total ST or VPA with HR, lnSDNN, or lnRMSSD (each p>0.05; *d* range: 0.01 – 0.02) were observed. However, small, favorable, and statistically significant relationships between MPA with lnSDNN (p=0.011 and 0.022, respectively; *d*=0.06 for each) and lnRMSSD (p=0.024 and 0.015; *d*=0.05 and 0.06, respectively), but not with HR (p>0.05; *d*=0.04) were detected. Adjusting for mediators and excluding women with potential underlying medical conditions that could have affected HRV and women who did not meet ECG-derived respiration rate yielded similar results.

**Supplemental Table 4-4 Associations between Total ST and Moderate and Vigorous PA with Heart Rate and HRV.**

| Variables | Total ST | | Moderate PA | | Vigorous PA | |
|---|---|---|---|---|---|---|
| | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** | **B±SE (p-value)** | ***d*** |
| **HR (beats/ minute)** | 0.105±0.15 (0.497) | 0.01 | -0.390±0.26 (0.139) | 0.04 | -0.248±0.55 (0.649) | 0.02 |
| **lnSDNN** | -0.012±0.01 (0.169) | 0.02 | **0.030±0.01 (0.011)** | 0.06 | 0.007±0.02 (0.760) | 0.01 |
| **lnRMSSD** | -0.010±0.01 (0.299) | 0.02 | **0.035±0.02 (0.022)** | 0.06 | 0.014±0.04 (0.697) | 0.02 |

*d*: Cohen's *d*; HR: heart rate; ln: natural log-transformed; PA: physical activity; N/A: not applicable; RMSDD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time. Bold indicates significant associations (p<0.05).
All models adjusted for total ST and moderate and vigorous PA, age, race, education, working status, insurance, and smoking.

Supplemental Table 4-5 presents the associations of domain-specific MPA and VPA with HR and HRV. When adjusting for confounders, only leisure MPA was associated with statistically significant favorable lnSDNN (p=0.013; *d*=0.18). When further adjusting for mediators, only leisure MPA and VPA were associated with statistically significant favorable lnSDNN (p=0.007 and 0.026; *d*=0.18 and 0.26, respectively); leisure VPA was also associated with statistically significant favorable lnRMSSD (p=0.019; *d*=0.35). No statistically significant associations with HR were observed (p>0.05; *d* range: 0.06 – 0.28). Neither occupational MPA nor VPA were associated with HR or HRV (p>0.05; *d* range: 0.00 – 0.06). Repeated analyses after excluding women with potential underlying medical conditions that could have affected HRV and women who did not meet ECG-derived respiration rate yielded similar results.

**Supplemental Table 4-5 Associations between Leisure and Occupational ST and Moderate and Vigorous Leisure and Occupational PA with Heart Rate and HRV.**

| Variables | Model | Moderate leisure PA | | Vigorous leisure PA | | Moderate occupational PA | | Vigorous occupational PA | |
|---|---|---|---|---|---|---|---|---|---|
| | | B±SE (p-value) | *d* | B±SE (p-value) | *d* | B±SE (p-value) | *d* | B±SE (p-value) | *d* |
| **HR (beats/ minute)** | 1 | -0.637±0.96 (0.508) | 0.06 | -1.286±1.80 (0.475) | 0.13 | -0.388±0.33 (0.233) | 0.04 | -0.050±0.58 (0.932) | 0.01 |
| | 2 | -0.728±0.94 (0.439) | 0.07 | -2.783±1.61 (0.084) | 0.28 | -0.380±0.32 (0.242) | 0.04 | -0.018±0.56 (0.975) | 0.00 |
| **lnSDNN** | 1 | **0.088±0.03 (0.013)** | 0.18 | 0.037±0.07 (0.613) | 0.04 | 0.022±0.01 (0.101) | 0.04 | -0.010±0.03 (0.714) | 0.02 |
| | 2 | **0.089±0.03 (0.007)** | 0.18 | **0.132±0.06 (0.026)** | 0.26 | 0.019±0.01 (0.152) | 0.04 | -0.016±0.02 (0.516) | 0.03 |
| **lnRMSSD** | 1 | 0.064±0.05 (0.206) | 0.11 | 0.110±0.10 (0.291) | 0.18 | 0.034±0.02 (0.088) | 0.06 | -0.006±0.04 (0.878) | 0.01 |
| | 2 | 0.069±0.05 (0.151) | 0.12 | **0.212±0.09 (0.019)** | 0.35 | 0.029±0.02 (0.116) | 0.05 | -0.012±0.04 (0.740) | 0.02 |

*d*: Cohen's *d;* HR: heart rate; ln: natural log-transformed; PA: physical activity; N/A: not applicable; RMSDD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time. Bold indicates significant associations (p<0.05).
Model 1 adjusted for leisure and occupational ST, and moderate and vigorous leisure and occupational PA, age, race, education, working status, insurance, and smoking.

Model 2 adjusted for leisure and occupational ST and moderate and vigorous leisure and occupational PA, age, race, education, working status, insurance, smoking, hypertension, hypertensive medications, diabetes, diabetic medications, and waist-to-hip ratio

Supplemental Table 4-6 presents the comparisons between currently working (n=383) vs. currently not working (n=139) women. Compared to not currently working women, currently working women tended to have more favorable demographic characteristics, health profiles, and lifestyle behaviors.

## Supplemental Table 4-6 Characteristics of Women who Were Currently Working vs. not Working.

| **Total N = 522** | **Currently Working Women (N = 383) Mean (SD), Median ($25^{th}$– $75^{th}$), or N (%)** | **Currently Not Working Women (N = 139) Mean (SD), Median ($25^{th}$– $75^{th}$), or N (%)** | **p-value** |
|---|---|---|---|
| **Age (years)** | 37.95 (5.57) | 37.12 (6.12) | 0.140 |
| **Race** | | | |
| White | **239 (62.4)** | **69 (49.6)** | **0.030** |
| African American | **123 (32.1)** | **61 (43.9)** | |
| Other | **21 (5.5)** | **9 (6.5)** | |
| **Education** | | | |
| High School or Less | **74 (19.3)** | **54 (38.8)** | **<0.001** |
| Associate Degree | **180 (47.0)** | **64 (46.0)** | |
| College Degree | **129 (33.7)** | **21 (15.1)** | |
| **Insurance** | | | |
| Private | **107 (27.9)** | **82 (59.0)** | **<0.001** |
| Medicaid | **246 (64.2)** | **40 (28.8)** | |
| None | **30 (7.8)** | **17 (12.2)** | |
| **Current Smoking** | | | |
| No | **289 (75.5)** | **84 (60.4)** | **0.001** |
| Yes | **94 (24.5)** | **55 (39.6)** | |
| **Waist-to-Hip Ratio** | **0.80 (0.1)** | **0.82 (0.1)** | **0.001** |
| **Systolic Blood Pressure (mmHg)** | **113.16 (13.8)** | **116.17 (14.1)** | **0.027** |
| **Diastolic Blood Pressure (mmHg)** | **74.58 (11.1)** | **77.68 (10.7)** | **0.004** |
| **Hypertension** | | | |
| No | 318 (83.0) | 106 (76.3) | 0.080 |
| Yes | 65 (17.0) | 33 (23.7) | |
| Using Medication | 41 (63.1) | 21 (63.6) | 0.169 |
| Not Using Medication | 24 (36.9) | 12 (36.4) | |
| **Diabetes** | | | |
| No | 366 (95.6) | 131 (94.2) | 0.533 |
| Yes | 17 (4.4) | 8 (5.8) | |
| Using Medication | 7 (41.2) | 6 (75.0) | 0.107 |
| Not Using Medication | 10 (58.8) | 2 (25.0) | |
| **HR (beats/minute)** | **75.72 (9.4)** | **79.60 (11.5)** | **<0.001** |
| **lnSDNN** | **3.60 (0.4)** | **3.44 (0.5)** | **<0.001** |
| **lnRMSSD** | **3.43 (0.6)** | **3.24 (0.7)** | **0.002** |
| **Total ST (hours/day)§** | 6.5 (4.3 – 9.0) | 7.0 (5.0 – 10.5) | 0.078 |
| Leisure ST (hours/day)§ | **3.0 (2.0 – 4.5)** | **4.0 (2.5 – 7.0)** | **<0.001** |
| Occupational ST (hours/day)§ | 3.0 (1.0 – 6.0) | 3.0 (1.5 – 5.0) | 0.954 |
| **Total PA (hours/day)§** | **1.0 (0.3 – 3.6)** | **0.5 (0.1 – 3.2)** | **0.015** |
| Leisure MVPA (hours/day)§ | 0.4 (0.1 – 0.7) | 0.3 (0.03 – 0.8) | 0.321 |
| Occupational MVPA (hours/day)§ | **0.0 (0.0 – 2.6)** | **0.0 (0.0 – 2.6)** | **0.014** |

§: non-normally distributed; HR: heart rate; ln: natural log-transformed; mm/Hg: millimeters of mercury; MVPA: moderate-to-vigorous physical activity; ST: sedentary time. Bold indicates significant difference ($p < 0.05$). Independent t-test or z-test was performed to compare differences in normally and non-normally distributed continuous variables, respectively, and chi square (chi2) test was performed to compare differences in categorical variables between the currently working vs. not working women.

Supplemental Table 4-7 displays the associations of self-reported leisure and occupational ST and PA with HR and HRV following stratification of women based on current working status, adjusted for confounders. Small, favorable, and statistically significant associations were observed only between leisure MVPA with lnSDNN (p=0.021; *d*=0.25) and lnRMSSD (p=0.024; *d*=0.24) in women who were currently not working; no associations were observed among women who were currently working. Leisure ST and occupational ST and MVPA did not have statistically significant relationships with HR or HRV regardless of current working status.

**Supplemental Table 4-7 Associations between Leisure and Occupational ST and MVPA with Heart Rate and HRV in Currently (n = 383) vs. not Currently Working Women (n = 139).**

| Variables | Employment Status | Leisure ST | | Occupational ST | | Leisure MVPA | | Occupational MVPA | |
|---|---|---|---|---|---|---|---|---|---|
| | | β±SE (p-value) | *d* | β±SE (p-value) | *d* | β±SE (p-value) | *d* | β±SE (p-value) | *d* |
| **HR (beats/ minute)** | Working | 0.419±0.33 (0.203) | 0.04 | 0.021±0.26 (0.933) | 0.00 | -0.660±0.63 (0.293) | 0.06 | -0.128±0.35 (0.714) | 0.01 |
| | Not Working | 0.430±0.40 (0.278) | 0.04 | 0.149±0.61 (0.809) | 0.01 | -0.409±1.46 (0.779) | 0.04 | -1.156±0.62 (0.064) | 0.11 |
| **lnSDNN** | Working | -0.025±0.02 (0.172) | 0.06 | 0.003±0.01 (0.773) | 0.01 | 0.058±0.03 (0.052) | 0.13 | 0.018±0.02 (0.247) | 0.04 |
| | Not Working | -0.003±0.03 (0.906) | 0.01 | -0.039±0.03 (0.201) | 0.09 | **0.114±0.05 (0.021)** | 0.25 | 0.046±0.03 (0.066) | 0.10 |
| **lnRMSSD** | Working | -0.033±0.02 (0.147) | 0.05 | 0.008±0.02 (0.584) | 0.01 | 0.044±0.04 (0.282) | 0.07 | 0.028±0.02 (0.191) | 0.04 |
| | Not Working | -0.003±0.03 (0.914) | 0.01 | -0.042±0.04 (0.259) | 0.06 | **0.154±0.07 (0.024)** | 0.24 | 0.052±0.03 (0.101) | 0.08 |

*d:* Cohen's *d*; HR: heart rate; ln: natural log-transformed; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time. Bold indicates significant associations (p<0.05).
All models adjusted for leisure and occupational ST and MVPA, age, race, education, insurance, and smoking.

Supplemental Table 4-8 presents the comparisons between women who had preterm (n=133) vs. normal term (n=389) pregnancy. No statistically significant differences between preterm and normal term women regarding demographic characteristics, health profiles, and lifestyle behaviors.

## Supplemental Table 4-8 Characteristics of Women who Had Normal Term Birth vs. Preterm Birth.

| **Total N = 522** | **Women who had normal birth (N = 389) Mean (SD), Median ($25^{th}$– $75^{th}$), or N (%)** | **Women who had preterm birth (N = 133) Mean (SD), Median ($25^{th}$– $75^{th}$), or N (%)** | **p-value** |
|---|---|---|---|
| **Age (years)** | 37.58 (5.7) | 38.17 (5.9) | 0.305 |
| **Race** | | | |
| White | 220 (56.6) | 88 (66.2) | 0.144 |
| African American | 146 (37.5) | 38 (28.6) | |
| Other | 23 (5.9) | 7 (5.2) | |
| **Education** | | | |
| High School or Less | 93 (23.9) | 35 (26.3) | 0.628 |
| Associate Degree | 180 (46.3) | 64 (48.1) | |
| College Degree | 116 (29.8) | 34 (25.6) | |
| **Insurance** | | | |
| Private | 140 (36.0) | 49 (36.8) | 0.517 |
| Medicaid | 217 (55.8) | 69 (51.9) | |
| None | 32 (8.2) | 15 (11.3) | |
| **Current Smoking** | | | |
| No | 281 (72.2) | 92 (69.2) | 0.499 |
| Yes | 108 (27.8) | 41 (30.8) | |
| **Waist-to-Hip Ratio** | 0.80 (0.1) | 0.81 (0.1) | 0.442 |
| **Systolic Blood Pressure (mmHg)** | 114.17 (13.9) | 113.38 (13.1) | 0.565 |
| **Diastolic Blood Pressure (mmHg)** | 75.63 (10.9) | 74.76 (11.5) | 0.432 |
| **Hypertension** | | | |
| No | 320 (82.3) | 104 (80.5) | 0.300 |
| Yes | 69 (17.7) | 29 (19.5) | |
| Using Medication | 43 (62.3) | 19 (65.5) | 0.320 |
| Not Using Medication | 26 (37.7) | 10 (34.5) | |
| **Diabetes** | | | |
| No | 374 (96.4) | 123 (92.5) | 0.088 |
| Yes | 15 (3.6) | 10 (7.5) | |
| Using Medication | 8 (53.3) | 5 (75.0) | 0.277 |
| Not Using Medication | 7 (46.7) | 5 (25.0) | |
| **HR (beats/minute)** | 76.74 (10.5) | 76.79 (8.9) | 0.958 |
| **lnSDNN** | 3.57 (0.5) | 3.55 (0.5) | 0.751 |
| **lnRMSSD** | 3.38 (0.6) | 3.38 (0.6) | 0.932 |
| **Total ST (hours/day)§** | 7.0 (4.8 – 10.0) | 6.5 (4.0 – 9.0) | 0.237 |
| Leisure ST (hours/day)§ | 3.0 (2.0 – 5.0) | 3.0 (2.0 – 5.0) | 0.405 |
| Occupational ST (hours/day)§ | 3.0 (1.0 – 5.4) | 3.0 (1.5 – 4.5) | 0.506 |
| **Total MVPA (hours/day)§** | 0.8 (0.2 – 3.3) | 1.1 (0.3 – 4.1) | 0.078 |
| Leisure MVPA (hours/day)§ | 0.3 (0.1 – 0.7) | 0.4 (0.1 – 0.8) | 0.272 |
| Occupational MVPA (hours/day)§ | 0.0 (0.0 – 2.6) | 0.0 (0.0 – 3.3) | 0.112 |

§: non-normally distributed; HR: heart rate; ln: natural log-transformed; mm/Hg: millimeters of mercury; MVPA: moderate-to-vigorous physical activity; ST: sedentary time. Bold indicates significant difference ($p < 0.05$). Independent t-test or z-test was performed to compare differences in normally and non-normally distributed continuous variables, respectively, and chi square ($chi^2$) test was performed to compare differences in categorical variables between women who had normal term birth vs. preterm birth.

Supplemental Table 4-9 displays the associations of self-reported leisure and occupational ST and PA with HR and HRV following stratification of women based on pregnancy term, adjusted for confounders. Small, favorable, and statistically significant associations were observed only between leisure MVPA with lnSDNN (p=0.010; *d*=0.16) and lnRMSSD (p=0.045; *d*=0.14) in women who had normal term pregnancy; no associations were observed among women who preterm pregnancy. Leisure ST and occupational ST and MVPA did not have statistically significant relationships with HR or HRV regardless of pregnancy term.

**Supplemental Table 4-9 Associations between Leisure and Occupational ST and MVPA with Heart Rate and HRV in Women who Had Normal (n = 389) vs. Preterm Birth (n = 133).**

| Variables | Employment Status | Leisure ST | | Occupational ST | | Leisure MVPA | | Occupational VPA | |
|---|---|---|---|---|---|---|---|---|---|
| | | **β±SE (p-value)** | ***d*** | **β±SE (p-value)** | ***d*** | **β±SE (p-value)** | ***d*** | **β±SE (p-value)** | ***d*** |
| **HR (beats/ minute)** | Normal | 0.417±0.268 (0.120) | 0.04 | -0.156±0.256 (0.543) | 0.02 | -0.894±0.674 (0.186) | 0.09 | -0.264±0.342 (0.440) | 0.03 |
| | Preterm | 0.648±0.339 (0.058) | 0.07 | 0.238±0.471 (0.614) | 0.02 | -0.064±0.962 (0.947) | 0.01 | -0.831±0.481 (0.086) | 0.09 |
| **lnSDNN** | Normal | -0.026±0.016 (0.100) | 0.05 | 0.002±0.011 (0.863) | 0.00 | **0.081±0.031 (0.010)** | 0.16 | 0.016±0.014 (0.258) | 0.03 |
| | Preterm | -0.010±0.020 (0.633) | 0.02 | -0.037±0.027 (0.170) | 0.04 | -0.007±0.048 (0.882) | 0.01 | 0.023±0.026 (0.375) | 0.05 |
| **lnRMSSD** | Normal | -0.031±0.018 (0.095) | 0.05 | 0.008±0.014 (0.563) | 0.01 | **0.083±0.041 (0.045)** | 0.14 | 0.023±0.020 (0.254) | 0.04 |
| | Preterm | -0.017±0.024 (0.471) | 0.03 | -0.042±0.038 (0.262) | 0.07 | -0.011±0.059 (0.850) | 0.02 | 0.042±0.034 (0.224) | 0.07 |

*d*: Cohen's *d*: HR; heart rate; ln: natural log-transformed; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time. Bold indicates significant associations (p<0.05).
All models adjusted for leisure and occupational ST and MVPA, age, race, education, working status, insurance, and smoking.

Supplemental Table 4-10 presents the comparisons between African American (n=184) vs. non-African American (n=338) women. Compared to non-African American women, African American women were younger and tended have less favorable demographic characteristics, health profiles, and lifestyle behaviors.

**Supplemental Table 4-10 Characteristics of African American vs. Non-African American Women.**

| **Total N = 522** | **African American Women (N = 184) Mean (SD), Median ($25^{th}$– $75^{th}$), or N (%)** | **Non-African American Women (N = 338) Mean (SD), Median ($25^{th}$– $75^{th}$), or N (%)** | **p-value** |
|---|---|---|---|
| **Age (years)** | **35.43 (5.03)** | **38.98 (5.7)** | **<0.001** |
| **Education** | | | |
| High School or Less | **67 (36.4)** | **61 (18.0)** | **<0.001** |
| Associate Degree | **85 (46.2)** | **159 (47.0)** | |
| College Degree | **32 (17.4)** | **118 (35.0)** | |
| **Insurance** | | | |
| Private | **111 (60.3)** | **78 (23.1)** | **<0.001** |
| Medicaid | **56 (30.4)** | **230 (68.0)** | |
| None | **17 (9.3)** | **30 (8.9)** | |
| **Current Smoking** | | | |
| No | 123 (66.8) | 250 (74.0) | 0.085 |
| Yes | 61 (33.2) | 88 (26.0) | |
| **Waist-to-Hip Ratio** | 0.81 (0.1) | 0.80 (0.1) | 0.436 |
| **Systolic Blood Pressure (mmHg)** | **117.47 (15.3)** | **112.05 (12.4)** | **<0.001** |
| **Diastolic Blood Pressure (mmHg)** | **78.04 (11.3)** | **73.97 (10.7)** | **<0.001** |
| **Hypertension** | | | |
| No | **137 (74.5)** | **287 (84.9)** | **0.003** |
| Yes | **47 (25.5)** | **51 (15.1)** | |
| Using Medication | **33 (70.2)** | **29 (56.9)** | **0.002** |
| Not Using Medication | **14 (29.8)** | **22 (43.1)** | |
| **Diabetes** | | | |
| No | 178 (96.7) | 319 (94.4) | 0.228 |
| Yes | 6 (3.3) | 19 (5.6) | |
| Using Medication | 5 (83.8) | 8 (42.0) | 0.806 |
| Not Using Medication | 1 (6.2) | 11 (58.0) | |
| **HR (beats/minute)** | 77.43 (10.3) | 76.38 (10.05) | 0.260 |
| **lnSDNN** | 3.60 (0.5) | 3.54 (0.5) | 0.215 |
| **lnRMSSD** | **3.52 (0.6)** | **3.30 (0.6)** | **<0.001** |
| **Total ST (hours/day)§** | **7.0 (5.0 – 10.5)** | **6.5 (4.0 – 9.0)** | **0.011** |
| Leisure ST (hours/day)§ | **4.0 (2.5 – 6.0)** | **3.0 (2.0 – 4.0)** | **<0.001** |
| Occupational ST (hours/day)§ | 3.0 (1.5 – 5.5) | 3.0 (1.0 – 5.0) | 0.462 |
| **Total MVPA (hours/day)§** | **0.5 (0.1 – 2.7)** | **1.2 (0.3 – 3.7)** | **<0.001** |
| Leisure MVPA (hours/day)§ | **0.2 (0.03 – 0.5)** | **0.4 (0.2 – 0.8)** | **<0.001** |
| Occupational MVPA (hours/day)§ | **0.0 (0.0 – 2.0)** | **0.4 (0.0 – 3.2)** | **<0.001** |

§: non-normally distributed; HR: heart rate; ln: natural log-transformed; mm/Hg: millimeters of mercury; MVPA: moderate-to-vigorous physical activity; ST: sedentary time. Bold indicates significant difference ($p < 0.05$). Independent t-test or z-test was performed to compare differences in normally and non-normally distributed continuous variables, respectively, and chi square ($chi^2$) test was performed to compare differences in categorical variables between African American vs. non-African American women.

Supplemental Table 4-11 presents the associations of self-reported leisure and occupational ST and PA with HR and HRV following stratification of women based on race, adjusted for confounders. Small, unfavorable, and statistically significant associations were observed between leisure ST with HR (p=0.044; *d*=0.07) only in non-African American. In addition, small, favorable, and statistically significant associations of leisure MVPA with lnSDNN (p=0.028; *d*=0.13) were detected only in non-African American; no associations were observed among African American. Occupational ST and MVPA did not have statistically significant relationships with HR or HRV regardless of race.

**Supplemental Table 4-11 Associations between Leisure and Occupational ST and MVPA with HR and HRV in African American (n = 184) vs. Non-African American (n = 338) Women.**

| Variables | Employment Status | Leisure ST | | Occupational ST | | Leisure MVPA | | Occupational MVPA | |
|---|---|---|---|---|---|---|---|---|---|
| | | β±SE (p-value) | *d* | β±SE (p-value) | *d* | β±SE (p-value) | *d* | β±SE (p-value) | *d* |
| **HR (beats/ minute)** | African American | 0.088±0.23 (0.699) | 0.01 | -0.123±0.37 (0.741) | 0.01 | -1.947±1.56 (0.212) | 0.19 | -0.111±0.36 (0.759) | 0.01 |
| | Non-African American | **0.685±0.34 (0.044)** | 0.07 | -0.117±0.29 (0.688) | 0.01 | -0.573±0.66 (0.389) | 0.06 | -0.440±0.41 (0.280) | 0.04 |
| **lnSDNN** | African American | -0.009±0.02 (0.694) | 0.02 | -0.006±0.02 (0.742) | 0.01 | 0.101±0.06 (0.106) | 0.20 | 0.003±0.02 (0.843) | 0.01 |
| | Non-African American | -0.034±0.018 (0.061) | 0.07 | 0.001±0.01 (0.966) | 0.00 | **0.067±0.03 (0.028)** | 0.13 | 0.024±0.02 (0.166) | 0.05 |
| **lnRMSSD** | African American | -0.010±0.02 (0.668) | 0.02 | -0.004±0.02 (0.865) | 0.01 | 0.124±0.07 (0.109) | 0.21 | -0.006±0.02 (0.760) | 0.01 |
| | Non-African American | -0.041±0.02 (0.060) | 0.07 | 0.008±0.02 (0.599) | 0.01 | 0.062±0.04 (0.124) | 0.10 | 0.040±0.02 (0.094) | 0.07 |

*d*: Cohen's *d*; HR: heart rate; ln: natural log-transformed; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time. Bold indicates significant associations (p<0.05).
All models adjusted for leisure and occupational ST and MVPA, age, race, education, working status, insurance, and smoking.

# 5.0 Manuscript 3: Isotemporal Associations of Device-Measured Sedentary Time and Physical Activity with Cardiac-Autonomic Regulation in Women (Specific Aim III)

## 5.1 Abstract

Excessive sedentary time (ST) and low physical activity (PA) may increase cardiovascular disease (CVD) risk, potentially though cardiac-autonomic dysregulation. **PURPOSE:** To examine effects of statistically exchanging device-measured ST and physical activity on cardiac-autonomic regulation in women. **METHODS:** This cross-sectional, secondary analysis from the Pregnancy Outcomes and Community Health Study follow-up exam included 286 women (age=32.6±5.7 yrs; 68% white). ST and light (LPA), moderate (MPA), vigorous (VPA), and moderate-to-vigorous intensity physical activity (MVPA) were measured by ActiGraph GT3X. ST was further partitioned into long (≥30 minutes) and short (<30 minutes) bouts. MVPA was also partitioned into long (≥10 minutes) and short (<10 minutes) bouts. Cardiac-autonomic regulation was assessed by calculating HRV indices (resting HR, lnSDNN, and lnRMSSD) with Kubios software from a 5-minute seated ECG. Progressive isotemporal substitution models evaluated associations of exchanging ST and PA with HRV indices and adjusted for confounders (demographics, health-related factors), potential mediators (clinical variables), and then conducted sensitivity analysis after removing women with important underlying medical conditions and who did not meet respiration rate criteria. **RESULTS:** In fully adjusted models, exchanging ST, LPA, and MVPA resulted in nonsignificant associations with HRV indices ($p>0.050$). Similar nonsignificant associations were observed when partitioning MPA and VPA ($p>0.05$). However, replacing long-

and short-bout ST with long-bout MVPA resulted in favorable associations with lnRMSSD (B=0.063 and B=0.056, respectively; both $p<0.05$). The sensitivity analyses strengthened these associations and resulted in additional favorable significant associations with lnSDNN and lnRMSSD when replacing short-bout with long-bout MVPA (B=0.074 and B=0.091, respectively). **CONCLUSION:** An activity pattern with less ST and greater long-bout MVPA is a potential behavioral strategy to improve cardiac-autonomic function in women.

## 5.2 Introduction

Adults spend the majority of their waking time in sedentary behavior [237], defined as any activity that occurs in a lying, reclining, or seated posture and has an energy expenditure of ≤ 1.5 metabolic equivalents [64]. Sedentary behavior is emerging as a risk factor, independent of physical inactivity, for many unfavorable health outcomes including cardiovascular disease (CVD) [67, 180, 238]. Recently, international, quantitative and non-quantitative sedentary behavior guidelines have been established in response to this novel risk factor [57, 105, 239, 240]. A common strategy recommended across these guidelines is to generally replace sedentary time (ST) with physical activity (PA), i.e. to 'sit less and move more,' to improve health [57]. Yet, these guidelines often lack specific recommendations about whether intensity and duration of PA is important when replacing ST and whether reducing long bouts of ST is more important than reducing short bouts of ST.

Heart rate variability (HRV), the measurement of variation in time intervals between consecutive heartbeats, is a measure of cardiac autonomic regulation [24]. HRV is a subclinical

marker of CVD that has consistently been associated with CVD events and mortality [18]. Reflecting some preliminary epidemiological and experimental studies finding that higher/increased ST was correlated to lower/reduced HRV [6, 165, 169], lower HRV is proposed as an important linking mechanism between ST and CVD [116]. Notably, the current epidemiological studies that have examined associations between ST and HRV have yielded inconsistent findings (i.e., negative, positive, or no associations) [160-162, 165, 198, 241]. This inconsistency may be explained by important limitations in some of these studies, including not using ECG (the gold standard method for HRV measurement), not accounting for respiration rate which can significantly affect HRV, and not considering the interrelatedness of ST, light (LPA), and moderate-to-vigorous intensity physical activity (MVPA). The latter limitation is particularly crucial because experimental aerobic MVPA intervention studies have consistently found that exercise training improves HRV [4]. Therefore, research that considers these important limitations is needed to clarify associations between ST and HRV.

Emerging studies have demonstrated the importance of simultaneously considering all physical activity behaviors (i.e., MVPA, LPA, ST) to accurately understand the associations between behavior changes and better health. This approach recognizes that an increase in time spent performing one physical behavior must result in a decrease of time spent performing another physical behavior [242]. As such, determining which behavioral *exchanges* are associated with health benefits is important. This issue can be statistically addressed by using isotemporal substitution analysis, a statistical framework that estimates the effect of reallocating time spent in one behavior for an equal amount of time spent in another behavior [243]. Specifically, utilization of this statistical technique can allow for estimation of the hypothetical effects of replacing overall, longer bouts of ST, or shorter bouts of ST with an equal amount of time in various intensities of

physical activity on HRV. This is an important advantage of this statistical model because it could help inform more specific sedentary behavior guidelines to promote cardiovascular health.

Lastly, addressing these research gaps among women is important. Compared to men, women have lower levels of aerobic MVPA and have greater increases in CVD risk development as they progress from young adulthood to middle age [244]. Isotemporal associations between ST, LPA, MVPA, and HRV have not been examined in this population, yet could explain CVD risk development and inform intervention strategies during this critical period. Therefore, the primary aim of this study was to assess the effects of statistically substituting accelerometer-measured ST with LPA and MVPA on HRV in women. We hypothesized that replacing ST with both LPA and MVPA would be associated with higher (i.e., better) HRV. Additional aims evaluated whether associations differed when moderate (MPA) and vigorous (VPA) intensity physical activity were considered separately or when ST and MVPA were separated into shorter and longer bouts.

## 5.3 Materials and Methods

This study was a secondary, cross-sectional analysis of the POUCH Study [225]. Briefly, the POUCH Study enrolled 3019 women during pregnancy to prospectively examine the pathophysiological pathways that lead to preterm delivery. The POUCHmoms Study added follow-up data collection 7 to 15 years after delivery (between 2011-2014) among a subset of these women (n = 1371). The selection strategy for the follow-up study included all women who had a preterm delivery (<37 weeks gestation), all who were at high risk of a preterm delivery, and a

random sample of the remaining women. To be included in POUCHmoms, women could not have been currently pregnant or pregnant within the past six months.

### 5.3.1 Study Population

Of 1371 women who were invited, 678 women participated in the follow-up assessment [225]. To be included in the current analysis, participants additionally had to have valid accelerometry data measuring ST and physical activity along with HRV measurement of sufficient quality. All participants provided written informed consent. This follow-up study was approved by the Institutional Review Boards of the Michigan State University and the University of Pittsburgh.

### 5.3.2 Measurements

#### 5.3.2.1 ST and Physical Activity

The POUCHmoms study used a daytime waist-wear protocol to measure daytime activity behavior. A subset of willing participants (n = 416) received a tri-axial accelerometer (ActiGraph GTX3+, ActiGraph LLC, Pensacola, FL, USA), elastic waist belt, and an activity diary. According to the current guidelines [131], the participants were instructed to wear the monitor around their waist using the elastic belt for 7 days. They were instructed to take the monitor off only for bathing or showering. If the monitor was removed for more than 5 minutes, participants were instructed to record the exact time of removal in the activity diary. ActiLife® software was used to initialize the monitor and to process the collected data. The sampling rate of the monitor was set at 30 Hz;

the collected data were downloaded as 10-second epochs and integrated into 60-second epochs. Non-wear time was defined as consecutive periods of ≥ 60 minutes of zero counts per minute (cpm) [131].

Participants were required to have at least 3 days of 10 hours of waking wear time for the measurement to be considered valid [245]. ST and activity were calculated from accelerometry data using standard methods and cut points: epochs with < 100 cpm were considered "ST" [221]; epochs 101-2690 cpm were considered "LPA"; epochs 2691 - 6166 cpm were considered "MPA"; epochs ≥ 6167 cpm were considered "VPA"; thus, epochs with ≥ 2691 cpm were considered "MVPA" [130]. In addition, reflecting preliminary evidence that prolonged ST could be more harmful for cardiovascular health [111, 171], ST was partitioned into long-bout ST (bouts lasting ≥ 30 minutes) and short-bout ST (< 30 minutes). To evaluate the potential influence of physical activity patterns [246], we also partitioned MVPA into long-bout MVPA (bouts lasting ≥ 10 minutes) and short-bout MVPA (< 10 minutes).

#### 5.3.2.2 HRV

HRV was measured using ECG at the POUCHmoms follow-up visit. Participants were instructed to fast for at least 8 hours prior to the study visit. Upon arriving, several assessments were conducted, including blood sample collection and self-reported questionnaires, and followed by a 45–60-minute snack break. Thereafter, ECG measurements were obtained while participants were seated quietly in a chair with both feet flat on the floor. Two electrodes were placed on the participant's upper chest, and one electrode was placed on the participant's abdomen to record resting ECG signals using the Biopac MP36RWSW system (Goeta, CA). Sampling rate was set at 1000 Hz. Thereafter, 6 minutes of ECG signals were recorded and were later exported as AQC

files (Biopac AcqKnowledge). AQC files were imported into Kubios Premium HRV analysis software (version 3.3.1, MATLAB, The MathWorks, Inc) for processing and deriving HRV indexes.

Established guidelines were followed to calculate HRV from ECG signals [24]. Among participants with at least 5 minutes of data, the automatic correction was employed to detect artifacts. Any files that had > 5 % artifacts were immediately excluded. Thereafter, files that had ≤ 5 % artifacts underwent further visual evaluation for noise, distortion, missing or premature R waves, ectopic beats, arrhythmias, or irregular rhythms; abnormal samples were corrected if possible according to the guidelines [24] or otherwise excluded. To account for the potential effects of respiratory maneuvers, changes in or extremes of respiratory rate on HRV, Kubios Premium software estimated the respiration rate from ECG using the amplitude of R waves (ECG-derived respiration rate) [226] to use in sensitivity analyses (described below). We selected HRV indices that have a well-understood physiological and statistical basis and predict CVD outcomes. As such, HR, SDNN (representing the overall variability), and RMSSD (representing cardiac parasympathetic activity) were selected as outcomes of interest.

#### 5.3.2.3 Covariates

The POUCHmoms follow-up visit linked prospectively collected pregnancy data from the POUCH study and measured confounders and mediators of our hypothesized associations. Demographic, lifestyle, and health-related factors including age, race (i.e., non-Hispanic white, African American, or other), education (i.e., high school or less, some college, or college degree), type of health insurance (i.e., private, Medicaid, or none), and current smoking status (i.e., yes or no) were self-reported. In addition, waist and hip circumferences were measured in triplicate with

a Gulick tape measure. The average of hip and waist measurements was used to calculate WHR. Following five minutes of seated rest, SBP and DBP were measured three times using an Omron HEM-907 (Omron Healthcare, Inc.; Lake Forest, IL) with an appropriately sized cuff. The average of the second and third measurements was calculated as the resting blood pressure [227]. Women with SBP ≥ 140 mmHg or DBP ≥ 90 mmHg or who reported using anti-hypertensive medications were classified as hypertensive (HTN). Finally, the presence of DM and/or glucose-lowering medications were self-reported.

### 5.3.3 Analytical Method

Participant characteristics were summarized descriptively as means with standard deviations, medians with $25^{th}$ and $75^{th}$ percentiles, or numbers and percentages, as appropriate. Characteristics of included versus excluded women were compared using independent t-tests for continuous variables and $\chi^2$ test for categorical variables. Outcome variables that were not normally distributed (i.e., SDNN and RMSSD) were natural log transformed. Confounders and/or mediators were defined *a priori* by constructing DAG (Supplemental Figure 5-1). Pearson's correlations between R-R intervals with HR, lnSDNN and lnRMSSD were also checked [228].

To address our aims, isotemporal substitution models were constructed to examine the associations of statistically exchanging ST and PA (i.e., LPA and MVPA) on HR and HRV, while holding wear time constant [243]. To elaborate, we used accelerometer measured time spent in each activity behaviors (i.e., ST, LPA, MPA, VPA, and MVPA) as well as total activity time (i.e., wear time) for each woman. Regression models were constructed by adding wear time and all activity behaviors except, for one activity behavior at a time that was dropped out due to

collinearity. As such, these models held the total activity time constant and allowed the included activity behaviors to increase at the expense of the dropped activity behavior.

To facilitate the interpretations, we rescaled the time unit of each behavior to 30 minutes/day. Models were specified, for example, as **HR or $HRV_{index}$ = $\beta_0$ + $\beta_1$(LPA) + $\beta_2$(MVPA) + $\beta_3$(wear time) + $\beta_k$(confounders),** where $\beta_1$ represented the effects of replacing 30 minutes of ST with the same amount of LPA, and $\beta_2$ represented the effects of replacing 30 minutes of ST with the same amount of MVPA, while keeping the total wear time and confounders constant. Similar models replaced $\beta_1$ with ST to additionally estimate the effect of replacing LPA with MVPA, and so forth. We repeated the isotemporal modelling strategy used above in expanded analyses to evaluate i) differential effects of VPA and MPA (i.e., separately considering ST, LPA, MPA, and VPA), and ii) patterns of ST and MVPA accumulation (i.e., by partitioning the overall duration of ST and MVPA into time spent in shorter and longer bouts). Further models added adjustment for mediators (i.e., HTN, DM, antihypertensive medication, glucose-lowering medications, and WHR); as results were similar, only fully adjusted models (i.e., adjusting for confounders + potential mediators) are presented.

In sensitivity analyses, we excluded participants with underlying medical conditions that can affect autonomic function and HRV (e.g., hypoglycemia, PTSD), carpel tunnel syndrome, heart flutters, neuropathy, cardiac problems) and participants whose ECG-derived respiration rate was outside of the normal range (9-24 breaths/minute). Further, because most HRV indices have a positive correlation with heart period (i.e., as heart period increases, HRV indices also increase), some researchers have suggested that HRV should be adjusted for heart period or rate [228]. Therefore, adjusted HRV indices were calculated according to the current recommendations using the CV technique as following: $_{CV}$HRV index = 100 x HRV index / heart period [228]. Then, a

final sensitivity analysis was conducted using the adjusted HRV indices to evaluate the potential influence.

Because women who had preterm delivery or were at higher risk of preterm delivery were oversampled in POUCHmoms, sampling weights were applied to all analyses. Stata version 15.0 (StataCorp, College Station, TX) was used to conduct all statistical analyses. The significance level was set as $\alpha \leq .05$.

## 5.4 Results

A total of 678 women completed the POUCHmoms follow-up assessment visit (Figure 5-1). Of them, 604 women had sufficient ECG records for 5 minutes of HRV analysis. Of these women, 82 participants had invalid HRV records due to the following reasons that prevented HRV calculation: ECG distortion (n = 49), arrhythmia/irregular ECG (n = 20), >5% artifacts (n = 10), excessive noise (n = 2), and file error (n = 1). Thus, 522 women had valid 5 minutes of HRV data. Of these, women who refused to wear the accelerometer or had insufficient wear time were also excluded (n = 236). Overall, 286 women had both valid HRV records and accelerometer data and were included in the current analyses. Compared to included women, excluded women (n = 392) tended to be younger, non-white, less likely to smoke, had higher SBP and DBP, and more frequent use of anti-hypertensive medications, and a higher HTN prevalence (Supplemental Table 5-1).

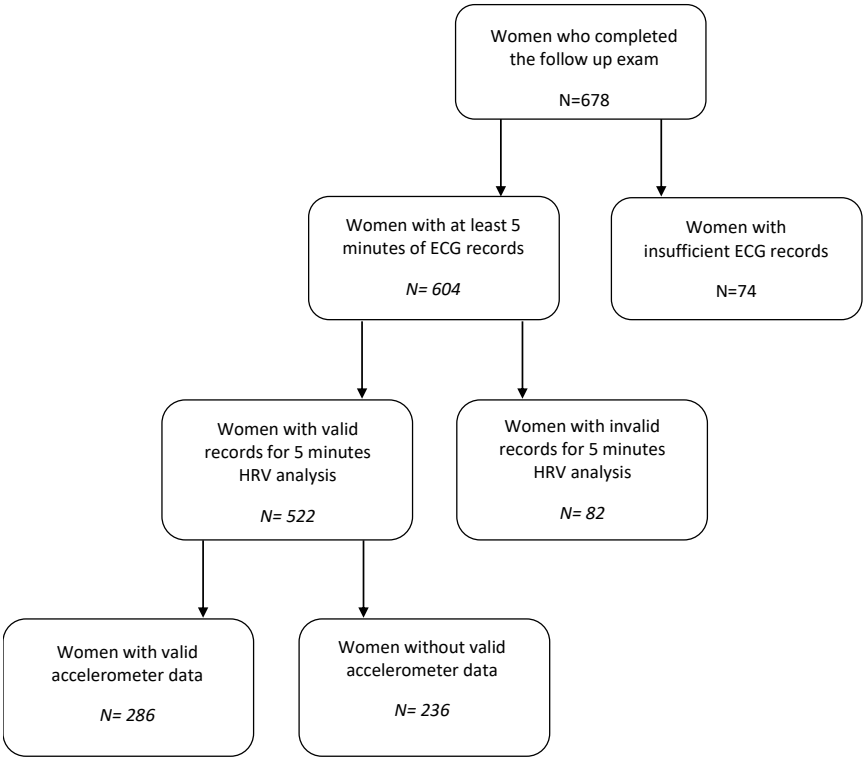



**Figure 5-1 Flowchart of Women who Completed ECG and Accelerometer Measurements.**

Table 5-1 presents characteristics of the sample. The majority were white (67.8%), non-smoking (79.4%), and had private insurance (59.8%). On average, SBP and DBP values were in the normal range, though some participants had HTN (15.4%). Few participants (4.2%) had DM. Median accelerometer wear time was 15.0 hours/day, median LPA was 7.7 hours/day, median MVPA was 0.8 hours/day, and median ST was 6.3 hours/day.

## Table 5-1 Characteristics of Participants (n=286).

| Characteristic | Mean (SD), Median ($25^{th}$– $75^{th}$), or n (%) |
|---|---|
| **Age (years)** | 32.6 (5.7) |
| **Race** | |
| White | 194 (67.8) |
| African American | 77 (26.9) |
| Other | 15 (5.2) |
| **Education** | |
| High School or Less | 53 (18.5) |
| Some College | 132 (46.2) |
| College Degree | 101 (35.3) |
| **Insurance** | |
| Private | 171 (59.8) |
| Medicaid | 87 (30.4) |
| None | 28 (9.8) |
| **Currently Smoking** | |
| No | 227 (79.4) |
| Yes | 59 (20.6) |
| **Waist-to-Hip Ratio** | 0.80 (0.1) |
| **Systolic Blood Pressure (mmHg)** | 112.71 (12.9) |
| **Diastolic Blood Pressure (mmHg)** | 74.52 (10.8) |
| **Hypertension** | |
| No | 242 (84.6) |
| Yes | 44 (15.4) |
| Using Medication | 24 (54.6) |
| Not Using Medication | 20 (45.5) |
| **Diabetes** | |
| No | 274 (95.8) |
| Yes | 12 (4.2) |
| Using Medication | 5 (41.7) |
| Not Using Medication | 7 (58.3) |
| **HR (beats/minute)** | 75.8 (9.4) |
| **lnSDNN** | 3.6 (0.4) |
| **lnRMSSD** | 3.4 (0.6) |
| **Wear Time (min/day)** | 901.8 (847.5 – 950.8) |
| **ST (min/day)** | 376.8 (324.5 – 435.3) |
| **LPA (min/day)** | 459.8 (395.2 – 528.0) |
| **MPA (min/day)** | 40.2 (28.5 – 61.8) |
| **VPA (min/day)** | 4.0 (2.0 – 8.8) |
| **MVPA (min/day)** | 46.2 (32.0 – 69.2) |

ln: natural logarithm; HR: heart rate; mmHg: millimeters of mercury; LPA: light physical activity; MPA: moderate physical activity; MVPA: moderate-to-vigorous physical activity; ms: millisecond; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; ST: sedentary time; VPA: vigorous physical activity.

Though replacing 30 minutes/day of ST with LPA, MPA, VPA, and MVPA yielded associations in a generally favorable direction with heart rate and HRV in fully adjusted models (Table 5-2 and 5-3), none of these associations reached statistical significance ($p>0.05$). Therefore, we failed to support our primary hypothesis. Yet in most cases, the magnitude of these associations appeared to be higher as the intensity of physical activity increased. In addition, exchanging 30 minutes/day of lower intensity physical activity with higher intensity physical activity tended to also have favorable, yet nonsignificant, associations with heart rate and HRV.

**Table 5-2 Isotemporal Associations of Replacing 30 Minutes/Day of ST, LPA, and MVPA with Heart Rate and HRV in Women (n=286).**

| Outcomes | HR | lnSDNN | lnRMSSD |
|---|---|---|---|
| | B±SE (p-value) | B±SE (p-value) | B±SE (p-value) |
| **Replacing ST with LPA** | -0.150±0.225 (0.504) | 0.006±0.012 (0.596) | 0.014±0.009 (0.125) |
| **Replacing ST with MVPA** | -0.309±0.365 (0.398) | 0.018±0.023 (0.418) | 0.007±0.018 (0.676) |
| **Replacing LPA with MVPA** | -0.158±0.418 (0.705) | -0.006±0.021 (0.759) | 0.012±0.026 (0.644) |

Bold indicates significant difference ($p\leq0.05$). B: beta coefficient; HR: heart rate; LPA: light physical activity; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; SE: standard error; ST: sedentary time.

All models adjusted for age, race, education, insurance, smoking, hypertension, diabetes, antihypertensive medication, glucose-lowering medications, and waist-to-hip ratio.

**Table 5-3 Isotemporal Associations of Replacing 30 Minutes/Day of ST, LPA, MPA, and VPA with Heart Rate and HRV in Women (n=286).**

| Outcomes | HR | lnSDNN | lnRMSSD |
|---|---|---|---|
| | **B±SE (p-value)** | **B±SE (p-value)** | **B±SE (p-value)** |
| **Replacing ST with LPA** | -0.167±0.231 (0.471) | 0.018±0.010 (0.075) | 0.009±0.013 (0.475) |
| **Replacing ST with MPA** | -0.168±0.787 (0.831) | -0.031±0.045 (0.491) | -0.007±0.056 (0.902) |
| **Replacing ST with VPA** | -0.624±1.377 (0.651) | 0.094±0.066 (0.153) | 0.075±0.088 (0.391) |
| **Replacing LPA with MPA** | -0.002±0.863 (0.998) | -0.049±0.051 (0.330) | -0.016±0.062 (0.796) |
| **Replacing LPA with VPA** | -0.457±1.343 (0.734) | 0.076±0.061 (0.219) | 0.066±0.084 (0.432) |
| **Replacing MPA with VPA** | -0.455±2.026 (0.822) | 0.125±0.106 (0.239) | 0.082±0.137 (0.549) |

Bold indicates significant difference ($p \leq 0.05$). B: beta coefficient; HR: heart rate; LPA: light physical activity; MPA: moderate physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; SE: standard error; ST: sedentary time; VPA: vigorous physical activity.
All models adjusted for age, race, education, insurance, smoking, hypertension, diabetes, antihypertensive medication, glucose lowering medications, and waist-to-hip ratio.

Similar results were observed when these models excluded women with potential underlying medical conditions that could have affected HRV and women who did not meet ECG-derived respiration rate criteria (Supplemental Table 5-2 and 5-3). Comparable associations were also observed when the adjusted cvHRV indices were utilized (data not shown).

Finally, to examine the role of activity patterns (our secondary objective), we also partitioned MVPA and ST into time accumulated in shorter and longer bouts and repeated the isotemporal substitution models (Table 5-4). Replacing 30 minutes/day of long-bout ST with other behaviors generally resulted in more favorable, but statistically nonsignificant, associations with HR and HRV compared to replacing short bouts ST. Yet, replacing 30 minutes/day of long-bout ST and short-bout ST with long-bout MVPA resulted in statistically significant associations with

greater lnRMSSD (B=0.060; p=0.038 and B=0.055; p=0.039, respectively), supporting our secondary hypothesis.

**Table 5-4 Isotemporal Associations of Replacing 30 Minutes/Day of Long- and Shout-Bout ST, LPA, and Short- and Long-Bout MVPA with Heart Rate and HRV in Women (n=286).**

| Outcomes | HR | LnSDNN | lnRMSSD |
|---|---|---|---|
| | B±SE (p-value) | B±SE (p-value) | B±SE (p-value) |
| **Replacing Long-Bout ST with Short-Bout ST** | -0.102±0.137 (0.456) | 0.006±0.007 (0.369) | 0.006±0.009 (0.523) |
| **Replacing Long-Bout ST with LPA** | -0.264±0.284 (0.352) | 0.021±0.011 (0.070) | 0.013±0.015 (0.382) |
| **Replacing Long-Bout ST with Short-Bout MVPA** | -0.019±0.507 (0.970) | -0.013±0.029 (0.647) | -0.011±0.036 (0.768) |
| **Replacing Long-Bout ST with Long-Bout MVPA** | -0.863±0.529 (0.104) | 0.041±0.022 (0.060) | **0.060±0.029 (0.038)** |
| **Replacing Short-Bout ST with LPA** | -0.162±0.222 (0.465) | 0.015±0.009 (0.095) | 0.007±0.012 (0.522) |
| **Replacing Short-Bout ST with Short-Bout MVPA** | 0.121±0.497 (0.808) | -0.019±0.029 (0.518) | -0.016±0.037 (0.663) |
| **Replacing Short-Bout ST with Long-Bout MVPA** | -0.761±0.475 (0.111) | 0.035±0.020 (0.082) | **0.055±0.026 (0.039)** |
| **Replacing LPA with Short-Bout MVPA** | 0.283±0.571 (0.620) | -0.034±0.032 (0.287) | -0.023±0.039 (0.50) |
| **Replacing LPA with Long-Bout MVPA** | -0.598±0.491 (0.224) | 0.021±0.023 (0.360) | 0.047±0.029 (0.109) |
| **Replacing Short-Bout MVPA with Long-Bout MVPA** | -0.881±0.648 (0.175) | 0.054±0.037 (0.139) | 0.071±0.046 (0.123) |

Bold indicates significant difference (p≤0.05). B: beta coefficient; HR: heart rate; LPA: light physical activity; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; SE: standard error; ST: sedentary time.
All models adjusted for age, race, education, insurance, smoking, hypertension, diabetes, antihypertensive medication, glucose-lowering medications, and waist-to-hip ratio.

Moreover, when we repeated analyses after excluding women with potential underlying medical conditions that could have affected HRV and women who did not meet ECG-derived respiration rate criteria, the statistically significant associations when exchanging long- and short-bout ST with long-bout MVPA persisted (B=0.063; p=0.036 and B=0.056; p=0.040, respectively) (Table 5-5). Further, replacing 30 minutes/day of short-bout MVPA with long-bout MVPA became significantly associated with greater lnSDNN (B=0.074; p=0.047) and lnRMSSD

(B=0.091; p=0.050) and nearly significant with lower resting HR (B=-1.258; p=0.051) in these sensitivity analyses. Comparable associations were also observed when the adjusted cvHRV indices were utilized (data not shown).

**Table 5-5 Isotemporal Associations of Replacing 30 Minutes/Day of Long- and Shout-Bout ST, LPA, and Short- and Long-Bout MVPA with HR and HRV in Women (n=264) with Sensitivity Analyses.**

| Outcomes | HR | LnSDNN | lnRMSSD |
|---|---|---|---|
| | B±SE (p-value) | B±SE (p-value) | B±SE (p-value) |
| **Replacing Long-Bout ST with Short-Bout ST** | -0.139±0.144 (0.335) | 0.008±0.007 (0.274) | 0.007±0.009 (0.431) |
| **Replacing Long-Bout ST with LPA** | -0.291±0.290 (0.317) | 0.022±0.011 (0.057) | 0.014±0.015 (0.356) |
| **Replacing Long-Bout ST with Short-Bout MVPA** | 0.201±0.505 (0.691) | -0.029±0.027 (0.285) | -0.028±0.034 (0.416) |
| **Replacing Long-Bout ST with Long-Bout MVPA** | -1.057±0.540 (0.051) | 0.044±0.023 (0.055) | **0.063±0.030 (0.036)** |
| **Replacing Short-Bout ST with LPA** | -0.152±0.226 (0.502) | 0.014±0.009 (0.112) | 0.007±0.012 (0.570) |
| **Replacing Short-Bout ST with Short-Bout MVPA** | 0.340±0.495 (0.493) | -0.037±0.028 (0.194) | -0.035±0.036 (0.326) |
| **Replacing Short-Bout ST with Long-Bout MVPA** | -0.918±0.479 (0.056) | 0.037±0.021 (0.084) | **0.056±0.027 (0.040)** |
| **Replacing LPA with Short-Bout MVPA** | 0.492±0.571 (0.390) | -0.051±0.031 (0.095) | -0.042±0.038 (0.274) |
| **Replacing LPA with Long-Bout MVPA** | -0.766±0.504 (0.130) | 0.022±0.024 (0.347) | 0.049±0.030 (0.107) |
| **Replacing Short-Bout MVPA with Long-Bout MVPA** | -1.258±0.641 (0.051) | **0.074±0.037 (0.047)** | **0.091±0.046 (0.050)** |

Bold indicates significant difference ($p \leq 0.05$). B: beta coefficient; HR: heart rate; LPA: light physical activity; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; SE: standard error; ST: sedentary time.

All models adjusted for age, race, education, insurance, smoking, hypertension, diabetes, antihypertensive medication, glucose-lowering medications, and waist-to-hip ratio and participants with diseases (n = 12) and who did not meet breathing frequency (n = 10) were excluded.

## 5.5 Discussion

This study examined the effects of statistically reallocating ST and various intensities of physical activity on cardiac-autonomic regulation, including resting HR, lnSDNN, lnRMSSD, in women. Our main findings were that exchanging 30 minutes/day of ST, LPA, MPA, VPA, or MVPA was not significantly associated with HRV indices. However, once partitioned into short and long bouts, statistically significantly beneficial relationships with lnRMSSD were detected when short-bout and long-bout ST were replaced with long-bout MVPA. Moreover, sensitivity analyses excluding women with underlying medical conditions and non-standard respiration rates strengthened these associations and resulted in additional, favorable associations with lnSDNN and lnRMSSD when replacing short- with long-bout MVPA.

Many epidemiological studies, in addition to meta-analyses of experimental interventions, have demonstrated beneficial effects of physical activity, especially MVPA, on various HRV indices in adults [224, 247, 248]. On the other hand, epidemiological studies associating ST with HRV indices are rare, mostly limited to cross-sectional designs, and have reported inconsistent results (i.e., unfavorable, favorable, or no associations) [160-162, 165]. The source of this discrepancy is not entirely clear and could potentially be due to differences in sample size and characteristics, differences in assessment methodology (e.g., utilized ST and HRV measurement approaches), and highly variable study designs and statistical approaches to evaluating associations.

The most comparable studies to ours are those using a compositional and/or isotemporal substitution analysis approach to evaluate associations between ST and PA with HRV. In one example of a cross-sectional study, a compositional analysis of data from Canadian adults (n =

6,322) found that a higher proportion spent in MVPA relative to ST, LPA, or sleep was associated with lower resting HR [198]. Comparably, a similar association with resting HR was detected when 60 minutes/day of ST was reallocated to MVPA but not to LPA measured using a thigh-worn accelerometer among 93 older adults [241]. Furthermore, a recent publication involving middle-aged adults (n = 1,668) from the CARDIA study revealed significant favorable associations with lnRMSSD when one standard deviation of waist-accelerometer-measured ST was replaced with LPA and VPA but not with MPA. In the same study, only replacement of ST with VPA resulted in a significant favorable correlation with lnSDNN [249]. Herein, we found nonsignificant favorable associations with HRV indices when 30 minutes/day of ST was reallocated to LPA, MPA, VPA, or MVPA. Thus, research has consistently associated MVPA with favorable HRV while studies associating ST with HRV have reported mixed and inconclusive results. Future longitudinal and experimental investigations are needed to draw stronger conclusions about the relationship between ST and HRV.

We also considered the intensity of the reallocated physical activity as a potentially important factor for healthier HRV. Indeed, this hypothesis is supported by several randomized controlled trials that reported greater HRV improvements following higher vs. lower intensity of physical activity programs [4, 250, 251]. In harmony with this, the previously mentioned CARDIA study observed higher favorable associations with lnRMSSD and lnSDNN when ST was replaced with VPA compared to MPA or LPA [249]. Although nonsignificant potentially due to our smaller sample size, we found similar results where the associations with HRV indices were of their greatest magnitude when ST was reallocated to VPA. Altogether, these findings may indicate that the potential benefit to HRV when reallocating ST to PA is intensity dependent. Yet, further

research that experimentally replaces ST with different intensities of PA is needed to confirm this hypothesis.

In addition to the total time spent in these behaviors, emerging evidence suggests that different patterns of activity behaviors may differentially influence health outcomes [232, 252]. Noteworthy is that the 2018 PA Guidelines for Americans recommended, for the first time, that 'any bout of MVPA counts' and to 'sit less and move more' to improve health [57]. Yet, the lack of evidence regarding the role of ST bout length and the importance of breaking up prolonged sitting, along with the importance of comparing long- and short-bout PA in future research, were also highlighted in the Guidelines Committee final report as areas in need of future research [72]. Herein, we found significant favorable associations with lnRMSSD when replacing any type of ST (long-bout [≥30 minutes] and short-bout [<30 minutes]) specifically to long-bout (≥10 minutes) MVPA. Furthermore, our sensitivity analyses revealed additional significant favorable associations with both lnSDNN and lnRMSSD when exchanging short- (<10 minutes) for long-bout MVPA. We are aware of only one other study in older adults (n = 93) that examined the role of bouts that found a significant favorable association with resting HR when ST was reallocated to short-bout, but not long-bout, MVPA [241]. Together, these findings suggest that different patterns of activity behaviors may differently affect HRV indices. Further research examining the role of bouts in the associations of MVPA and ST with HRV indices is needed to impart specific MVPA and ST prescriptions.

Several physiological mechanisms have been proposed to explain the influences of ST and MVPA on HRV. MVPA is believed to improve HRV mainly through increasing cardiac vagal activity [151]. This vagal improvement may be ascribed to increased nitric oxide (NO) bioavailability, heightened oxytocin concentration, and/or suppressed angiotensin II, all of which

can exert direct and/or indirect favorable effects on the vagal nerve [151, 235, 236]. MVPA can also enhance blood/plasma volume, which may induce baroreflex-mediated increased vagal activity [205]. However, one hypothesis suggests that these physiological benefits may be reversed by excessive ST. To elaborate, frequent exposure to sedentary behavior is suggested to cause chronic reductions in shear stress and, eventually, decreased NO bioavailability [209]. In addition, sedentary behavior, especially prolonged sitting, may lead to decreased blood/plasma volume [253]. These reduction in NO bioavailability and blood/plasma volume may lead to attenuated vagal activity and, thus, lower HRV. Our significant results are consistent with these proposed mechanisms, where reallocating long- and short-bout ST specifically with long-bout MVPA was associated with higher resting cardiac-vagal activity that is modulated by respiration (i.e., higher lnRMSSD). Yet, further experimental studies examining and confirming these physiological mechanisms are warranted.

Our study has several strengths that are worth highlighting. Our unique sample was a cohort of multiracial women, which improves the generalizability of our findings. We used the gold standard field-base measurement of PA (i.e., accelerometer), allowing us to evaluate associations across various intensities of activity, to compare total, long-bout, and short-bout ST and MVPA, and to account for the interrelation between ST and PA via isotemporal substitution. Lastly, we also used gold standard assessment of HRV by ECG and carefully implemented robust guidelines to process the ECG data.

Still, several limitations should be considered when interpreting our results. Our study was observational and cross-sectional, making it susceptible to biases such as reverse causality and residual confounding. Future studies with longitudinal designs that establish temporality or experimental studies that manipulate ST, LPA, and MVPA are needed. In addition, most of the

women included in this analysis exceeded MVPA guidelines; thus, our results may not apply to less active populations. This relates to the loss of participants due to health-related selection and compliance-related selection; notably, however, our current results were robust to control for participating individuals that did have health-related concerns. Estimated, but not directly measured, respiration rate is another limitation to our study and should be considered in future research [24]. Lastly, HRV only reflects overall and cardiac-parasympathetic activity when resting. As such, the associations of reallocating total and short- and long-bout ST to LPA and MVPA with cardiac-sympathetic activity remain to be evaluated.

## 5.6 Conclusion

Altogether, our results provide limited evidence suggesting that ST unfavorably affects HRV indices in women. Fortunately, replacing ST with long-bout MVPA may counteract these effects and elicit a beneficial influence on HRV indices. Moreover, additional benefit to HRV may be achieved by reallocating short- to long-bout MVPA in healthy women without existing cardiovascular or other conditions that could impact HRV. Our findings may contribute mechanistic insight into the pathway between high ST, low MVPA, and CVD risk development; cardiac-autonomic dysregulation may be a potential linking mechanism between ST, MVPA, and CVD in women. Lastly, our study also provides insight into specific MVPA and ST prescriptions. In addition to the current recommendations, women may reduce ST and replace it with MVPA accumulated in bouts ≥10 minutes in length to achieve better cardiac-autonomic health.

## 5.7 Supplemental Tables and Figures

### Supplemental Table 5-1 Characteristics of Included vs. Excluded Women from the Analyses.

| **Total N = 678** | **Included Participants (N = 286) Mean (SD), Median ($25^{th}$– $75^{th}$), or N (%)** | **Excluded Participants (N = 392) Mean (SD), Median ($25^{th}$– $75^{th}$), or N (%)** | **p-value** |
|---|---|---|---|
| **Age (years)** | **38.61 (5.7)** | **37.03 (5.7)** | **<0.001** |
| **Race** | | | |
| White | **194 (67.8)** | **196 (50.0)** | **<0.001** |
| African American | **77 (26.9)** | **173 (44.1)** | |
| Others | **15 (5.2)** | **23 (5.9)** | |
| **Education** | | | |
| High School or Less | **53 (18.5)** | **126 (32.1)** | **<0.001** |
| Some College | **132 (46.2)** | **176 (44.9)** | |
| College Degree | **101 (35.3)** | **90 (23.0)** | |
| **Insurance** | | | |
| Private | **171 (59.8)** | **200 (51.0)** | **0.005** |
| Medicaid | **87 (30.4)** | **166 (42.4)** | |
| None | **28 (9.8)** | **26 (6.6)** | |
| **Current Smoking** | | | |
| No | **227 (79.4)** | **261 (66.6)** | **<0.001** |
| Yes | **59 (20.6)** | **131 (33.4)** | |
| **Waist-to-Hip Ratio** | 0.80 (0.06) | 0.81 (0.06) | 0.2730 |
| **Systolic Blood Pressure (mmHg)** | **112.71 (12.9)** | **116.65 (14.7)** | **<0.001** |
| **Diastolic Blood Pressure (mmHg)** | **74.51 (10.8)** | **77.16 (11.1)** | **0.002** |
| **Hypertension** | | | |
| No | **242 (84.6)** | **297 (75.8)** | **0.005** |
| Yes | **44 (15.4)** | **95 (24.2)** | |
| Using Medication | **24 (54.5)** | **65 (68.4)** | **0.005** |
| Not Using Medication | **20 (45.5)** | **30 (31.6)** | |
| **Diabetes** | | | |
| No | 274 (95.8) | 366 (93.4) | 0.173 |
| Yes | 12 (4.2) | 26 (6.6) | |
| Using Medication | 5 (41.7) | 19 (73.1) | 0.062 |
| Not Using Medication | 7 (58.3) | 7 (26.9) | |

Bold indicates significant difference ($p \leq 0.05$). mm/Hg: millimeters of mercury.

## Supplemental Table 5-2 Isotemporal Associations of Replacing 30 Minutes/Day of ST, LPA, and MVPA with HR and HRV in Women (n=264).

| Outcomes | HR | lnSDNN | lnRMSSD |
|---|---|---|---|
| | **B±SE (p-value)** | **B±SE (p-value)** | **B±SE (p-value)** |
| **Replacing ST with LPA** | -0.140±0.231 (0.545) | 0.013±0.009 (0.146) | 0.006±0.012 (0.643) |
| **Replacing ST with MVPA** | -0.271±0.368 (0.462) | -0.001±0.018 (0.942) | 0.009±0.022 (0.675) |
| **Replacing LPA with MVPA** | -0.13±0.425 (0.758) | 0.001±0.021 (0.972) | 0.004±0.026 (0.889) |

Bold indicates significant difference (p≤0.05). B: beta coefficient; HR: heart rate; LPA: light physical activity; MVPA: moderate-to-vigorous physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; SE: standard error; ST: sedentary time.

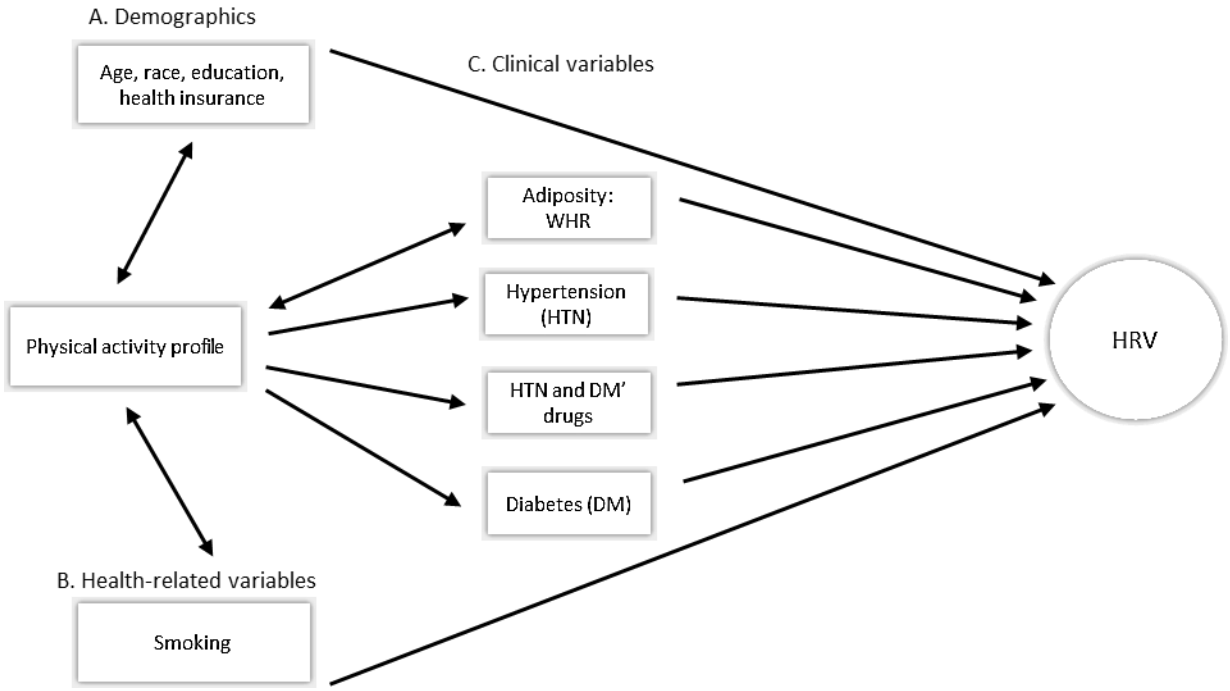
All models adjusted for age, race, education, insurance, smoking, hypertension, diabetes, antihypertensive medication, glucose-lowering medications, and waist-to-hip ratio. Participants with conditions that could impact HRV (e.g., cardiac disease; n = 12) and whose estimated respiration rate were outside of the normal range (i.e., 9-20 breaths/minute; n = 10) were excluded from these analyses.

## Supplemental Table 5-3 Isotemporal Associations of Replacing 30 Minutes/Day of ST, LPA, MPA, and VPA with HR and HRV in Women (n=264).

| Outcomes | HR | lnSDNN | lnRMSSD |
|---|---|---|---|
| | **B±SE (p-value)** | **B±SE (p-value)** | **B±SE (p-value)** |
| **Replacing ST with LPA** | -0.167±0.237 (0.483) | 0.018±0.010 (0.080) | 0.009±0.013 (0.496) |
| **Replacing ST with MPA** | -0.041±0.810 (0.959) | -0.043±0.046 (0.346) | -0.019±0.057 (0.738) |
| **Replacing ST with VPA** | -0.789±1.418 (0.579) | 0.093±0.066 (0.161) | 0.074±0.090 (0.413) |
| **Replacing LPA with MPA** | 0.125±0.888 (0.888) | -0.061±0.051 (0.233) | -0.028±0.064 (0.657) |
| **Replacing LPA with VPA** | -0.622±1.385 (0.654) | 0.074±0.062 (0.231) | 0.065±0.086 (0.455) |
| **Replacing MPA with VPA** | -0.748±2.089 (0.721) | 0.135±0.107 (0.208) | 0.093±0.141 (0.511) |

Bold indicates significant difference (p≤0.05). B: beta coefficient; HR: heart rate; LPA: light physical activity; MPA: moderate physical activity; RMSSD: root mean square of successive differences; SDNN: standard deviation of normal R-R intervals; SE: standard error; ST: sedentary time; VPA: vigorous physical activity.

All models adjusted for age, race, education, insurance, smoking, hypertension, diabetes, antihypertensive medication, glucose-lowering medications, and waist-to-hip ratio. Participants with conditions that could impact HRV (e.g., cardiac disease; n = 12) and whose estimated respiration rate were outside of the normal range (i.e., 9-20 breaths/minute; n = 10) were excluded from these analyses.




**Supplemental Figure 5-1 Directed Acyclic Graph (DAG) for Potential Confounders and Mediators between Physical Activity (PA) Profile (i.e., Sedentary Behavior and Physical Activity) and Heart Rate Variability (HRV).**

## 6.0 Summary of Findings

This dissertation sought to advance the scientific knowledge regarding the associations of sedentary time (ST) with heart rate (HR) and variability (HRV) with three manuscripts. First, we undertook a systematic review and meta-analysis to summarize the literature and identify the current research gaps. Then, we conducted two original analyses in a cohort of women and addressed some, but not all, of these gaps. In all three manuscripts, we hypothesized that higher time spent in different aspects of sedentary behavior would be associated with unfavorable HR and HRV.

In the first manuscript (the systematic review and meta-analysis), we found that the existing observational studies associating ST with HR and HRV are typically of low quality, only have cross-sectional designs, and use a variety of methods that make synthesis of findings challenging. Yet, collectively, these studies suggest an unfavorable, but not clinically meaningful, association between ST and HR in males only. Moreover, the available limited evidence does not suggest a correlation between ST and HRV. Importantly, several crucial limitations in the current literature were identified that limit confidence in these conclusions. A prominent limitation was that most studies did not use gold standard approaches to assess ST and HRV, i.e. a thigh-worn monitor and ECG, respectively. Rather, most studies measured ST by self-report, did not consider domain-specific ST, did not considering the inter-relatedness between ST and moderate-to-vigorous intensity physical activity (MVPA) in analyses, and did not evaluate the roles of patterns, bouts, or intensities of ST and physical activity (PA). Further, the exclusive use of cross-sectional designs limited our ability to establish temporality of ST and MVPA occurring prior to changes in HRV.

These considerable limitations prevent strong inferences about the associations of ST with HR and HRV. Based on these observations, we suggested that more research addressing these limitations was needed to draw a strong and comprehensive conclusion regarding the relationship between ST and overall activity with HRV.

In our second manuscript, we conducted an analysis on young to middle-aged women (N=522) and addressed many, but not all, of the limitations we observed in the systematic review and meta-analysis (i.e., manuscript 1). The gold standard approach of assessing HRV was implemented, the inter-relatedness between ST and MVPA was accounted for using statistical adjustment, and both total and domain-specific ST and MVPA were evaluated. The results of this manuscript suggest no relationships between total and domain-specific ST or MVPA with HR in women. Yet, the results do suggest that leisure MVPA may be related to more favorable HRV, while leisure ST may be associated with unfavorable HRV among women who do not meet the leisure physical activity guidelines. Of importance, the effect sizes observed for significant associations were small. Overall, these findings suggest that leisure-time activity behaviors may have a small effect on cardiac-parasympathetic health and, therefore, cardiac-parasympathetic dysregulation may be a linking mechanism between high leisure ST/low leisure MVPA with cardiovascular disease (CVD) in women.

The third manuscript, which describes the results of analyses on a sample of 286 young to middle-aged women, further addressed some of the limitations observed in the systematic review and meta-analysis. The gold standard approaches to assess HRV and MVPA were used, the inter-relatedness between ST and MVPA was accounted for by including both variables in statistical models, and the roles of intensity (i.e., light [LPA], moderate [MPA], vigorous [VPA] intensity physical activity, and MVPA) of PA and the pattern (i.e., short and long bouts) of ST and PA were

evaluated. Furthermore, this manuscript has uniquely utilized isotemporal substitution analysis as the statistical approach, which allowed us to assess the hypothetical effects of statistically exchanging time spent in various activity behaviors on HR and HRV. The results of this manuscript suggested no cross-sectional effects of exchanging ST, LPA, MPA, VPA, or MVPA on HR or HRV in this sample of women. Yet, when examining the role of the activity patterns, our results suggest that replacing ST with long bouts of MVPA that are accumulated in bouts of 10 minutes or more may lead to beneficial effects on HRV. Importantly, our findings did not suggest that reducing ST by increasing LPA or short-bout MVPA was associated with favorable effects on HRV. In sensitivity analyses, we found that additional HRV benefits may also be achieved by replacing short bouts of MVPA with long bouts of MVPA, specifically in women free of cardiovascular or other HRV-related conditions. These findings suggest that the patterns of activity behaviors may be important for cardiac-parasympathetic health. Therefore, while high ST is suggested to have negligible unfavorable impact on HRV, specifically replacing ST with longer bouts of MVPA may counteract these effects and elicit a slight favorable influence on HRV. These findings further support that cardiac-parasympathetic dysregulation at best only contributes a small portion to the linking mechanisms between high ST and low MVPA with CVD in women.

In summary, this dissertation advances our understanding of the association between ST and cardiac-parasympathetic regulation measured via resting HR and HRV. We found that high ST as well as low MVPA may have a small impact on cardiac-parasympathetic regulation. In agreement with the current ST and PA guidelines, our results indicate that reducing ST and increasing MVPA may beneficially influence cardiac-parasympathetic health. Importantly, our findings indicate that the greatest cardiac-parasympathetic benefits may be gained when leisure ST is replaced with long bouts of leisure MVPA.

# 7.0 Significance and Future Directions

High sedentary time (ST) is a novel cardiovascular disease (CVD) risk factor, independent of physical inactivity. Cardiac-autonomic dysregulation has been hypothesized as a linking mechanism between ST and CVD. Resting heart rate variability (HRV), and potentially heart rate (HR), are non-invasive measures of cardiac-parasympathetic activity. This dissertation attempted to assess the associations of ST with HR and HRV using observational study designs. Overall, our systematic literature review and new statistical investigations suggest limited evidence of associations of ST with HR. Specifically, most of the existing literature (and our pooled analysis) found largely null associations and we identified only a few new, unfavorable effects of leisure ST on HRV among inactive women when measured by self-report and only when exchanging ST for long-bout moderate-to-vigorous intensity physical activity (MVPA) when using accelerometry. Thus, the results of this dissertation do not strongly support the hypothesis that cardiac-parasympathetic dysregulation is a strong linking mechanism between ST and CVD. Importantly, many limitations should be addressed in future research to draw stronger and more comprehensive conclusions of the associations between ST and HR or HRV.

Perhaps most importantly, all available observational studies associating ST with HR and HRV, including the two new analyses in this dissertation, have cross-sectional designs. This type of study is susceptible to biases such as residual confounding and reverse causality which prevents the confirmation of the temporal relationships between ST and HR or HRV. As such, future studies with robust study designs such as longitudinal cohort studies or randomized controlled trial (RCT) designs that measure ST and activity prior to the outcome or experimentally manipulate ST and

activity patterns are warranted. Indeed, our laboratory is conducting an ongoing RCT that is assessing the effects of reducing ST on HR and HRV in desk workers. The results of this study will allow us to draw stronger conclusions about the cause-effect associations of ST with HR and HRV.

Another remaining limitation of the existing literature is the infrequent utilization of gold standard assessment methodology for ST and HRV. Though our second and third manuscripts uniquely measured domains and patterns of ST, the gold standard approach (i.e., thigh-worn monitors) was not employed. Noteworthy, a growing number of international cohort studies and ongoing RCTs (including ours) are utilizing thigh-worn monitors (i.e., activPAL) which will allow to accurately measure different aspects of ST, including the pattern of ST, along with light-intensity physical activity (LPA) and MVPA. Furthermore, respiration rate, which can significantly affect HRV, was measured indirectly in our second and third manuscripts. Thus, though we were able to improve upon the available literature using self-reported ST and no direct measure of respiration rate, future studies with even higher quality assessment methodology are needed to clarify associations.

Furthermore, the included samples in the currently available observational studies are mostly healthy, young-to-middle aged adults. Yet, we would hypothesize that the associations of ST with HR and HRV may be stronger among individuals with existing CVD (e.g., coronary heart disease patients) and/or in individuals at high risk of CVD (e.g., older adults). The association between ST and HR or HRV remain to be investigated in such samples and should be a priority for future studies.

Lastly, an important limitation to HRV measured at rest (as in this project) can only measure cardiac-parasympathetic regulation and lacks the ability to assess the cardiac-sympathetic

regulation. Limited experimental evidence suggests that uninterrupted prolonged sitting increases overall sympathetic activity (e.g., assessed by measuring plasma norepinephrine) [254]. Yet, to our knowledge, no epidemiological or experimental studies have evaluated the association of ST specifically with cardiac-sympathetic regulation. Thus, the relationship between ST and cardiac-sympathetic regulation should be investigated in future research to achieve a more comprehensive understanding of the role of cardiac-autonomic dysfunction in the associations between ST and CVD.

In short, this dissertation suggests marginal, unfavorable associations between ST and cardiac-parasympathetic activity that is regulated by respiration. However, several important limitations in the current literature remain. Thus, future investigations addressing these gaps are needed to better characterize and strengthen conclusions regarding associations between ST and HRV along with the role of cardiac-autonomic dysfunction in the association between ST and CVD.

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