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Sleep Disturbance and Autonomic Dysregulation as Pathways of Mortality and Cardiovascular Risk in Depression

By

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Sleep Disturbance and Autonomic Dysregulation as Pathways of Mortality and Cardiovascular Risk in Depression

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Sc.M., Johns Hopkins University, 2013

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An abstract of

A dissertation submitted to the Faculty of the
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Abstract

Sleep Disturbance and Autonomic Dysregulation as Pathways of
Mortality and Cardiovascular Risk in Depression
By Minxuan Huang

Depression is a prevalent psychiatric condition, and remains a risk factor for adverse health outcomes, including mortality and cardiovascular disease (CVD). However, little is known about the pathophysiology underlying depression and its associated consequences. Sleep disturbance is a modifiable behavior that is a common symptom of depression; autonomic dysregulation has been linked to depression. Both factors independently contribute to higher risk of mortality and CVD. The objective was to elucidate the complex roles of sleep disturbance and autonomic dysregulation on the pathways linking depression and adverse outcomes, using a co-twin control design. This dissertation leveraged data from the Emory Twin Study, which included 283 pairs (n=566) from members of the Vietnam Era Twin Registry.

In **Aim 1**, we conducted a cross-sectional evaluation of the association of depressive symptoms, assessed by the Beck Depression Inventory-II (BDI), with sleep disturbance, assessed by in-lab polysomnography, at-home actigraphy, and the self-rated Pittsburgh Sleep Quality Index. We found that depression was associated with longer rapid eye movement sleep disruption, sleep fragmentation and variability. Depression was consistently not associated with sleep architecture or sleep-disordered breathing.

In **Aim 2**, we evaluated the temporal relationships between sleep and autonomic dysregulation indexed by heart rate variability (HRV). We found that the associations of daytime HRV with sleep stages, cumulative hypoxic burden and sleep continuity measures were bidirectional. Autonomic function during wakefulness and sleep disturbance are closely interrelated and their influence on each other may extend beyond 24 hours.

In **Aim 3**, we assessed the prognostic implications of baseline HRV and depression with adverse outcomes during 12-year follow-up. We found that depression and reduced HRV at baseline were associated with higher risk of all-cause mortality and CVD during follow-up.

The findings of this dissertation extend the existing literature by providing substantial evidence that depression, sleep disturbance and autonomic dysregulation are closely interrelated, and they together contribute to a higher risk of adverse outcomes. Our results contribute to clarify the link between depression and its outcomes, and help inform future research on strategies targeting sleep and HRV in lowering mortality and cardiovascular risk in depressed individuals.

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CHAPTER 1: INTRODUCTION

1.1 Background

Depression is a prevalent psychiatric condition, with a lifetime prevalence of 16% in the United States, translating into 33 to 35 million adults with depression some time in their lives.[1-3] It remains a recognized risk factor for adverse health outcomes, such as all-cause mortality, as well as the development and progression of cardiovascular disease (CVD).[4-9] However, the exact pathophysiology and mechanisms underlying the association between depression and adverse health outcomes, including mortality and CVD, still remain unclear. Despite many proposed mechanisms (e.g., inflammation, neuroendocrine dysregulation, and lifestyle behaviors), they only partly account for the magnitude of the associations (i.e. roughly 2-fold increased risk from depression). Furthermore, evidence from our lab and others suggests a shared genetic predisposition underlying both depression and adverse health consequences such as mortality and CVD,[10-14] which suggests that genetic factors could be a potential confounder on this association. Identification of new modifiable risk pathways is critical to the design and implementation of effective intervention strategies to reduce risk related to mortality and CVD.

Among the many proposed mechanisms underlying the association between depression and adverse health outcomes, sleep disturbance is a modifiable behavior that is a common symptom of depression, as well as part of the diagnostic criteria for major depressive disorder (MDD).[15] Sleep disturbance is an umbrella term that describes a range of sleep disorders, including disorders of initiating and maintaining sleep, disorders of excessive somnolence, disorders of sleep-wake schedule, and dysfunctions associated with sleep, sleep stages, or particle arousals. Research have shown that sleep disturbance is independently associated with all-cause mortality and cause-specific mortality related to CVD.[16-18] Sleep disturbance also

contributes to cardiovascular risk, including CVD risk factors such as hypertension, obesity, and metabolic syndrome, and CVD incidence.[19-24] Thus, sleep disturbance could be an important pathway linking depression and adverse events, including mortality and CVD.

The exact mechanisms linking sleep disturbance with adverse outcomes among depressed patients remain undetermined. The autonomic nervous system (ANS) could be a biological mediator amongst depression, sleep disturbance, and adverse outcomes. Sleep disturbance, such as poor self-reported sleep quality or daytime sleepiness, has been linked to nighttime ANS dysregulation.[25-27] Heart rate variability (HRV) is a measure of beat-to-beat heart rate fluctuations over time and represents a noninvasive index of cardiac ANS regulation.[28,29] A relationship between depression and HRV, and other measures of autonomic dysregulation, is well known.[30,31] In a recent study by the same research team here at Emory University, we has demonstrated that such association is bidirectional.[32] It is likely that the association between sleep and HRV is also bidirectional. An evaluation of the directionality of such association can help better understand the complex mechanisms of depression, sleep disturbance, autonomic dysregulation, and adverse health outcomes.

1.2 Study Motivation

To date, no prior research has comprehensively evaluated the complex associations between depression, sleep disturbance, autonomic dysregulation, and adverse health outcomes including mortality and CVD. Such research is necessary to help elucidate and better understand the complex pathophysiological mechanisms and pathways linking depression and adverse health consequences, in order to help reduce risk of mortality and CVD, especially among depressed individuals.

Previous research have been limited by a lack of comprehensive evaluation of clinical diagnosis of depression, as well as a full spectrum of objective sleep dimensions. Even though depression has shown an association with self-reported sleep problems,[15,33-36] the relationship between depression and objectively measured sleep disturbance is far less consistent in some objectively measured sleep dimensions, such as sleep architecture and sleep apnea, using objective tools including polysomnography (PSG) and actigraphy. While some studies have reported an association,[37-40] others have not.[41-43] In addition, the sleep dimensions that are associated with depression vary from study to study. For instance, some studies have linked depressive symptoms with prolonged stage 2 sleep and less rapid eye movement (REM) sleep,[44,45]while others have not identified such association between depression and sleep architecture.[41] The inconsistencies of prior studies may results from the limited sample sizes, differences in their measurement of depression (clinical diagnosis vs. self-rated questionnaires), or a lack of comprehensive evaluation of objective sleep dimensions.[33,46-48]

As for the association between sleep disturbance and autonomic dysregulation, the existing literature has suggested that such association may be bidirectional, but the directionality of the association and the temporal dynamics still remain undetermined given the cross-sectional design of most previous studies.[26,49,50] To date, the pathways linking ANS function and sleep still remain unclear. Some studies have suggested that sleep disturbance, including obstructive sleep apnea and sleep quality measures, such as sleep duration and latency, may result in autonomic imbalance by triggering a dominance of sympathetic over parasympathetic activity.[51-53] In contrast, other studies have demonstrated that ANS regulation, measured by HRV, predicts subsequent alterations in sleep quality and architecture.[54-56] However, no prior studies have comprehensively evaluated their temporal dynamics and directionality of association using a full

spectrum of objectively measured sleep dimensions. More information is also needed on the temporal dynamics between HRV and sleep, i.e., how long and the extent to which their influence on each other is maintained over time, since prior studies assessed primarily short-term associations.[55,57,58]

In addition to limited information regarding the temporal directionality of the association between sleep disturbance and autonomic dysregulation, most prior studies recruited participants with specific clinical problems, such as chronic fatigue syndrome, narcolepsy, and obstructive sleep apnea.[53,54,59,60] Literature in predominantly healthy populations is limited and results have not been consistent.[58,61,62] Furthermore, most prior studies used laboratory-based methods to measure sleep.[56,59,63,64] While this provides a controlled environment, it may not illuminate sleep problems in normal life.[65,66]

Autonomic dysregulation as indexed by reduced HRV and other electrocardiographic (ECG) metrics, including deceleration capacity, is an independent predictor of adverse health outcomes, such as all-cause mortality.[67-70] However, few studies have evaluated whether ECG metrics of autonomic dysfunction predict future incident CVD events. In addition, to our knowledge, no previous study has assessed nighttime HRV (which could relate to sleep disturbance) separately from daytime HRV in predicting CVD risk. Understanding such association has important clinical and public health implications.

Prior twin studies suggest that the link between depression and sleep may be partially explained by shared genetic and familial factors.[71-74] However, these findings were limited to self-reported sleep measures. Similarly, it is undetermined whether sleep disturbance, autonomic dysregulation, as well as mortality and CVD risk, may share common pathophysiology.[75-79] By using a co-twin matched study design and within-pair analysis of twin pairs discordant on

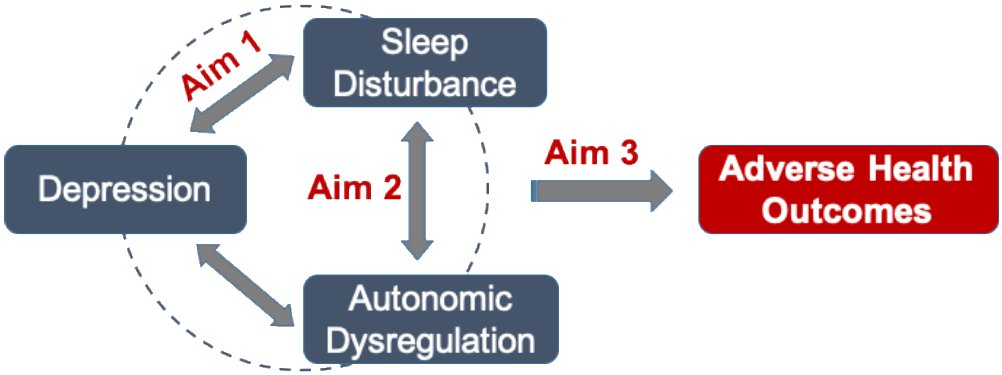
exposure variables, we are able to control for potential genetic and early familial confounding. The inclusion of both monozygotic (MZ) and dizygotic (DZ) twins also allows an evaluation of genetic and familial factors and their effects on the association.

1.3 Objective and Specific Aims

My dissertation research is aimed at elucidating the complex underpinnings of depression, sleep disturbance, autonomic dysregulation, and adverse health outcomes, and will address the limitations of previous studies. The objective of this dissertation is to elucidate the associations between depression, sleep disturbance and autonomic dysregulation, as they may represent pathophysiological pathways linking depression and adverse health outcomes including mortality and CVD.

This dissertation includes the following aims/hypotheses. The interrelationships of the dissertation aims are summarized in **Figure 1.1.**

Figure 1.1 Interrelationships among the specific aims of the dissertation research



Specific Aim #1

Examine the association of depressive symptoms with objectively and subjectively measured sleep disturbance. Depressive symptoms were measured using the Beck Depression Inventory-II (BDI) score, and major depression was assessed using structured clinical interview. Sleep disturbance was measured objectively using one-night in-lab PSG and 7-day in-home actigraphy, and was measured subjectively using the self-rated Pittsburgh Sleep Quality Index. The approach to this aim was to use multivariable mix-effects regression model with random effect for twin pair. The hypothesis for this aim is that individuals with more depressive symptoms (or depression diagnosis) have more sleep disturbance compared to individuals with fewer depressive symptoms (or no depression diagnosis). We also hypothesized that depression is more consistently associated with subjective than objective sleep measures, and that genetic and familial factors play a role in this association.

Specific Aim #2

Evaluate the temporal relationships and directionality of association between objectively measured sleep disturbance and autonomic dysregulation. Sleep disturbance was objectively measured using one-night in-lab PSG and 7-day in-home actigraphy. Autonomic dysregulation indexed by HRV was obtained using 24-hour Holter ECG and 7-day ECG monitoring with a wearable patch. Multivariable mixed-effects regression models and vector autoregressive models with Granger causality tests were used to examine the temporal dynamics and directionality of the association between sleep and HRV. We hypothesized that the association between sleep and HRV is bidirectional and that the influence of these phenotypes on each other would be relatively brief, within 24 hours.

Specific Aim #3

Evaluate the prognostic implications of depression and autonomic dysregulation at baseline with risk of all-cause mortality and incident CVD events during follow-up. Depressive symptoms were measured using the Beck Depression Inventory-II (BDI) score, and major depression was assessed using structured clinical interview. At baseline assessment, autonomic dysregulation indexed by HRV was measured through 24-hour ECG monitoring, and were segmented into daytime and nighttime data. During an average of 12-year follow-up, mortality data were collected via National Death Index database, and within a subset of twins, incident CVD events data were obtained during in-person visit or phone interview, and were further verified and adjudicated via a thorough medical chart review. The approach to this aim was to use Kaplan-Meier figures to illustrate the survival probabilities by depression status or HRV values, and to use multivariable frailty models with random effect for twin pair to examine the hazard ratios for mortality and CVD events within twin pairs. We hypothesized that fewer depressive symptoms (or no depression diagnosis) and higher values of both daytime and nighttime HRV are associated with decreased risk of mortality and CVD, and genetic and familial factors play a role in this association.

1.4 Data Source

We will leverage the Emory Twin Study (ETS) and its follow-up study (the Emory Twin Study Follow-up, or ETSF). The participants of this study were recruited from the Vietnam Era Twin Registry (VETR), which is a national sample of >7,000 male MZ and DZ twin pairs who served on active duty during the Vietnam war (1964-1975).[80] The ETS included 566 twins (283 pairs), and its objective was to evaluate the role of biological, psychological, and behavioral risk

factors in the development of subclinical CVD.[81,82] The ETSF followed these twins either in-person or by phone, who were initially without a history of CVD, for incident CVD events and subclinical coronary heart disease on average 12 years after the baseline assessment. The study over-sampled twin pairs that are discordant of depression or posttraumatic stress disorder in order to address questions related to these conditions. About half of the twin pairs are discordant for major depressive disorder (MDD). In a subgroup of 112 twin pairs (n=224) at follow-up we have collected objective sleep (polysomnography and 7-day actigraphy) and ANS data through both in-lab and at-home monitoring, thus allowing to gather data in a controlled laboratory environment as well as in the "real-world".

A co-twin control study provides a natural "counterfactual" design to examine phenotypic associations with intrinsic adjustment for potential confounders, as twins are matched for genetic and early familial factors.[83] The study of MZ and DZ twins provides information on common etiological pathways linking phenotypes of interest. Because MZ twin pairs share 100% of their genes while DZ twin pairs only share 50% on average, if a larger association of interest is found within DZ pairs than within MZ pairs, this suggests that genetic factors may play a role in this association. Our co-twin control study design has improved internal validity and precision by intrinsically adjusting for unknown or unmeasured confounders, such as genetic, familial and environmental factors.

1.5 Public Health Importance

This dissertation contributes to a better understanding of the complex relationships among depression, sleep disturbance, autonomic dysregulation and adverse health outcomes, including mortality and CVD.

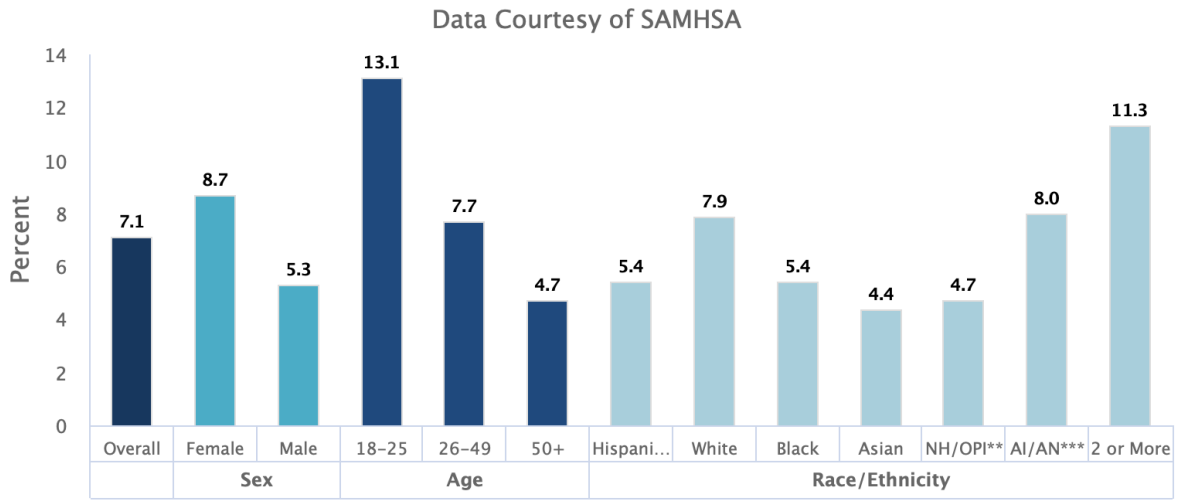
Specifically, this research project provides a comprehensive evaluation on the association of depression with both objective and subjective sleep disturbance, in a full spectrum of sleep dimensions. It contributes to clarify the link between depression and sleep disturbance, and their roles in the pathophysiology of adverse health events. This study elucidates the temporal dynamics and directionality of association between sleep and HRV, which helps inform future research directions on prevention and treatment strategies to mitigate sleep disturbance and autonomic dysregulation among depressed individuals. In addition, this dissertation project sheds light on the prognostic implications of alterations in HRV and depressive symptoms in predicting mortality and CVD, which suggest the utility of HRV monitoring in preventing and treating adverse health consequences, especially among individuals with depression and/or sleep disturbance. Our research helps understand whether screening for potential sleep disorders and/or autonomic dysregulation among patients with depression is effective in identifying individuals at the highest risk for adverse outcomes. Our study supports the sleep hygiene education as an adjunct treatment for depression, as well as exercise interventions, which are shown to improve autonomic dysfunction, as an adjunct treatment for sleep improvement. In addition, pharmacological therapies to restore autonomic balance and/or treat poor sleep may have an effect on adverse health outcomes among depressed individuals. Furthermore, capitalizing on the twin sample, this study helps evaluate the role of genetic predisposition and familial factors as opposed to unshared environment in the underlying pathways from depression to sleep disruption, autonomic imbalance, mortality and CVD.

CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

2.1. Depression and Adverse Health Outcomes

Depression is a prevalent psychiatric condition with a lifetime prevalence of 16% in the United States, translating into 33 to 35 million adults who will develop depression at some point in their life time.[1-3,32,84] During 2013 and 2016, it was estimated that 8.1% of American adults aged 20 and over had major depressive disorder (MDD) in a given 2-week period.[85] Depression also has substantial gender, age and racial differences. For example, women are almost twice as likely as are men to have had depression; people in younger age groups (such as 18-25 years) have higher prevalence of depression than people in older age groups (such as >50 years). As for racial differences, although racial minorities are less likely to report acute episodes of MDD than white, they are more like to suffer from chronic depression with heavier consequences on daily functioning.[86] **Figure 2.1** shows the prevalence of major depressive episode as well as the differences among US adults in 2017. Research has reported that the prevalence of depression has increased drastically, especially among adolescents and young adults in the recent years.[87,88] The increasing prevalence and the adverse health outcomes that are associated with depressive disorders highlight the importance of depression prevention and intervention.

Figure 2.1 Prevalence of major depressive episode among US adults (2017)



Depression remains a recognized risk factor for disability, mortality, as well as the development and progression of CVD.[1,3,7,9,89-91] Numerous studies have been conducted on the association of depression with increased risk of all-cause mortality in general population as well as various patient groups. For example, a 2014 meta-analysis that included 293 studies with more than 1.8 million participants from 35 countries showed that depression was associated with a 1.5 times increased risk of all-cause mortality.[7] For cause-specific mortality, studies have suggested that individuals in the general community with depression may be at increased risk of mortality due to CVD, even several decades after depression assessment.[92-94] As for cancer-specific mortality, even though depression is linked to disease development and progression in cancer patients,[95] the association between depression and cancer mortality is weaker compared to CVD mortality, and decreases when the follow-up period exceeds 5 years.[92,94,96-98] The excess mortality risk associated with depression is not explained by excess suicide deaths.[99]

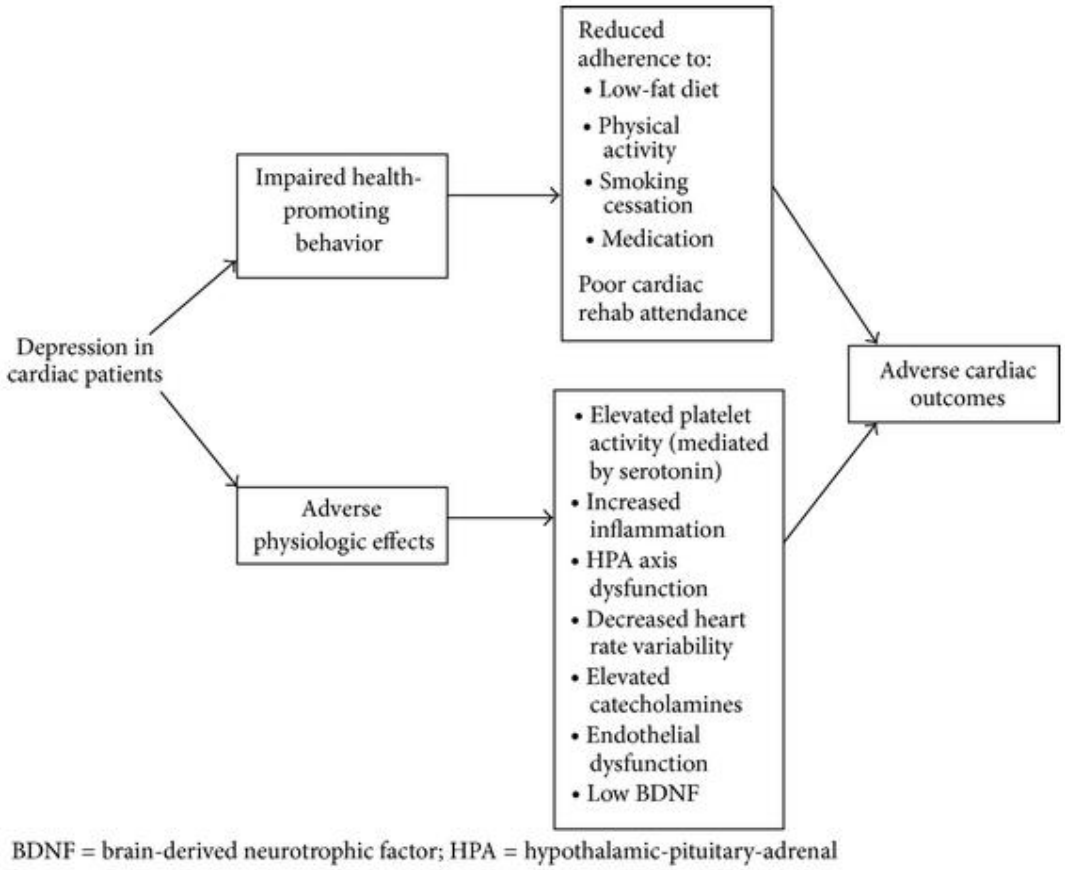
The association of MDD or depressive symptoms with CVD morbidity is well established among subjects with and without CVD.[6,90,91,100] For example, a systematic review and meta-

analysis of prospective cohort studies showed that depression is associated with a significant increase in the risk of coronary heart disease and myocardial infarction by about 30%, which may have implications for CVD etiological research and psychological treatments.[90] Research has continually shown that the severity of depressive symptoms is proportional to the risk of developing CVD.[101] As CVD is the leading cause of mortality in developed countries, when CVD and MDD present together, the prognosis for both worsen.[102-104] Evaluation of the potential mechanisms linking depression and adverse events such as CVD contributes to a better understanding of pathophysiological pathways, and helps inform prevention and treatment strategies for CVD risk.

The exact pathophysiology and mechanisms underlying the association between depression and mortality still remain unclear. It has been proposed that higher mortality found among depressed individuals might be attributable to the mechanisms specific to the existing diseases, behavioral pathways such as treatment adherence and health behaviors, and biological pathways (e.g. neuroendocrine and neuro-immunological systems, and the circadian rhythm).[7,105] Specifically, research has demonstrated that depression is associated with peripheral inflammation and oxidative stress, which are mechanisms that may contribute to higher risks of obesity and cardiometabolic conditions that lead to shorter time to mortality.[106-108] The association between depression and cardiovascular mortality may also be attributable to platelet function, endothelial function, inflammation, and autonomic balance, which play important roles in cardiovascular disease development and progression.[109,110] For cancer-specific mortality, one of the suggested mechanisms is stress affecting the development and progression of cancer by impacting the repair of damaged DNA and accelerating tumor cell growth, which may contribute to a shorter time to cancer death.[111,112]

As for mechanisms linking depression to CVD, studies have suggested multifactorial pathophysiological pathways through which depression can increase CVD risk, including neuroendocrine dysregulation, metabolic and immune-inflammatory disturbance, and unhealthy lifestyle behaviors (e.g., smoking, alcohol abuse, physical inactivity, sleep disturbance, and unhealthy diet).[19,20,32,100,113-115] **Figure 2.2** summarizes a few of the proposed mechanisms linking depression to adverse cardiac events.[116] However, these data have not been entirely consistent, and the above suggested mechanisms only partially explain the association between depression and CVD; and none has been shown to account for more than a small proportion of the CVD risk.[6] Identification of additional modifiable bio-behavioral mechanisms linking depression to CVD is critical to the design and implementation of intervention strategies to lower CVD risks among depressed individuals. Furthermore, evidence from our lab and others suggests a shared genetic predisposition underlying both depression and CVD,[10,11] pointing to common pathophysiological mechanisms yet to be discovered. In the current application, we will test the hypothesis that sleep disturbance and nighttime autonomic dysregulation are key interrelated pathways linking depression and CVD.

Figure 2.2 Proposed mechanisms of adverse cardiac outcomes in cardiac patients with depression



Among the many proposed mechanisms and potential pathophysiology underlying the pathways from depression to adverse health outcomes, such as mortality and CVD, sleep disturbance and autonomic dysregulation may explain the excess risk of adverse outcomes among depressed individuals in addition to the traditional risk factors and other known mechanisms. However, there is a lack of comprehensive evaluation of both these factors and their relationships with mortality and CVD in the context of major depression. Thus, both sleep disturbance and autonomic dysregulation are the main factors to be evaluated in this dissertation project.

2.2 Depression and Sleep Disturbance

Among the many proposed mechanisms underlying the association between depression and adverse outcomes, sleep disturbance is a modifiable behavior that is a common symptom of depression.[15] As sleep disturbance has been shown to be independently associated with all-cause mortality and cause-specific mortality related to CVD,[16-18] it is likely a potential mediating factor that may explain the excess risk of mortality and CVD events in major depression.

Sleep is an essential component of physiological regulation and is critical for optimal brain and bodily functions. Sleep disturbance affects 87 million adults in the US annually.[117,118] Sleep disturbance is an umbrella term that describes a range of sleep disorders, including disorders of initiating and maintaining sleep, disorders of excessive somnolence, disorders of sleep-wake schedule, and dysfunctions associated with sleep, sleep stages, or particle arousals. **Table 2.1** summarizes the main categories of sleep disturbance, including insomnias, hypersomnias, and disorders of the sleep-wake schedule.[119]

Table 2.1 Classification of sleep disturbance

Classification of sleep disturbance
I. Insomnias: Disorders of initiating and maintaining sleep
A. Psychophysiologic–situational or persistent
B. Associated with psychiatric disorders, particularly affective disorders
C. Associated with drugs and alcohol
1. Tolerance to or withdrawal from CNS depressants
2. Sustained use of CNS stimulants
3. Sustained use of or withdrawal from other drugs
4. Chronic alcoholism
D. Associated with sleep-induced respiratory impairment
1. Sleep apnea syndrome
2. Alveolar hypoventilation syndrome
E. Associated with sleep-related (nocturnal) myoclonus and "restless legs"
F. Miscellaneous—other medical, toxic, or environmental conditions
II. Hypersomnias: Disorders of excessive somnolence
A. Psychophysiologic—situational or persistent
B. Associated with psychiatric disorders, particularly affective disorders
C. Associated with drugs and alcohol
D. Associated with sleep-induced respiratory impairment (as in D above)
E. Narcolepsy—cataplexy
F. Miscellaneous—other medical, toxic, environmental, or idiopathic conditions
III. Disorders of the sleep–wake schedule
A. Transient—jet lag, work shift
B. Persistent
1. Delayed sleep phase syndrome
2. Advanced sleep phase syndrome
3. Non-24-hour sleep–wake syndrome
IV. Parasomnias: Dysfunctions associated with sleep, sleep stages, or partial arousal
A. Sleepwalking
B. Sleep terrors and dream anxiety attacks
C. Enuresis
D. Nocturnal seizures
E. Other sleep-related dysfunctions

Previous research that evaluated the association between depression and sleep have focused on a few specific sleep dimensions, such as insomnia, sleep-disordered breathing, hypersomnolence disorders, and disruption in the sleep-wake schedule. Among common types of sleep disturbance, insomnia is a prevalent and persistent sleep problem in which a person has difficulty falling asleep or staying sleep throughout the night. It has been estimated that up to

10% to 30% of adults live with some form of insomnia.[120] Most cases of insomnia have been attributed to poor sleep habits, depression, anxiety, lack of exercise, chronic illness, or certain medications.[121] Sleep-disordered breathing (SDB) describes a chronic condition in which partial or complete cessation of breathing occurs throughout the night, and may result in daytime sleepiness or fatigue.[121] Sleep apnea is the most common form of SDB, in which breathing briefly and repeatedly stops and starts during sleep. Hypersomnolence disorders refer to feelings of sleepiness and fatigue during the day despite a healthy circadian rhythm and an adequate amount of sleep during the previous night.[121] Specifically, narcolepsy describes a condition that is characterized by extreme sleepiness during the day and falling asleep suddenly during the day. Sleep-wake disorders occur when the body's internal clock does not work properly or is out of sync with the surrounding environment.[122] Jet lag disorder and shift work disorder are the two common types of sleep-wake disorders. Parasomnia is a collective term for unusual behaviors that occur prior to sleep, during sleep, or during the transition period between sleep and waking.[123] Other sleep dimensions of interest may include sleep fragmentation that is characterized by repetitive short interruptions of sleep, restless legs syndrome which is a type of sleep-related movement disorder, and disruptions in the sleep stages and architecture.[121]

Sleep disturbance is a common symptom of depression.[15,124] Studies have shown that more depressive symptoms are positively associated with various self-reported indices of sleep disturbance, including worse sleep quality, more sleep onset difficulties, and more frequent awakenings.[15,33,34,125] Numerous studies have shown an association between depression and self-reported sleep problems.[15,33-36] For example, in a community sample of elderly Asian population, depressive symptoms, evaluated by a self-rated measure of depression in older adults, were significantly associated with sleep disturbance measured by self-reported questionnaire, the

Pittsburgh Sleep Quality Index (PSQI).[33] In another patient population with heart disease, more depressive symptoms, measured by the Beck Depression Inventory (BDI) score, were significantly associated with poor sleep indexed by PSQI components in subjective sleep quality and daytime dysfunction. Because of the low cost and convenience of administration, self-reported sleep measures such as the PSQI score remain the most frequently used methods to measure sleep disturbance.

There have been quite a few studies that evaluated the association between depression and objectively measured sleep disturbance, using measures such as polysomnography (PSG) or actigraphy. However, their results have not been consistent, in terms of directionality and magnitude of association, or sleep dimensions that are shown to be associated with depression. For example, some studies have reported an association between more depressive symptoms and longer rapid eye movement (REM) sleep latency and higher REM density,[37,38] while other studies have not found such association.[41,42] In addition, the existing literature is far from consistent regarding the sleep dimensions that may be affected by depression. For example, some studies have linked depressive symptoms with prolonged stage 2 sleep and less rapid eye movement (REM) sleep,[44,45] while others have not found an association between depression and sleep architecture.[41] The inconsistencies of results in prior studies may be due to the fact that several studies were limited in sample size, differed in their measurement of depression (self-rated or clinical diagnosis), or lacked a comprehensive evaluation of objective sleep dimensions.[33,46-48] As for SDB, most previous studies that examined the relationship of depression with SDB-related measures did not find a significant association.[42,126-131] For actigraphy-measured sleep disturbance, prior studies have reported a significant association between depression and worse sleep quality and continuity, such as lower sleep efficiency (SE)

and higher fragmentation.[132-135]

A growing body of literature has pointed out a potential bidirectional association of clinical depression or depressive symptoms with various indices of sleep disturbance (e.g., longer sleep onset latency, more numbers of awakenings, and lower sleep efficiency).[15,136-138] For example, more than 90% of depressed patients have reported having one or more sleep issues, such as insomnia and hypersomnia.[34] Specifically, a study by Lovato et al suggested that depressed individuals had significantly longer sleep onset, more wake after sleep onset, and lower sleep efficiency compared to individuals without depression.[136] These sleep problems may emerge as a symptom of major depression or as a side effect from treatment of depression. On the other hand, sleep disorders often occur prior to the onset or recurrent episode of major depression. It has been reported that among individuals with major depression, 40% reported having insomnia before depression.[139] Other studies reported as many as 24% to 58% of individuals with sleep-disordered breathing were also diagnosed with depression, suggesting that sleep disturbance may be involved in the pathogenesis of depression.[140,141]

2.3 Depression and Autonomic Dysregulation

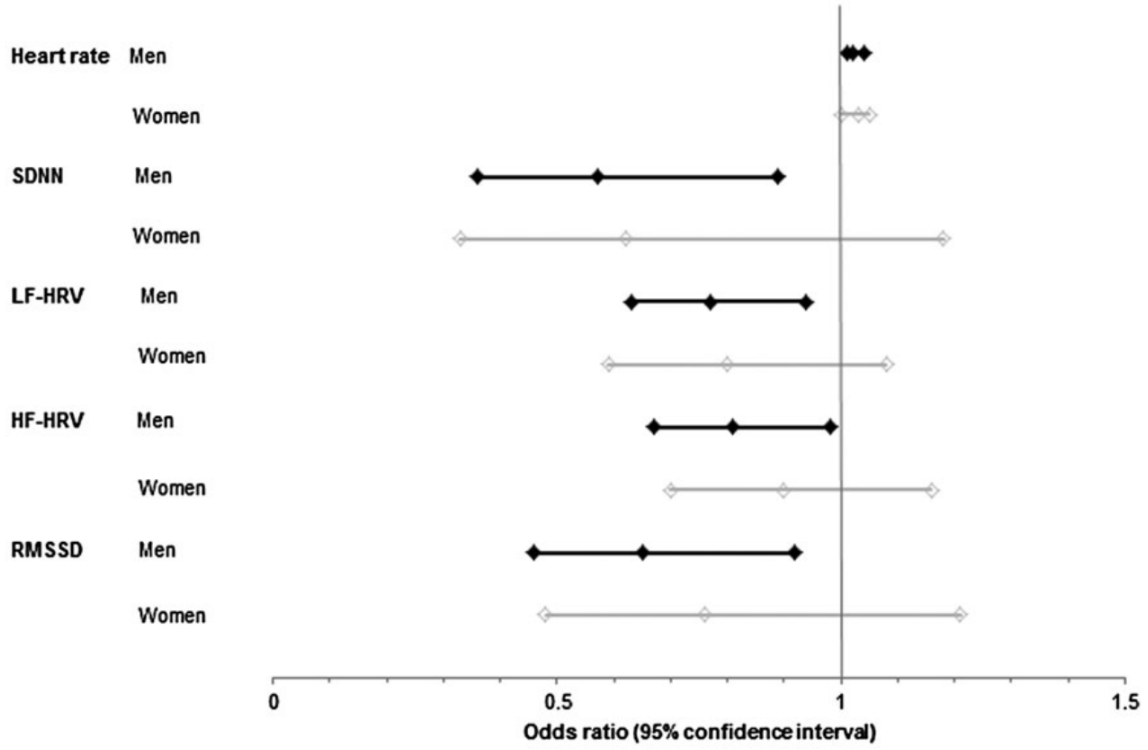
Autonomic nervous system (ANS) controls basic bodily functions such as heartbeat, digestion, respiration and blood pressure regulation. The dysregulation of the ANS system can be a complication of many diseases, and is associated with various pathological conditions, such as higher blood pressure, incident cardiovascular disease (CVD), and mortality.[67,142-144] Autonomic dysregulation can be measured noninvasively using heart rate variability (HRV), which provides a measure of beat-to-beat heart rate fluctuations over time and represents a noninvasive index of cardiac autonomic regulation.[28,29]. Reduced HRV is indicative of an imbalance between

sympathetic and parasympathetic modulation, i.e. increase in the sympathetic nervous system (SNS) and/or a decrease in the parasympathetic nervous system (PNS) modulation,[114] and is suggestive of increased morbidity and mortality. Deceleration capacity (DC) is a novel and powerful HRV metric of parasympathetic activity that provides an average speed of heart rate deceleration, and is potentially more robust and predictive than other HRV metrics in evaluating parasympathetic function and predicting adverse outcomes.[70] Specifically, compared to HF HRV, one advantage of DC is that it is not influenced by respiration of body position, which makes it a more stable and reproducible metric that depends less on the activity in general. Reduced HRV predicts CVD morbidity and all-cause mortality, and vagal function indexed by HRV may provide a structural link connecting psychological moments to morbidity and mortality.[28,29,67,143,145,146]

ANS dysregulation indexed by a reduced HRV is likely a critical biological mediator linking depression, sleep disturbance, and adverse health outcomes.[6,25-27,30,31,100] Prior studies have consistently shown an association of depression with reduced HRV among individuals with CVD.[6,100] Predominantly cross-sectional studies have reported an inverse association between more depressive symptoms and reduced HRV in multiple domains.[147-149] Due to the small number of longitudinal investigations on the directionality of the association between depression and HRV, the temporal relationships between these two phenotypes still remain undetermined. The existing studies have been inconsistent in terms of the directions of association between depression and unfavorable HRV indices and notably reduced cardiac vagal modulation. Of note, a recent study, using a cross-lagged analysis over a 10-year period, has demonstrated that higher baseline HRV measures were associated with a lower likelihood of incident depressive symptoms at follow-up in men, but depressive symptoms at baseline were not associated with

HRV at follow-up.[150] **Figure 2.3** shows the association between baseline heart rate and HRV with the risk of incidence depressive symptoms 10 years later in men and women without depressive episodes at baseline.[150]

Figure 2.3 Baseline heart rate and HRV and risk of incidence depressive symptoms 10 years later in men and women without depressive episode at baseline



Using our own twin dataset, the candidate has also recently investigated the temporal directionality of the association between depression and autonomic dysregulation indexed by reduced HRV.[32] This study demonstrated that depressive symptoms are bidirectionally associated with a reduced HRV in all frequency domains, including ultra-low frequency (ULF), very low frequency (VLF), low frequency (LF), and high frequency (LF). In addition, results showed consistent associations between baseline HRV and depressive symptoms at follow-up

across all HRV domains and models, which were not explained by antidepressants or other participant characteristics. The magnitude of the association was similar in the opposite pathways linking baseline depressive symptoms to HRV at follow-up, and it can be mediated by antidepressant use. The associations were slightly stronger in dizygotic twins, suggesting a potential role of genetic predisposition on the association of depression with HRV. Our findings agree with the previous longitudinal study that evaluated the temporal directionality, and expand previous predominantly cross-sectional studies of the inverse association between depressive symptoms and HRV. This evidence supports the hypothesis that ANS disturbances, as reflected by reduced HRV metrics, may have bidirectional association with depression, and the causal pathway from depression to ANS disturbances can be mediated by use of antidepressants.[150]

The exact mechanisms linking depression and ANS dysfunction are still unknown. Emotional regulation and social behavior, which are involved in the risk of depression, have been associated with brain areas that regulate vagal modulation and cardiac ANS control, such as prefrontal cortex and the amygdala.[151,152] This suggests that there could be shared pathophysiology underlying disturbed ANS functions and links both depression vulnerability and cardiac ANS regulation. Other research also suggested that chronic stress and prolonged negative emotions, often experienced among depressed individual, can lead to increased sympathetic and reduced parasympathetic modulation.[115] The effect of depression on ANS dysfunction may be partly explained by antidepressant use, as supported by our own findings as well as other studies.[32,153,154] For example, in a longitudinal study, depressed individuals who started to use different antidepressant medications exhibited a decreased cardiac vagal control, compared with antidepressant naïve individuals with depression or individuals who stopped using antidepressants.[153]

2.4 Sleep Disturbance and Autonomic Dysregulation

Similar to the bidirectional association between depression and autonomic dysregulation indexed by reduced HRV, it is likely that the association between sleep disturbance and HRV is also bidirectional. However, to date, given the cross-sectional design of most previous studies, the directionality of associations between sleep disturbance and autonomic dysregulation still remains unclear.[26,49,50]

Some studies have suggested that sleep disturbance may precede autonomic dysregulation. For example, studies have suggested that sleep disturbance, including obstructive sleep apnea and measures of sleep quality, may cause autonomic imbalance by triggering a dominance of sympathetic over parasympathetic activity.[51-53] REM sleep is characterized by decreased parasympathetic modulation, whereas during non-REM sleep efferent sympathetic nerve activities diminish and parasympathetic modulation increases, indexed by HF HRV.[155,156] Marked autonomic dysfunction involving the parasympathetic system has been described in patients with sleep disorders.[25-27,49,50,156] For example, in a children population aged between 5 to 11 years old, long sleep latency predicted lower HF HRV, while nocturnal awakenings, sleep latency, low sleep efficiency, and low corrected sleep duration were related to higher LF/HF, which is an indicator for sympathetic and parasympathetic imbalance.[52] Similarly, another study showed that patients with obstructive sleep apnea (OSA) are characterized by reduced HRV, compared to individuals without OSA.[53] The findings of this study also indicates that a higher apnea/hypopnea index (AHI), which is a primary indicator for sleep apnea, constitutes an independent predictor of reduced HRV, both in the sympathetic and parasympathetic components, as well as the sympathetic-parasympathetic balance.

In contrast, other studies have proposed that ANS regulation, measured by HRV, is a

predictor of subsequent sleep quality and sleep architecture.[54-56] In a study of chronic fatigue syndrome, researchers found that low HF HRV domain strongly predicted subjective sleep quality, such as repeated awakenings during the study night.[54] In a population of healthy individuals, researchers found that higher values of parasympathetic indices, such as root mean square of successive RR intervals (RMSSD) and percentage of successive RR that differ by more than 50 ms (pNN50), predicted poor sleep such as lower values in wake after sleep onset (WASO).[55] Other studies also suggest these parasympathetic indices, collected during a short wakefulness resting period, are associated with better one-week sleep efficiency.[157]

Despite the above-mentioned investigations that longitudinally evaluated the association between sleep and HRV, no prior studies have comprehensively evaluated the temporal directionality of these associations using a full spectrum of objective sleep measures. More information is also needed on the temporal dynamics between HRV and sleep, i.e., the extent to which their influence is maintained over time, since prior studies assessed primarily short-term effects.[55,57,58] Most existing data are also based on patients with specific clinical problems, such as chronic fatigue syndrome, narcolepsy, and obstructive sleep apnea.[53,54,59,60] and literature in healthy populations has been limited and results have differed.[58,61,62] An evaluation of the temporal relationships between autonomic dysregulation and objectively measured sleep disturbance is necessary to better clarify their temporal dynamics and directionality.

The exact underlying mechanisms linking sleep disturbance and autonomic dysregulation have not been completely understood. For the pathway from sleep disturbance to autonomic dysfunction, it has been reported that sleep shortage might increase sympathetic activity by higher levels of the catecholamines norepinephrine and epinephrine through activation of the stress system.[51,158,159] It has been noted that low sleep duration and poor sleep continuity can

increase sympathetic over parasympathetic dominance, which may be reflected in reduced HRV.[51,52,160] Sleep disturbance may also lead to decreased sensitivity of hormonal receptors such as corticotropin-releasing hormone and serotonin receptors, which may result in dysregulation of stress responses and autonomic function.[51] It has also been suggested that decreased HRV could be interpreted as a state of autonomic hypervigilance, which is consistent with documented effects of daily stress on HRV during sleep.[161,162] Overall, autonomic dysregulation has been shown to be a potential pathway linking sleep disturbance with the common pathophysiology of hypertension, diabetes, cardiovascular disease, and mortality risk.[20,163]

As for the pathway from autonomic dysregulation to sleep disturbance, previous research suggests that cardiac vagal control, indexed by HF HRV domain, is implicated in flexible regulation of arousal, which has important clinical implications in supporting better sleep quality.[57] This study also found that cardiac vagal control during wakefulness was only related to a variety of sleep quality variables such as sleep latency, and number of arousals during sleep, and not with variables that are related to sleep quantity or architecture, which agrees with other reports.[164,165] Similarly, another study found that HRV before falling asleep can be used as a predictor for sleep efficiency.[56] This may be due to sympathetic activation which then result in the relative dominance of the HRV LF band power over the HF band power, which has been shown to affect sleep quality, such as sleep efficiency. A comprehensive evaluation of the association between autonomic dysregulation in a full spectrum of sleep dimensions is necessary to better elucidate the mechanisms and pathophysiology underlying these two phenotypes.

2.5 Sleep Disturbance and Adverse Health Outcomes

Overall, prior studies have reported a positive relationship between sleep disturbance and risk of mortality.[16-18]. Sleep disturbance has also emerged as an independent contributor to higher cardiovascular risk, including CVD risk factors such as hypertension, obesity, and metabolic syndrome, and higher CVD incidence.[19-23,166] Thus, sleep disturbance could be an important pathway of mortality and CVD risk, especially for depressed individuals.

A systematic review and meta-analysis of prospective cohort studies pointed out that both short and long sleep duration (e.g. <5 or >9 hours) are associated with an increased risk of all-cause mortality (i.e. a U-shape association).[166] Longer sleep onset latency, lower sleep efficiency, duration of REM sleep, use of sleep medication, and severity of obstructive sleep apnea have all been shown to be associated with higher risk for mortality.[18,167-169] However, prior literature on the association between sleep disturbance and mortality and CVD risk has not been completely consistent. In addition, there seems to be a disparity in the dimensions of sleep that are associated with risk of adverse health outcomes. For example, some studies reported a significant association of sleep dimensions such as shorter sleep duration and OSA with mortality,[18,170,171] but other studies reported that insomnia was not significant associated with excess mortality hazard.[170] The discrepancies in prior findings may result from differences in study design, study population and participant characteristics, and sleep measurements (i.e. whether sleep was objectively or subjectively measured).

Prior literature has also been inconsistent on the association between sleep disturbance and cause-specific mortality. Some studies have shown a significant association between sleep disturbance and cardiovascular mortality,[17] but others have not found a clear association.[167] As for cancer-related mortality, some reported there was no noteworthy association of sleep duration

and sleep quality with mortality due to cancer or other causes,[17] but others reported that sleep apnea was significantly associated with incident cancer and cancer mortality.[18]

There are several potential mechanisms that may contribute to the relationships between sleep disturbance and increased risk for mortality and CVD. First, sleep disturbance may have multiple effects on endocrine and metabolic function, such as decreased levels of testosterone and melatonin secretion, as well as vascular damage, which may be implicated with mortality or cardiovascular events.[172-176] Second, sleep deprivation or sleep irregularity may result in circadian misalignment, which may aggravate cardiovascular risk.[177] Third, experimental studies have shown that sleep disturbance may increase systemic inflammation and insulin resistance,[178,179] and other observational studies supported the role of sleep disturbance as a risk factor for diabetes, obesity and hypertension, which are established risk factors for mortality and cardiovascular outcomes.[180-182] In addition, it has been shown that the hypothalamic-pituitary-adrenal (HPA) axis may be another potential mechanisms that is associated with both sleep disturbance and poor health outcomes.[183] The positive association between sleep disturbance and increased risk for mortality and CVD events may also be explained by pre-existing psychiatric conditions, such as depression and posttraumatic stress disorder (PTSD).

It is necessary to comprehensively evaluate the association between objectively measured sleep disturbance and risk of mortality and CVD events. Such research is needed to further elucidate the sleep dimensions of that are most strongly associated with mortality and CVD, and it may shed light on the potential prevention strategies for excess mortality risk and CVD by targeting on sleep disturbance as a modifiable behavioral factor. However, our twins dataset is limited by a lack of objective sleep disturbance assessment at baseline, thus we are unable to assess the prognostic implications of sleep disturbance in predicting adverse health outcomes

including mortality and CVD in this population. In our own dataset, we were only able to assess the association of baseline depression and autonomic dysregulation in predicting mortality and CVD events during follow-up. Future investigations are necessary to evaluate the predictive values of a full spectrum of objectively measured sleep dimensions in predicting adverse events, especially among depressed individuals, using a twin difference design.

2.6 Autonomic Dysregulation and Adverse Health Outcomes

Autonomic dysregulation as indexed by reduced HRV and other ECG metrics, such as deceleration capacity, is an independent predictor of adverse health outcomes including all-cause mortality and CVD, mostly among patients with CVD.[67-70] For example, a meta-analysis of 28 cohort studies in patients with known CVD showed that individuals with a lower HRV had 112% and 46% higher risk of all-cause death and cardiovascular events, respectively.[184] A few prior studies have also assessed the prognostic significance of ECG-derived autonomic metrics in predicting CVD events,[185-187] however data are limited among individuals without known CVD. Prior community-based research in middle-aged to elderly participants,[28,188-190] including individuals without known CVD,[67] also suggested that reduced HRV is associated with an adverse cardiovascular risk profile and an elevated risk of mortality and CVD events, and the elevated risk of mortality could not be attributable to a specific cause.

Prior investigations showed that, among all HRV frequency bands, LF power is the strongest HRV predictor with regard to mortality, and the prognostic implications of baseline HRV still remain after 5 years.[145,188] The power in the LF HRV domain is modulated mainly through sympathetic nervous system (SNS) as a response to oscillations in blood pressure.[191] The reduction in parasympathetic function may be an early sign of autonomic dysregulation, but it

has been hypothesized that impaired sympathetic modulation indexed by reduced LF power may imply a more severe involvement of autonomic nervous system.[145,192-194] Studies also found significant prognostic values in VLF and HF domains in predicting all-cause mortality.[188] Studies that evaluated the predictive values of DC among patients with CVD demonstrated that DC at baseline significantly predicted all-cause mortality.[70,195]

As for cause-specific mortality, prior research showed that the association between reduced HRV and increased risk of mortality may not be specific to CVD causes, and can be largely explained by non-CVD causes, such as cancer.[67,189] It has been reported that sympathetic activation, linked with reduced HRV, may have direct effects on the number, function, and subset distribution of circulating lymphocytes, which play a major role in the immune function and cancer risk.[196,197] Other studies also found significant predictive values of HRV for CVD mortality.[198,199]

Lower HRV in multiple domains has also been linked to increased risk for incident cardiovascular events, in patients with or without known CVD at baseline, and the elevated risk could not be attributable to other risk factors.[28,67,184] Of note, one study has shown that lower HRV significantly predicted a higher risk in patients with MI but not in patients with CHF.[184] Reduced VLF HRV was linked to an increased risk of major adverse CVD events and hospitalizations in maintenance of hemodialysis patients.[185] Similarly, a systematic review found that lower HF and LF domains were associated with higher risk of first CVD events among individuals without known CVD.[186] Another study also showed that one-unit increase in log-ULF was significantly associated with 3.66 increased hazard of cardiovascular events.[200]

However, no prior study has evaluated and compared the prognostic implications of both daytime and nighttime HRV in predicting mortality and CVD events. Physical activity and

mental stressors can influence the measurement of HRV, thus nighttime HRV measuring during sleep may provide useful information compared with HRV assessment based on 24-hour Holter monitoring.[201,202] However, to date no prior study has evaluated and compared the prognostic values of daytime and nighttime HRV frequency domains in predicting mortality. For CVD event, a previous study found that nighttime HRV but not 24-hour HRV was associated with stroke events.[202] It is necessary to investigate and compare both daytime and nighttime HRV and their prognostic implications in predicting adverse health outcomes. Evaluation of the prognostic values of both daytime and nighttime autonomic dysregulation in predicting adverse health outcomes has important clinical and public health implications in implementing better preventive strategies and reducing healthcare burden.

The pathophysiological mechanisms linking reduced HRV and risk for mortality and CVD events still remain unclear. HRV represents the adaptive responses in heart rate caused by fluctuations of both SNS and PNS activities of the autonomic nervous system. Dysfunction of the autonomic nervous system, indexed by reduced HRV, reflects sympathovagal imbalance.[203] It has been hypothesized that sympathovagal imbalance or an overshooting sympathetic activation may be linked to higher risk of mortality and cardiovascular events.[195,204] The higher mortality and CVD incidence associated with lower HRV may also be due to subclinical coronary artery disease. It has also been hypothesized that lower HRV is an indicator of unfavorable general health, such as immune function, which plays a major role in tumor formation and progression.[189] This may explain the association of HRV with increased risk of cancer-specific mortality in previous investigations. DC indicates a measure of cardiac vagal modulation, and prior literature has shown a cardioprotective role of vagal activity, which is linked to a reduced risk of mortality and CVD.[205,206]

2.7 Preliminary Work

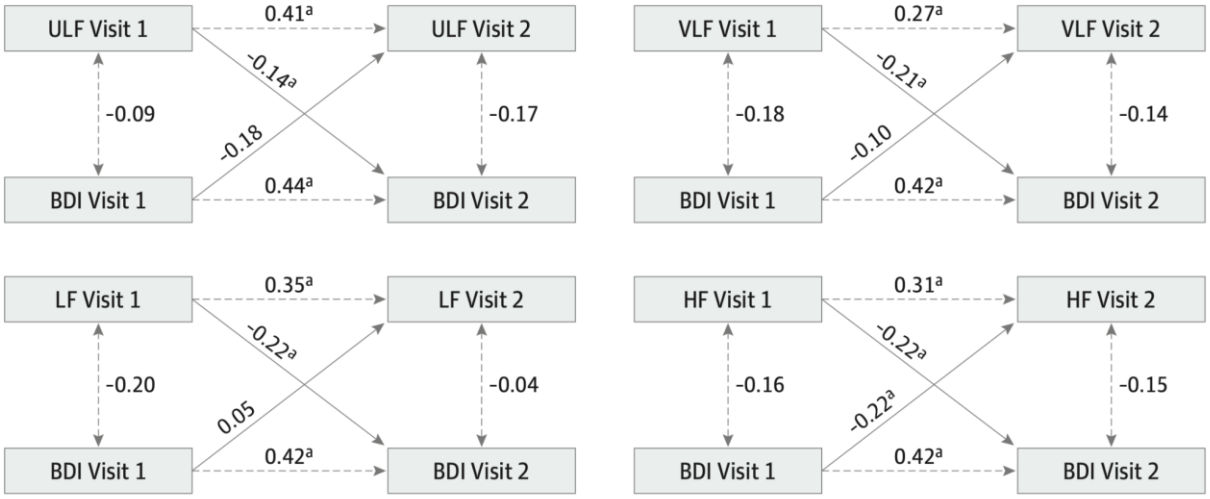
To investigate the temporal directionality of association between depressive symptoms and HRV, the candidate recently conducted a longitudinal cross-lagged twin study to investigate the association in a subsample of our twin study population with repeated measures on these factors.[32]

The study was based on a follow-up of a subgroup of the Vietnam Era Twin Registry twin pairs who participated in the Emory Twin Study.[81,207] A total of 83 pairs completed the in-person follow-up, on average 6.6 years after the baseline visit. Both depressive symptoms and HRV data were collected at baseline assessments from March 2002 to March 2006 (visit 1) and at a 7-year in-person follow-up (visit 2). A total of 73 pairs (n=146) had available depression and HRV data at both visit, thus they represent the analytical population for this study. At both visit, depressive symptoms were measured using the Beck Depression Inventory-II (BDI-II) score, and HRV was measured using 24-hour electrocardiogram (ECG) monitoring. A cross-lagged analysis approach was used to assess the directionality of the association between depressive symptoms and HRV, and within-pair differences in multivariable mixed-effects regression models were examined. We also standardized all β coefficients for both pathways (i.e. from depression to HRV, and from HRV to depression) to allow a comparison of the magnitude of associations. The associations were also evaluated separately in monozygotic and dizygotic twins to examine the role of potential genetic predisposition.

Results showed a consistent association between baseline HRV and depression at follow-up, which was not explained by use of antidepressants at baseline or any other participant characteristics. The magnitude of the association was similar in the opposite pathway linking baseline depression to reduced HRV at follow-up, but this association was largely explained by

antidepressant use. **Figure 2.4** illustrates the cross-lagged association between all HRV domains with BDI score. The associations in the DZ twins were slightly stronger compared to those in the MZ twins, suggesting a potential role of genetic factors on the association.

Figure 2.4 Illustration of the cross-lagged association between all HRV domains and BDI score in fully adjusted models



This research suggests that depressive symptoms and autonomic dysregulation indexed by reduced HRV are bidirectionally associated. The pathway from depression to autonomic dysregulation could be partly mediated by use of antidepressants. These findings highlight the important potential role of the autonomic nervous system in the pathophysiology from depression to adverse health outcomes, and contribute new understanding of the mechanisms underlying the comorbidity of depression and CVD. Our results also suggest that future interventions modulating autonomic nervous system regulation may be useful for the prevention and treatment of cardiovascular events among patients with depression. More investigations are needed to further assess the link between sleep disturbance and autonomic dysregulation, as well

as the role of sleep disturbance on the pathways from depression to adverse outcomes.

In conclusion, preliminary work from our twin study supports our hypotheses of a relationship between depression and autonomic dysregulation. What is needed now is a comprehensive evaluation of such associations using objective measures of sleep and differentiation between daytime and nighttime ANS function; an evaluation of the directionality of the association between autonomic dysregulation and sleep; and a demonstration that depression and autonomic dysregulation, both during the day and night, predicts adverse health outcomes, such as mortality and CVD events.

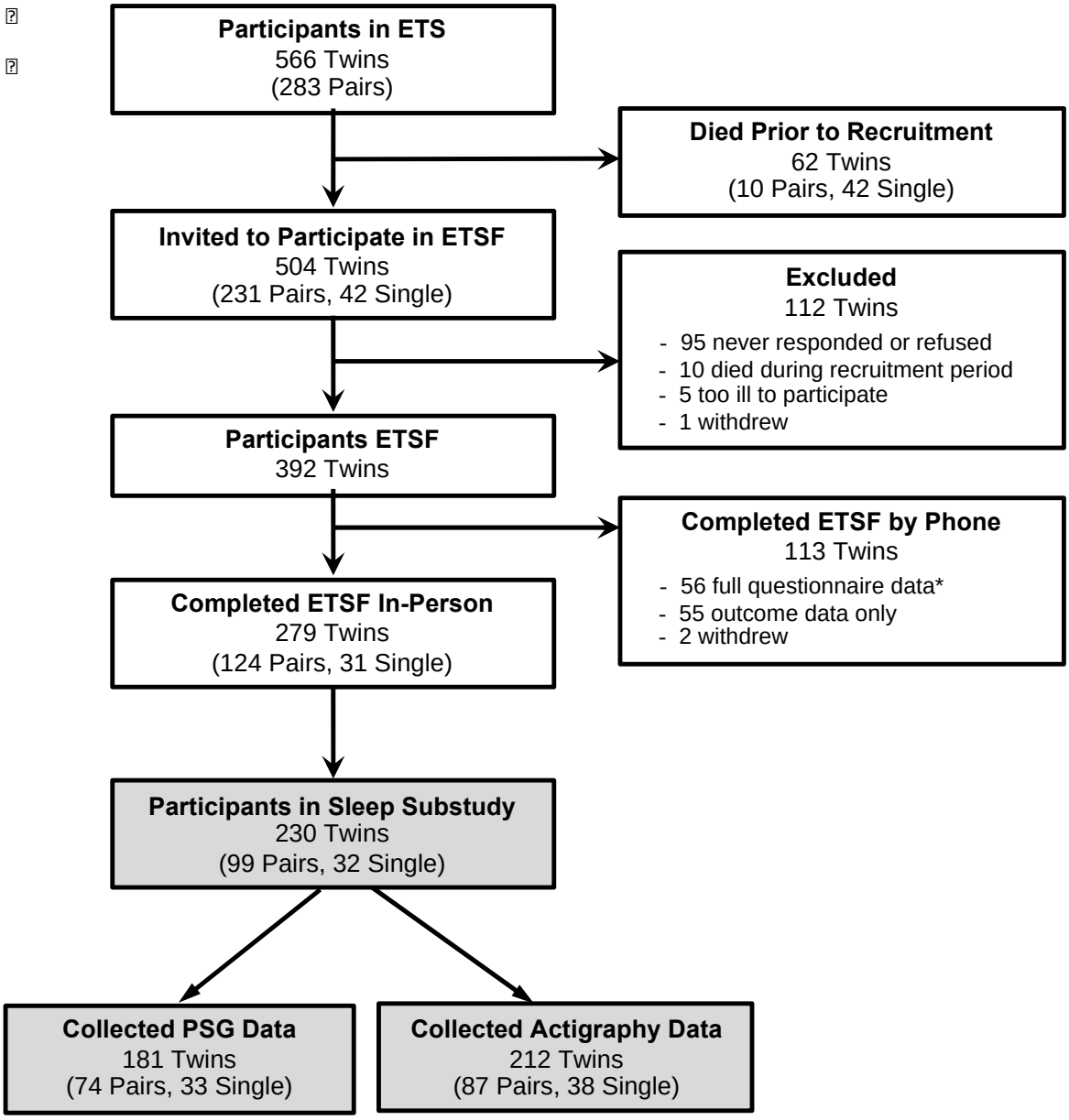
CHAPTER 3: METHODS

3.1 Data Source

The participants in this study were recruited form the Vietnam Era Twin (VET) Registry, which is a national samples of adult male twins from all military branches who served on active duty during the Vietnam War (1964-1975).[208] The present study is based on the 566 twins (283 pairs) recruited from VET Registry and participated in the Emory Twin Study (ETS).[81] The objective of the ETS was to evaluate the role of biological, psychological, and behavioral risk factors in the development of subclinical CVD.[81,82] We included twin pairs who were born between 1946 and 1956, and excluded twin pairs if either member of the twin pair self-reported history of CVD based on previous survey data obtained by the Registry in 1990.[207,209] The twin pairs were discordant for depression or posttraumatic stress disorder (PTSD), or free of these psychiatric conditions as control pairs. The twin pairs were in person (in pairs) examined together between 2002 and 2010, when their mean age was 55 years.[209]

Of the 283 ETS twin pairs, we invited 504 twins to participate in the ETSF, followed for clinical outcomes, for an in-person or phone evaluation that was conducted on average 12 years after the initial assessment. A total of 392 twins participated in ETSF, and among them 279 twins (including 124 pairs and 31 singles) completed the second in-person visit. Of these, 230 twins (99 pairs, 32 singles) were included in the sleep substudy which collected objective sleep data. Self-reported sleep disturbance was available in the entire sample of 124 pairs (248 twins) who completed the in-person ETSF visit. **Figure 3.1** shows the construction of the study population.

Figure 3.1 Participant flow diagram



* 5 additional twins are still in the recruitment phase
Abbreviations: ETS: Emory Twin Study; ETSF: Emory Twin Study Follow-up; PSG: polysomnography

At both ETS and ETSF visits, twin pairs were examined together at Emory University on the same day using identical assessment protocols to minimize measurement error. We obtained twins' comprehensive medical history data during a two-day admission under controlled conditions, and collected blood samples, autonomic function data, anthropometric measurements, behavioral and psychosocial assessments using identical protocols and similar schedule for the two twins. Polysomnography (PSG) sleep data were obtained in the Emory Sleep Center. At the end of the visit, a research coordinator placed the ECG patch and the wrist actigraphy device on each twin for a 7-day home monitoring of ECG and sleep—both devices were returned by mail. Zygosity was obtained and verified by DNA typing.[210] We obtained written informed consent from all twins, and the Emory University institutional review board approved this research.

3.2 Measurements of Depression

At both ETS and ETSF visits, the Beck Depression Inventory-II (BDI-II) was administered to assess the severity of depressive symptoms. The BDI is a validated scale providing a continuous measure of depressive symptoms, including 21 items each scored from 0 to 3, with a total score ranging from 0 to 63.[211-213] A higher BDI indicates more depressive symptoms. In the analysis related to Specific Aim #1, we removed the sleep item in BDI to eliminate the potential influence it may have on the association of BDI with sleep disturbance. At both visit, we also administered the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorder, 4th Edition (DSM-4), or SCID, to obtain a clinical diagnosis of major depressive disorder (MDD). Depression data at the ETSF visit were used for Aims 1 & 2 analyses, and depression data at the baseline visit were used for Aim 3 sensitivity analysis.

3.3 Measurements of Sleep Disturbance

We used both objective and subjective methods to obtain a comprehensive assessment of sleep disturbance. We conducted one-night in-lab PSG at the Emory Sleep Center using a Natus N7000 recording system (Natus, Inc.). PSG is an objective multi-parametric test usually performed overnight that continuously monitors the biophysiological changes that occur during sleep.[214] PSG is necessary to derive measures of sleep stages and architecture, oxygen desaturation, and nighttime muscle activity, and it remains the gold standard for measuring sleep apnea, which is the most common form of SDB. PSG consisted of measurements of frontal, central and occipital electroencephalography, bilateral electrooculography and mentalis surface electromyography to derive measures of sleep architecture and continuity. PSG also included measures of respiratory airflow, respiratory effort, breathing sounds, electrocardiography and surface electromyography recorded above the anterior tibialis of the left and right legs. All scoring was performed by registered technologists who were blind to the participants' clinical data following guidelines of the American Academy of Sleep Medicine.[215] A summary of the PSG and actigraphy variables and their definitions are summarized in **Table 3.1**.

Since prior research was especially inconsistent on whether depression is associated with alterations in sleep architecture, periodic limb movements (PLMS), and sleep-disordered breathing (SDB),[40,41,44,45,126,216,217] we focused on these measures in Aim 1 analysis. We quantified the proportion of total sleep time (TST) spent asleep (sleep efficiency, SE), and the proportion of TST spent in N1, N2, N3, and REM sleep, as well as REM latency (time from the first epoch of sleep to the first epoch of REM including intervening wakefulness). We also analyzed the PLMS index (number of movements per hour of TST). SDB was analyzed using different indices: the apnea/hypopnea index (AHI), respiratory disturbance index (RDI), oxygen

desaturation index (ODI), and percentage of TST with saturated oxygen below 90%. Both AHI and ODI definitions required a drop in saturated oxygen of 4% or greater. The AHI was our primary measure of SDB.

For Aim 2 analysis, we used the following PSG measures: (1) sleep architecture variables: proportions of total sleep time (TST) spent in N1, N2, N3, and REM sleep; (2) the PLMS index; and (3) SDB-related indices, including the apnea/hypopnea index (AHI) and the percentage of TST with oxygen saturation <90%. Although PSG also generates data on sleep efficiency (SE), and wake after sleep onset (WASO), we did not use these data derived from PSG because of their low short-term stability in the context of a single lab night. Instead, we relied on actigraphy, derived from up to 7 nights of data, to provide more stable estimates for these parameters. A total of 181 twins had usable PSG data for Aim 1 & Aim 2 analyses, including 71 pairs (n=142) who were included in the within-pair analysis in Aim 1 analysis.

Table 3.1 Definitions of PSG and actigraphy variables

PSG variables	Definitions
Total sleep time	Total amount of sleep time from sleep onset to sleep offset, in minutes
REM latency	The time from the sleep onset to the first epoch of REM sleep, in minutes
Sleep efficiency	Percentage of total time in bed actually spent in sleep; calculated as sum of stages N1, N2, N3, and REM sleep, divided by the total time in bed and multiplied by 100
Percentage of total sleep time in N1	Percentage of total sleep time in stage N1 sleep; calculated as sleep time in stage N1 divided by the total sleep time and multiplied by 100
Percentage of total sleep time in N2	Percentage of total sleep time in stage N2 sleep; calculated as sleep time in stage N2 divided by the total sleep time and multiplied by 100
Percentage of total sleep time in N3	Percentage of total sleep time in stage N3 sleep; calculated as sleep time in stage N3 divided by the total sleep time and multiplied by 100
Percentage of total sleep time in REM	Percentage of total sleep time in REM sleep; calculated as sleep time in REM divided by the total sleep time and multiplied by 100
Total arousal index	Total number of arousals (i.e. interruptions of sleep lasting 3 to 15 seconds) adjusted per hour of sleep
Periodic leg movement index	Total number of period leg movements (i.e. in series of at least 4 consecutive movement each lasting 0.5-5 seconds and separated by intervals of 4-90 seconds) adjusted per hour of sleep
Apnea/hypopnea index	The number of apnea (i.e. pauses in breathing for at least 10 seconds) and hypopnea (i.e. partial loss of breath for at least 10 seconds) events adjusted per hour of sleep
Respiratory disturbance index	The number of respiratory events, including apnea and hypopnea and respiratory-effort related arousals (RERAs), adjusted per hour of sleep
Oxygen desaturation index	The number of desaturation episodes (i.e. decrease in the mean oxygen saturation of ≥4% over the last 120 seconds that lasts for at least 10 seconds), adjusted per hour of sleep
Percentage of sleep duration with SaO2 <90%	Percentage of total sleep time where arterial oxygen saturation was below 90%
Actigraphy Variables	
Total sleep time	Total amount of sleep time from sleep onset to sleep offset, in minutes
Sleep onset latency	Time between the start of the nocturnal sleep period and the onset of sleep, in minutes
Sleep efficiency	Percentage of total time in bed actually spent in sleep
Wake after sleep onset	Time of wakefulness during the sleep period after sleep onset, in minutes
Fragmentation index	The number of interruptions of sleep by physical movement calculated as 100 x the number of groups of consecutive mobile 30s epochs/by the total number of immobile epochs
SD of sleep duration	Standard deviation of total sleep time over 7-day period
SD of sleep onset timing	Standard deviation of sleep onset timing over 7-day period

Following the PSG, each participant returned home with a wrist-worn actigraph, i.e. Actiwatch Spectrum Pro (Philips-Respironics, Inc.), to derive objective sleep metrics in a naturalistic environment. All participants were instructed to wear the devices on their non-dominant wrist for 7 days. Wrist actigraphy is an accepted measure of rest-activity patterns, and

has been recognized as a useful adjunctive tool in sleep medicine.[65,66] It measures body movement using a motion sensor, which allows inference of various measures of sleep continuity and daytime napping.[218] We used the Actiwatch Spectrum Pro (Philips-Respironics, Inc.), which contains a calibrated accelerometer that records physical movement in 1-minute epochs. Raw actigraphy data (including activity counts and event markers) were first adjudicated using a sleep diary kept by each participant, and then we applied a standardized scoring algorithm to the data,[219] using Actiwatch software (version 6.0), primarily to determine the onset and offset of intended nighttime sleep periods. We derived objective nap data from the actigraphy with the aid of the sleep diary. Both number and duration of naps were obtained.

For selected variables, we derived the mean and within subject standard deviation for analyses. For Aim 1 analysis, our measures of interest included TST (minutes), sleep onset latency (SOL, minutes), SE (%), wake after sleep onset (WASO) (minutes), fragmentation index (%), and two additional SD measures for sleep irregularity: 7-day SD in sleep duration (minutes) and 7-day SD in sleep onset timing (minutes).[220] For Aim 2 analysis, our primary actigraphy measures included: (1) TST, defined as the total number of minutes spent asleep during the night (not including daytime naps); (2) SE, the percentage of the nocturnal sleep period spent asleep; and (3) WASO, the total minutes of wakefulness during the sleep period after sleep onset. A total of 212 twins had usable actigraphy data for Aim 1 and Aim 2 analyses, including 87 pairs (n=174) who were included in the within-pair analysis. Among twins included in the within-pair analysis (n=174), all twins had at least 4 days of actigraphy data; most (n=151, 87%) had the whole 7 days of data, and almost all (n=170, 98%) had at least 6 days of data. Thus, all twins were included in the analysis to maximize sample size.

We also assessed subjective sleep disturbance using the Pittsburgh Sleep Quality Index

(PSQI), a self-reported 19-item questionnaire with excellent psychometric properties, yielding 7 subscales (with a score 0 to 3) and a single total score (with a score 0 to 21).[221] A higher PSQI indicates more sleep disturbance over the previous month. The 7 subscales are: (1) subjective sleep quality; (2) SOL; (3) sleep duration; (4) SE; (5) sleep disturbance; (6) need any medications to sleep; and (7) sleep issues causing any daytime dysfunction. The entire sample of 124 pairs (n=248) who completed the in-person ETSF visit had usable PSQI data.

3.4 Measurements of Autonomic Dysregulation

At ETS, twin wore an ambulatory electrocardiogram monitor for 24 hours. We followed previously published procedures to maximize accuracy of recordings and minimize potential confounding.[222] Both twins in the same pair were evaluated at the same time, and their recording times, schedule, and activity level during each recording were similar. Twins were refrained from smoking, drinking alcohol, and having coffee during measurements. We used frequency-domain methods to analyze the HRV data, utilizing customized software to assign bands of frequency and then count the number of beat-to-beat intervals that match each band.[11,223] Each tape of Holter recordings was digitally processed and analyzed using methods as previously described in the literature,[11,223] and was further segmented into daytime (6am to 10pm) and nighttime (10pm to 6am) periods as determined by time stamps on Holter recording. The HRV spectrum was computed using a fast Fourier transform with a Parzen window on the 24-hour R-R interval file.

We evaluated 24-hour average, as well as daytime and nighttime average values for four discrete frequency bands, including ultra-low frequency (ULF, <0.003 Hz), very low frequency (VLF, 0.0033-0.04 Hz), low frequency (LF, 0.04-0.15 Hz), and high frequency (HF, 0.15-0.40

Hz).[68,143] We also calculated deceleration capacity (DC), which provides an average speed of heart rate deceleration, which is a potentially more useful indicator than other HRV metrics in evaluating parasympathetic nervous function and predicting adverse health outcomes.[70] The HRV data processing was performed blindly to twins' characteristics.

During the clinic visit at ETSF visit, twins wore an ambulatory Holter electrocardiogram (ECG) monitor for 24 hours. We followed previously published procedures to maximize accuracy of recordings and minimize potential confounding.[222] Both twins in the same pair were evaluated at the same time, and their recording times, schedule, and activity level during recording were similar. We used manufacturer's custom-built validated software to extract the raw signal and convert it into WFDB format.[224] Then we extracted RR intervals and computed the frequency domains using a previously validated HRV toolbox from the Clifford lab.[225] Specifically, a signal to quality index (SQI) based on beat detection was computed for each ECG signal.[226] Non-sinus rhythm and beats with SQI lower than 90% were removed to obtain a normal to normal (NN) interval time series. The power spectra of the NN time series was generated using the Lomb periodogram, and frequency domain HRV metrics were calculated on 5 minutes 30 seconds sliding windows on the NN time series signal. Each tape of Holter recordings was digitally processed and analyzed, and was further segmented into sleep (nighttime) and wake (daytime) periods as determined by the beginning and end of the in-lab PSG recording (i.e. day 1, night, and day 2).

We evaluated four discrete frequency domains, including ultra-low frequency (ULF, <0.0033 Hz), very low frequency (VLF, 0.0033-0.04 Hz), low frequency (LF, 0.04-0.15 Hz), and high frequency (HF, 0.15-0.40 Hz).[68,143] We also calculated deceleration capacity (DC), which provides an average speed of heart rate deceleration, and it is potentially more useful than other

HRV metrics in evaluating parasympathetic nervous function and predicting adverse events.[70] A total of 151 twins had usable Holter HRV, including 53 pairs who were included in the within-pair analysis.

During the home monitoring at the ETSF visit, we used the CardeaSolo™ patch, which is a non-invasive and wearable ambulatory ECG monitoring adhesive patch monitor. A study coordinator applied the device over the left pectoral region of each participant's chest, and instructed him to wear the patch for 7 days. ECG data were extracted and processed using the manufacturer's custom-built validated software in the methods that used to process the 24-hour Holter ECG data.[224,225] On each day, four frequency domains (i.e. ULF, VLF, LF, and HF) and DC were obtained, similar to 24-hour Holter recording. Data were also further separated into sleep (nighttime) and wake (daytime) periods as determined by the adjudicated actigraphy data for up to 7 days. Twins with low quality data (e.g. loss of electrode contact, movement artifacts, low SQI <90%, or twins with <75% data) were excluded, reducing the number of subjects with usable HRV home monitoring data to 115 twins, including 34 twin pairs (n=68) who were included in the within-pair analysis. There were no differences between twins who did (n=115) and did not have (n=97) complete HRV assessments in terms of sociodemographic and health-related characteristics.

3.5 Measurements of Adverse Health Outcomes

Vital status data during follow-up, including mortality dates and causes of deaths (e.g. cancer, CVD, etc.), were collected and verified by National Death Index database through December 31st, 2017. All-cause mortality was the primary outcome of this study. Comprehensive medical history data, including all cardiovascular events and hospitalization dates, were obtained

among twins who completed ETSF, either in-person or over the phone. Data on CVD outcomes, including dates and reasons for hospitalizations, were objectively measured and adjudicated by a thorough medical chart review. As a secondary outcome, we evaluated a composite measure of major CVD events, including myocardial infarction (MI), congestive heart failure (CHF), and stroke.

3.6 Other Measurements

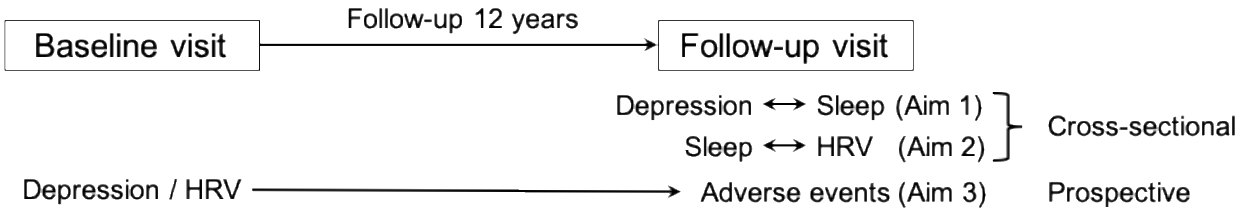
At both ETS and ETSF visits, a thorough assessment including medical history and physical examination were obtained by a research nurse or physician assistant. Sociodemographic and anthropometric data, health behaviors, fasting blood glucose and lipid profile were measured as previously described.[81,207] Habitual physical activity was measured using the Baecke Questionnaire of Habitual Physical Activity. This is a 16-question instrument recording physical activity levels at work, during sports and non-sports activities, rendering a global physical activity score.[227,228] History of hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or self-reported use of anti-hypertensive medications, following the Joint National Committee (JNC)-7 classification for Stage 1 hypertension which was the accepted staging at the time.[229] History of coronary artery disease that might have occurred from the time of the initial screen was also assessed. Diabetes mellitus was defined as having a measured fasting glucose of more than 126 mg/dL or any current treatment with antidiabetic medications. Current use of beta-blockers, antidepressants, statins, and angiotensin-converting enzyme inhibitors were also recorded. A continuous measure of depressive symptoms was assessed by the Beck Depression Inventory-II (BDI-II) score, which includes 21 items each scored from 0 to 3, with a total score ranging from 0 to 63. A clinical

diagnosis of major depression and PTSD (lifetime and current), as well as alcohol abuse disorder, were obtained using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorder, 4th Edition (DSM-IV), or SCID.

3.7 Data Analysis Plan

The available data at baseline visit and during follow-up for each aim were illustrated in **Figure 3.2**. The statistical analysis plan for each aim is also described as following.

Figure 3.2 Available data at baseline and follow-up visits and for each aim



Specific Aim #1

We conducted descriptive analyses by summarizing participants' characteristics, including sociodemographic factors, health-related factors, medication use and sleep measures. We also compared study variables by tertiles of BDI, using ANOVA for continuous variables and chi-squared tests for categorical variables. In a secondary analysis, we compared the same variables in twins with vs. without MDD.

Our primary analysis focused on the associations between the within-pair difference in the BDI and the within-pair difference in sleep disturbance represented by PSG, actigraphy, and PSQI. In a study of twins, within-pair differences intrinsically control for potential confounding by shared genetic and familial influences, as well as environmental factors during the clinic visit

as twins were examined together. For all analyses, we fitted multivariable mixed-effects models and accounted for twin pair as random effect. The BDI was rescaled so that the β coefficients would describe the within-pair change in sleep metrics, per 5-unit difference in within-pair BDI. In the primary analysis, we removed the sleep item from the BDI to eliminate its potential influence on the association with sleep disturbance.

We constructed a series of models to examine the impact of sets of *a priori* selected variables on the association of interest. The base model (model 1) was unadjusted, including only the within-pair difference of the independent variable. We then progressively adjusted for sociodemographic and behavioral variables (education, employment, smoking status, and alcohol abuse) in model 2, and further adjusted for CVD risk factors most likely to be related to both sleep and depression (BMI and history of hypertension) in model 3. In the analysis of actigraphy data, we further adjusted for number of naps and average nap duration during the 7-day period in model 4.

To assess potential shared genetic influence on depression and sleep, we examined MZ and DZ twins separately to examine effect modification by zygosity. Because MZ twin pairs share 100% of their genes while DZ twin pairs only share 50% on average, if a larger association of depression with sleep disturbance is found within DZ pairs than within MZ pairs, this suggests that genetic factors may play a role in the association.

We also conducted a series of sensitivity analyses to expand our primary analytic approach. First, we repeated the analysis including the use of antidepressants as an additionally adjusted variable. Second, we replaced the BDI score with a lifetime history of MDD measured with the SCID. Third, we examined whether the results remained robust after adjustment for PTSD symptoms measured with the PCL-4 or a SCID diagnosis of lifetime history of PTSD.

Fourth, we repeated the analysis using the full BDI, without removal of the sleep item. Finally, we conducted stratified analyses separating somatic and cognitive dimensions of BDI, and used standardized estimates of beta-coefficients to compare these two depression dimensions.

Specific Aim #2

To clarify the data analysis structure for Aim 2, we divided the analysis into 2 different parts: (1) Part I used the clinic visit day data, including the HRV data collected by 24-hour Holter recording, and sleep disturbance data measured by one-night in-lab PSG; (2) Part II used the 7-day at monitoring data, including the HRV data obtained by the adhesive patch ECG monitoring, and sleep disturbance data measured by 7-day at-home wrist actigraphy.

Using PSG and Holter data collected during clinic visit day (Study I), we analyzed the temporal directionality of associations of the within-pair difference in PSG metrics with the within-pair difference in HRV metrics. In a study of twins, within-pair differences intrinsically control for potential confounding by shared genetic factors and early familial background, as well as environmental factors during ambulatory monitoring as twins were examined together. We first evaluated the association of the average within-pair difference in HRV during day 1 (i.e. from start of data collection to nighttime sleep onset) with PSG findings during nighttime. Second, we examined the reverse, i.e., the association of nighttime PSG findings with HRV during day 2, (i.e. from sleep offset to end of data collection), with adjustment for average nighttime HRV data. Illustration of this analysis is shown in Figure 2. For all analyses, we fitted multivariable mixed-effects regression models and accounted for twin pair as a random effect. All models were adjusted for potential confounding factors (smoking status, habitual physical

activity, BMI, history of hypertension, and history of depression, PTSD and alcohol abuse). As HRV data were skewed, logarithmic transformations were used to normalize the distributions.

Using actigraphy and ECG data collected during 7-day home monitoring (Study II), we further evaluated the temporal relationships between sleep measures and HRV in a naturalistic environment. The within-pair analysis included a total of 362 observations (days) reflecting data from 68 twins, and on average, each twin contributed 5.3 observations (days) of data. We fit bivariate vector autoregressive (VAR) models to analyze the longitudinal data.[230] We included each combination of HRV (ULF, VLF, LF, HF, and DC) and sleep metric (TST, SE, and WASO) in separate models. Then for each combination of HRV and sleep measures, we built bivariate VAR models that adjusted for the same potential confounders as in Study I.

In Study II, the recording of multiple 24-hour periods of simultaneous ECG and actigraphy allowed us to model potential temporal causality using time-lagged models. To determine the length of time the association between HRV and sleep was maintained, we built a series of models by adding lagged values of the dependent variables. The Bayesian Information Criterion (BIC) was used for order selection, with lower BIC values indicating better model fit. To formally test whether the associations between HRV and sleep persist beyond a single 24-hour period, we used likelihood ratio tests to compare VAR models with the lowest BIC with the first order models.

After determining the appropriate lag order, to evaluate the temporal directionality of the associations between HRV and sleep, we conducted *F* tests of Granger causality. In a Granger causality test, if the *F*-value is statistically significant, it means that the past values of predictor *X* contain information that helps predict outcome *Y* in addition to the information contained in the past values of *Y* alone, after controlling for other covariates; in other words, *X* "Granger-causes"

or predicts *Y*. We individually tested if each of the HRV metric predicted each of the sleep measure, and vice versa, and conducted VAR models separately for wake and sleep periods to evaluate the day and night difference in the relationship between HRV and sleep. We further conducted mixed-effects regression models to clarify the direction of significant effects after controlling for the same set of covariates as in the VAR models. In these models, predictor variables were expressed as daily variation from individual's averages.

To assess potential shared genetic influence on the association between HRV and sleep disturbance, we examined the associations separately in MZ and DZ twins to evaluate effect modification by zygosity. Because MZ twin pairs share 100% of their genes while DZ twin pairs only share 50% on average, if a larger effect of HRV on sleep or vice versa is observed within DZ pairs than in MZ pairs, then it may suggest that genetic factors play a role in this association.

Specific Aim #3

We conducted descriptive analyses by summarizing participants' characteristics at the baseline visit, sociodemographic factors, health-related factors, medication use, depression status, and 24-hour average HRV. Continuous variables were described as mean and standard deviation (SD), and categorical variables as frequencies (percentage). The HRV data were log-transformed owing to non-normality. We also compared the characteristics among twins who deceased during follow-up to those who survived, using two sample t-test (for continuous variables) and chi-squared test (for categorical variable). Kaplan-Meier curves for all-cause mortality and a composite measure of major CVD events were computed by within-pair difference of depression (i.e. within-pair difference of BDI or MDD >0 vs. <0) or log-HRV (i.e.

within-pair difference of log-HRV >0 vs. <0), and log rank tests were used to compare the survival curves.

Our primary analysis focused on the associations between the within-pair difference in depression and log-HRV with time to all-cause mortality. For all analyses, BDI, MDD and 24-hour average HRV metrics were used as primary predictors of interest, and we also examined daytime and nighttime average HRV metrics. As a secondary analysis, we also evaluated the associations between depression and log-HRV and time to first major CVD events, including MI, CHF, and stroke. In a study of twins, within-pair differences intrinsically control for potential confounding by shared genetic and early familial confounding, as well as environmental factors during ambulatory monitoring as twins were examined together. For all analyses, we fitted multivariable frailty models and accounted for twin pair as random effect. The frailty models are the extensions of the Cox proportional hazard models, with random effect to account for heterogeneity in clustered data (such as in twins dataset).[231] To allow comparisons between different HRV metrics, the HRV metrics were standardized so that the β coefficients can be interpreted as hazard ratios for all-cause mortality or CVD events, per 1-SD increment in log-HRV metrics.

To avoid model overfitting, we constructed a series of models to examine the impact of sets of a priori selected variables on the association of interest. The base model, or model 1, was unadjusted, and only included between-pair difference of HRV metrics. We then progressively adjusted for sociodemographic and behavioral variables (education, employment status, ever smoking status, alcohol abuse, and physical activity) in model 2, and further adjusted for CVD risk factors that are likely related to depression, HRV and adverse health outcomes (BMI, history

of hypertension, history of coronary artery disease, and diabetes) in model 3.[163] In model 4, we additionally adjusted for medication use, including beta-blockers and antidepressants.

To assess potential shared genetic influence on the HRV and adverse health outcome, we examined the associations separately in MZ and DZ twins to evaluate effect modification by zygosity. Because MZ twin pairs share 100% of their genes while DZ twin pairs only share 50% on average, if a larger effect of HRV on adverse health outcomes is observed within DZ pairs than in MZ pairs, then it may suggest that genetic factors play a role in this association.

We also conducted a series of sensitivity analyses to expand our primary analytic approach. First, we examined the association between HRV and cause-specific mortality including cancer and CVD, and further accounted for competing risk due to non-cancer or non-CVD mortality, respectively, using Fine & Gray subhazard models.[232] Second, we repeated all analyses by examining twins as individuals instead of within-pair, to allow an evaluation of potential familial and environmental influence on the associations of interest. Third, we examined whether the results remained robust after additionally adjusting for depression and PTSD diagnosis or symptoms, as well as adjusting for 24-hour average heart rate, as prior research pointed out that the correlation between HRV and mortality could be partially attributable to concurrent change in HR.[233] We also tested the effect modification between depression and HRV by including an interaction term between the two variables in all models.

For analyses in all the Specific Aims, missing data were rare (<5%) for all variables, thus we used all available data without imputation. We checked linearity assumptions of all continuous variables, as well as potential multicollinearity by variance inflation factors. A two-sided p-value of less than 0.05 was used to indicate statistical significance, and hazard ratios and associated 95% confidence intervals (CI) were calculated for model parameters. All statistical

analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC) and Stata 14.0 (StataCorp, College Station, TX).

CHAPTER 4: AIM 1: THE ASSOCIATION BETWEEN DEPRESSIVE SYMPTOMS AND SLEEP DISTURBANCE

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4.1 ABSTRACT

Background: Few studies have comprehensively evaluated the association of depression with both objective and subjective sleep disturbance using a controlled twin study design.

Methods: We studied 246 members of the Vietnam Era Twin Registry. We measured depressive symptoms using the Beck Depression Inventory-II (BDI), and assessed major depression using structured clinical interview. Twins underwent one-night polysomnography and 7-day actigraphy to derive measures of objective sleep disturbance, and completed the Pittsburgh Sleep Quality Index for subjective sleep. Multivariable mixed-effects regression models were used to examine the association of depression with sleep disturbance within twin pairs.

Results: Twins were all male, mostly white (97%), with mean (SD) age of 68 (2) years. The mean (SD) BDI was 5.9 (6.3), and 49 (20%) met criteria for major depression. For polysomnography, each 5-unit higher BDI, within-pair, was significantly associated with 19.7 minutes longer rapid eye movement (REM) sleep latency, and 1.1% shorter REM sleep after multivariable adjustment. The BDI was not associated with sleep architecture or sleep-disordered breathing. For actigraphy, a higher within-pair difference in BDI was significantly associated with lower sleep efficiency, more fragmentation and higher variability in sleep duration. The BDI was associated with almost all dimensions of self-reported sleep disturbance. Results remained consistent using major depression diagnosis instead of BDI and were independent of presence of comorbid posttraumatic stress disorder and antidepressant use.

Conclusions: Depression is associated with REM sleep disruption in lab and sleep fragmentation and sleep variability at home, but not with sleep architecture or sleep-disordered breathing.

4.2 Introduction

Depression is a prevalent psychiatric condition with a lifetime prevalence of 16% in the US.[1-3] Sleep disturbance is a commonly reported symptom of depression,[15,234] and numerous studies have shown an association between depression and self-reported sleep problems.[15,33-36] However, the relationship between depression and sleep disturbance is less consistent when using objective tools to measure sleep, such as polysomnography (PSG) or actigraphy. While some of these studies have reported an association,[37-40] others have not.[41-43] Furthermore, the existing literature is far from consistent regarding the sleep dimensions that may be affected by depression. For example, some studies have linked depressive symptoms with prolonged stage 2 sleep and less rapid eye movement (REM) sleep,[44,45] while others have not found an association between depression and sleep architecture.[41] These inconsistencies may be due to the fact that several studies were limited in sample size, differed in their measurement of depression, or lacked a comprehensive evaluation of objective sleep dimensions.[33,46-48]

A co-twin control study provides a natural "counterfactual" design to examine phenotypic associations, as twins are matched for genetic and early familial factors.[83] The inclusion of both monozygotic (MZ) and dizygotic (DZ) twin pairs allows to examine the genetic influence on the association of interest. Prior twin studies suggest that the link between depression and sleep may be partially explained by shared genetic and familial factors.[71-74] However, these findings were limited to self-reported sleep measures. Currently, it is unknown whether shared genes and familial factors explain the association between depression and objectively measured sleep disturbance.

In a twin study of older veterans, a population highly affected by both depression and sleep disturbance,[235-237] we sought to evaluate whether depressive symptoms and major

depression are related to sleep disturbance utilizing a comprehensive set of subjective and objective sleep measures, and whether genetic and familial factors play a role in the association. We hypothesized that depression is more consistently associated with subjective than objective sleep measures, and that familial factors play a role in this association.

4.3 Methods and Materials

Study Cohort

The subjects of this study were recruited from the Vietnam Era Twin Registry, which is a national sample of 7,369 male twins who served on active duty during the Vietnam war (1964-1975).[80] The present study is based on the sleep substudy of the Emory Twin Study Follow-up (ETSF). ETSF conducted a second visit of the Registry twins who participated in the Emory Twin Study (ETS).[81,207] ETS initially included 283 twin pairs (n=566) born between 1946 and 1956, with no prior history of cardiovascular disease who were examined in person between 2002 and 2010 when their mean age was 55 years.[209] The study included twin pairs discordant for depression or posttraumatic stress disorder (PTSD) based on previous registry surveys, as well as control pairs free of these psychiatric conditions. Of the 283 ETS twin pairs, we invited 504 twins to participate in the ETSF for an in-person or phone evaluation that was conducted on average 12 years after the initial assessment. A total of 392 twins participated in ETSF, and among them 278 twins (including 123 pairs and 32 singles) completed the visit and had available depression data. Of these, 230 twins (99 pairs, 32 singles) were included in the sleep substudy which collected objective sleep data. Self-reported sleep disturbance was available in the entire sample of 123 pairs (246 twins) who completed the in-person ETSF visit. Thus, the latter

represented the analytical sample for this study. **Figure 4.1** shows the construction of the study population.

At the ETSF, all twin pairs were examined together at Emory University on the same day to minimize measurement error. Medical history, anthropometric measurements, behavioral and psychosocial measures, and sleep data were collected using identical protocols and similar schedule for the two twins. Zygosity data were obtained and verified by DNA typing.[210] We obtained written informed consent from all twins, and the Emory University institutional review board approved this study.

Measurements of Depressive Symptoms and Major Depression

The Beck Depression Inventory-II (BDI-II) was administered to assess the severity of depressive symptoms. The BDI is a validated scale providing a continuous measure of depressive symptoms, including 21 items each scored from 0 to 3, with a total score ranging from 0 to 63.[211-213] A higher BDI indicates more depressive symptoms. In this study, we removed the sleep item in BDI to eliminate the potential influence it may have on the association of BDI with sleep disturbance. We also administered the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorder, 4th Edition (DSM-4), or SCID, to obtain a clinical diagnosis of major depressive disorder (MDD). Given the small number of twin pairs discordant for lifetime MDD (37 pairs, n=74, or 30%), we chose the BDI instead of MDD for our primary analysis.

Measurements of Sleep Disturbance

We used both objective and subjective methods to obtain a comprehensive assessment of sleep disturbance. We conducted one-night in-lab PSG at the Emory Sleep Center using a Natus N7000 recording system (Natus, Inc.). PSG consisted of measurements of frontal, central and occipital electroencephalography, bilateral electrooculography and mentalis surface electromyography to derive measures of sleep architecture and continuity. PSG also included measures of respiratory airflow, respiratory effort, breathing sounds, electrocardiography and surface electromyography recorded above the anterior tibialis of the left and right legs. All scoring was performed by registered technologists who were blind to the participants' clinical data following guidelines of the American Academy of Sleep Medicine.[215] Since prior research was especially inconsistent on whether depression is associated with alterations in sleep architecture, periodic limb movements (PLMS), and sleep-disordered breathing (SDB),[40,41,44,45,126,216,217] we focused on these measures. We quantified the proportion of total sleep time (TST) spent asleep (sleep efficiency, SE), and the proportion of TST spent in N1, N2, N3, and REM sleep, as well as REM latency (time from the first epoch of sleep to the first epoch of REM including intervening wakefulness). We also analyzed the PLMS index (number of movements per hour of TST). SDB was analyzed using different indices: the apnea/hypopnea index (AHI), respiratory disturbance index (RDI), oxygen desaturation index (ODI), and percentage of TST with saturated oxygen below 90%. Both AHI and ODI definitions required a drop in saturated oxygen of 4% or greater. The AHI was our primary measure of SDB. A total of 178 twins had usable PSG data, including 71 pairs (n=142) who were included in the within-pair analysis.

Following the PSG, each participant returned home with a wrist-worn actigraph to derive objective sleep metrics in a naturalistic environment. All participants were instructed to wear the devices on their non-dominant wrist for 7 days. Wrist actigraphy is an accepted measure of rest-activity patterns. It measures body movement using a motion sensor, which allows inference of various measures of sleep continuity and daytime napping.[218] We used the Actiwatch Spectrum Pro (Philips-Respironics, Inc.), which contains a calibrated accelerometer that records physical movement in 1-minute epochs. Raw actigraphy data were adjudicated using a sleep diary and a standardized scoring algorithm, primarily to determine the onset and offset of intended nighttime sleep periods. We derived objective nap data from the actigraphy with the aid of the sleep diary. Both number and duration of naps were obtained. For selected variables, we derived the mean and within subject standard deviation for analyses. Our measures included TST (minutes), sleep onset latency (SOL, minutes), SE (%), wake after sleep onset (WASO) (minutes), fragmentation index (%), and two additional SD measures for sleep irregularity: 7-day SD in sleep duration (minutes) and 7-day SD in sleep onset timing (minutes).[220] A total of 212 twins had usable actigraphy data, including 87 pairs (n=174) who were included in the within-pair analysis. All twins had at least 4 days of actigraphy data, thus were all included in the analysis; most (n=151, 87%) had the whole 7 days of data, and almost all (n=170, 98%) had at least 6 days of data.

We assessed subjective sleep disturbance using the Pittsburgh Sleep Quality Index (PSQI), a self-reported 19-item questionnaire with excellent psychometric properties, yielding 7 subscales (with a score 0 to 3) and a single total score (with a score 0 to 21).[221] A higher PSQI indicates more sleep disturbance over the previous month. The 7 subscales are: (1) subjective sleep quality; (2) SOL; (3) sleep duration; (4) SE; (5) sleep disturbance; (6) need any

medications to sleep; and (7) sleep issues causing any daytime dysfunction. The entire sample of 123 pairs (n=246) who completed the in-person ETSF visit had usable PSQI data.

Other Measurements

Medical history and physical examination were obtained by a research nurse or physician assistant. Anthropometric data, fasting blood glucose, lipid profile, and health behaviors were measured as previously described.[11] We used standardized questionnaires to obtain data on sociodemographic factors and health behaviors. Physical activity was measured using the Baecke Questionnaire of Habitual Physical Activity. History of hypertension was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg, or self-reported use of anti-hypertensive medications. History of coronary artery disease that might have occurred from the time of the initial screen, was defined as a previous diagnosis of myocardial infarction or angina pectoris, or previous coronary revascularization procedures. Diabetes mellitus was defined as having a measured fasting glucose of more than 126 mg/dL or being treated with antidiabetic medications. Current use of beta-blockers, antidepressants, statins, angiotensin-converting enzyme inhibitors, and sleep medications were also recorded. A continuous measure of PTSD symptoms was obtained using the PTSD Checklist for DSM-4 (PCL-4), and a binary measure of PTSD diagnosis (lifetime and current), as well as a diagnosis of alcohol abuse disorder, were obtained using the SCID.

Statistical Analysis

We conducted descriptive analyses by summarizing participants' characteristics, including sociodemographic factors, health-related factors, medication use and sleep measures.

We also compared study variables by tertiles of BDI, using ANOVA for continuous variables and chi-squared tests for categorical variables. In a secondary analysis, we compared the same variables in twins with vs. without MDD.

Our primary analysis focused on the associations between the within-pair difference in the BDI and the within-pair difference in sleep disturbance represented by PSG, actigraphy, and PSQI. In a study of twins, within-pair differences intrinsically control for potential confounding by shared genetic and familial influences, as well as environmental factors during the clinic visit as twins were examined together. For all analyses, we fitted multivariable mixed-effects models and accounted for twin pair as random effect. The BDI was rescaled so that the β coefficients would describe the within-pair change in sleep metrics, per 5-unit difference in within-pair BDI. In the primary analysis, we removed the sleep item from the BDI to eliminate its potential influence on the association with sleep disturbance.

We constructed a series of models to examine the impact of sets of *a priori* selected variables on the association of interest. The base model (model 1) was unadjusted, including only the within-pair difference of the independent variable. We then progressively adjusted for sociodemographic and behavioral variables (education, employment, smoking status, and alcohol abuse) in model 2, and further adjusted for CVD risk factors most likely to be related to both sleep and depression (BMI and history of hypertension) in model 3. In the analysis of actigraphy data, we further adjusted for number of naps and average nap duration during the 7-day period in model 4.

To assess potential shared genetic influence on depression and sleep, we examined MZ and DZ twins separately to examine effect modification by zygosity. Because MZ twin pairs share 100% of their genes while DZ twin pairs only share 50% on average, if a larger association

of depression with sleep disturbance is found within DZ pairs than within MZ pairs, this suggests that genetic factors may play a role in the association.

We also conducted a series of sensitivity analyses to expand our primary analytic approach. First, we repeated the analysis including the use of antidepressants as an additionally adjusted variable. Second, we replaced the BDI score with a lifetime history of MDD measured with the SCID. Third, we examined whether the results remained robust after adjustment for PTSD symptoms measured with the PCL-4 or a SCID diagnosis of lifetime history of PTSD. Fourth, we repeated the analysis using the full BDI, without removal of the sleep item. Finally, we conducted stratified analyses separating somatic and cognitive dimensions of BDI, and used standardized estimates of beta-coefficients to compare these two depression dimensions.

Missing data were rare (<5%) for all variables, thus we used all available data without imputation. We assessed linearity of all continuous variables, and evaluated multicollinearity by variance inflation factors. A two-sided p-value of less than 0.05 was used for statistical significance and 95% confidence intervals (CI) were calculated from model parameters. All statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC).

4.4 Results

Participants' Characteristics

To calculate within-pair differences in sleep disturbance, 246 twins (123 pairs) with any usable sleep data were included in the within-pair base sample (**Figure 4.1).** Among these twins, 238 (97%) were white, with a mean age ± SD of 68 ± 2 years at the in-person visit (**Table 4.1**). Of the 246 twins with any sleep data, 76 pairs (n=152, 62%) were MZ twins and 47 pairs (n=94, 38%) were DZ twins. The number of twin pairs discordant for MDD was relatively small, with

19 discordant pairs, 25 pairs, and 37 pairs, respectively, with PSG, actigraphy and PSQI. Thus, the analysis based on MDD discordance status was a secondary analysis.

Depression and Polysomnography Measures of Sleep Disturbance

Twins with higher BDI scores than their brothers, signifying more depressive symptoms, showed longer REM latency assessed with PSG across all models (**Table 4.2**). After multivariable adjustment, a 5-unit higher BDI score was associated with 19.7 minutes (95% CI: 8.6 to 30.8 minutes) longer REM latency. We also observed a tendency for a higher BDI score to be associated with lower REM% (β coefficients per 5-unit higher BDI: -1.1%, 95% CI: -2.1% to -0.1%). BDI was consistently not associated with any SDB-related variables.

These findings were consistent with the analysis that examined MDD instead of BDI. Among 19 MDD-discordant pairs, twins with MDD had significantly longer REM latency (β coefficient=58.7 minutes, 95% CI: 23.8 to 93.6 minutes) and lower REM% (β coefficient= -4.7%, 95% CI: -7.7% to -1.7%), compared to their co-twins without MDD after full covariates adjustment (**Supplement Table S.4.2**). MDD was also significantly associated with more sleep time with oxygen saturation below 90% (β coefficient= 11.1%, 95% CI: 5.1% to 17.2%).

Neither BDI nor MDD, however, were associated with sleep architecture defined as percentage of TST in N1, N2, and N3. Furthermore, neither BDI nor MDD were associated with AHI (**Table 4.2, Table S.4.2**). In contrast, health-related factors, such as higher BMI and history of hypertension were significantly associated with a higher AHI (**Tables S.4.3-S.4.4**).

Depression and Actigraphy Measures of Sleep Disturbance over 7 Days

Within twin pairs, a higher BDI was significantly associated with lower SE, higher fragmentation index, and more irregular sleep duration measured as the SD of sleep duration over 7 days. The BDI, however, was not associated with TST, SOL, WASO, or irregular sleep onset time (**Table 4.3**).

Similar to the PSG analysis, adjustment for other participant characteristics did not affect the results. After multivariable adjustment, a 5-unit increment in BDI was associated with 0.5% lower SE (95% CI: -1.1% to -0.1%), 1.2% higher fragmentation index (95% CI: 0.6% to 1.9%) and 5.8 minutes higher SD of sleep duration (95% CI: 1.7 to 9.9 minutes) (**Table 4.3**). Further adjustment for number of naps during home monitoring and the average nap duration did not materially change the results. The analysis that examined pairs discordant for MDD (n=50 twins) yielded consistent results: twins with MDD compared with their co-twins without MDD had 2.1% lowered SE (95% CI: -3.6% to -0.6%), 2.6% higher fragmentation index (95% CI: 0.5% to 4.8%) and 17.9 minutes higher SD of sleep duration (95% CI: 6.8 to 28.9 minutes) after full covariate adjustment (**Table S.4.5**).

Depression and Self-Reported Measures of Sleep Disturbance

Within twin pairs, a higher BDI was significantly associated with more sleep disturbance measured by self-report with the PSQI across almost all dimensions, after multivariable adjustment (**Table 4.4**). For example, a 5-unit higher BDI was associated with a 1.16-point higher PSQI total score (95% CI: 0.83 to 1.49), and higher PSQI subscales indicating poorer sleep quality, shorter sleep duration, lower SE, more sleep disturbance, need for more

medications to sleep, and more daily dysfunction due to poor sleep (β coefficients ranging from 0.11 to 0.27). Similar results were obtained when examining MDD (**Table S.4.6**).

Additional Analyses

In stratified analysis by zygosity, significant associations between BDI and sleep metrics, including PSG, actigraphy and PSQI, were observed in both MZ and DZ twins, with no material difference in the strength of the association by zygosity (results not shown). The interaction term between zygosity and depression was not significant in any of the models. When we examined different dimensions of BDI, the somatic and cognitive subscales showed similar associations with sleep disturbance (**Tables S.4.7-S.4.9**). Using full BDI without removal of the sleep item did not materially change the results. Further adjustment for antidepressant use, as well as adjustment for PTSD symptoms or lifetime history of PTSD weakened the association between BDI and percentage of TST in REM, which became marginally significant, but overall it did not affect the association between BDI and other sleep metrics (**Tables S.4.10-S.4.12**).

4.5 Discussion

In this co-twin control study, depressive symptoms and MDD were associated with a number of objectively measured indices of sleep disturbance. With PSG, twins with higher levels of depressive symptoms or a history of MDD showed more REM sleep abnormalities than their brothers with fewer depressive symptoms or without MDD, including longer REM latency and lower percentage of TST in REM. Depression measures were also associated with more actigraphy-measured irregular and fragmented sleep, such as lower SE, more fragmentation and higher day-to-day variability of sleep duration. These associations persisted with adjustment for

sociodemographic, behavioral and CVD risk factors. In contrast, depression measures were not associated with several other sleep abnormalities, including TST, indices of SDB, PLMS, sleep architecture, and WASO. The results were similar in both MZ and DZ twins, and the interaction with zygosity was not significant, suggesting the absence of shared genetic and familial influence on the association of depression with sleep abnormalities.

Our findings are consistent with prior PSG studies showing that more depressive symptoms are associated with longer REM latency[38,45] and shorter TST spent in REM.[44,45,238] Some previous studies,[44,45] but not others,[19,234,239] have reported an association of depressive symptoms with increased stage N2 sleep. In our study, none of the sleep architecture variables were associated with depression measures.

In our study, depressive symptoms were not associated with any of the SDB-related measures. This is in agreement with previous studies that examined the relationship of depression with PSG-derived indices of sleep disturbance.[42,126-131] Most of these prior investigations were limited by small sample sizes, inclusion of selected samples of symptomatic patients referred for evaluation, and lack of measures of clinical diagnosis of depression. Thus, we have confirmed these previous finding using a more rigorous design. In contrast to the in-lab PSG results, individuals with depression demonstrated worse actigraphy-measured sleep over a week, especially lower SE and higher fragmentation, as others have also reported.[132-135]

Taken together, our results suggest that depression is primarily related to metrics of sleep disturbance in a natural environment over several days (as measured through actigraphy), but not to disturbed sleep as measured via PSG. PSG conducted in a laboratory environment provides an artificial setting, and may not truly represent the typical sleep pattern of a person, though it is typically used to characterize sleep disorders such as SDB.[240] Our findings are consistent with

the notion that, insofar as depression is concerned, the most salient features of sleep are likely best appreciated over a longer interval of study in the home environment.

Even though we excluded the item on sleep problems from BDI, we observed a much more consistent association of depressive symptoms with subjective rather than objective sleep metrics. This was also reported by others,[41,241] and supports the theory that sleep complaints differ from objective sleep disturbance, and may be more strongly rooted in psychological disorders, such as depression.[242,243] Furthermore, self-reported sleep measures may introduce recall bias which could lead to overestimation of the association between depression and sleep disturbance. Thus, objectively measured sleep metrics may be more useful in measuring sleep disturbance than self-reported measures in the context of psychological disorders like depression.

There are a number of possible mechanisms linking depression with sleep disturbance. First, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis may be implicated, as the HPA axis plays a critical role in sleep regulation as well as in depression.[244,245] Second, some antidepressant drugs can increase the function of monoaminergic systems such as the norepinephrine and serotonin systems, which can suppress REM sleep and prolong REM latency.[216] This is consistent with our findings that adjusting for antidepressant use dampened the strength of the association between depressive symptoms and reduced REM sleep. Third, there could be a shared biological pathway linking depression and sleep, as suggested by previous twin studies based on self-reported sleep measures.[71-74] Using objective measures, however, we did not find evidence that genetic factors play a major role in this association. Fourth, cognitive characteristics of depression could play a role. According to the cognitive model of insomnia, a tendency to worry and ruminate during the day in depressed individuals may extend to the pre-sleep period, and may trigger autonomic arousal and emotional distress.[246] Lastly, the association

of depression with sleep abnormalities could be mediated by autonomic dysregulation. We have previously shown that depressive symptoms and autonomic dysregulation are bidirectionally associated.[32]

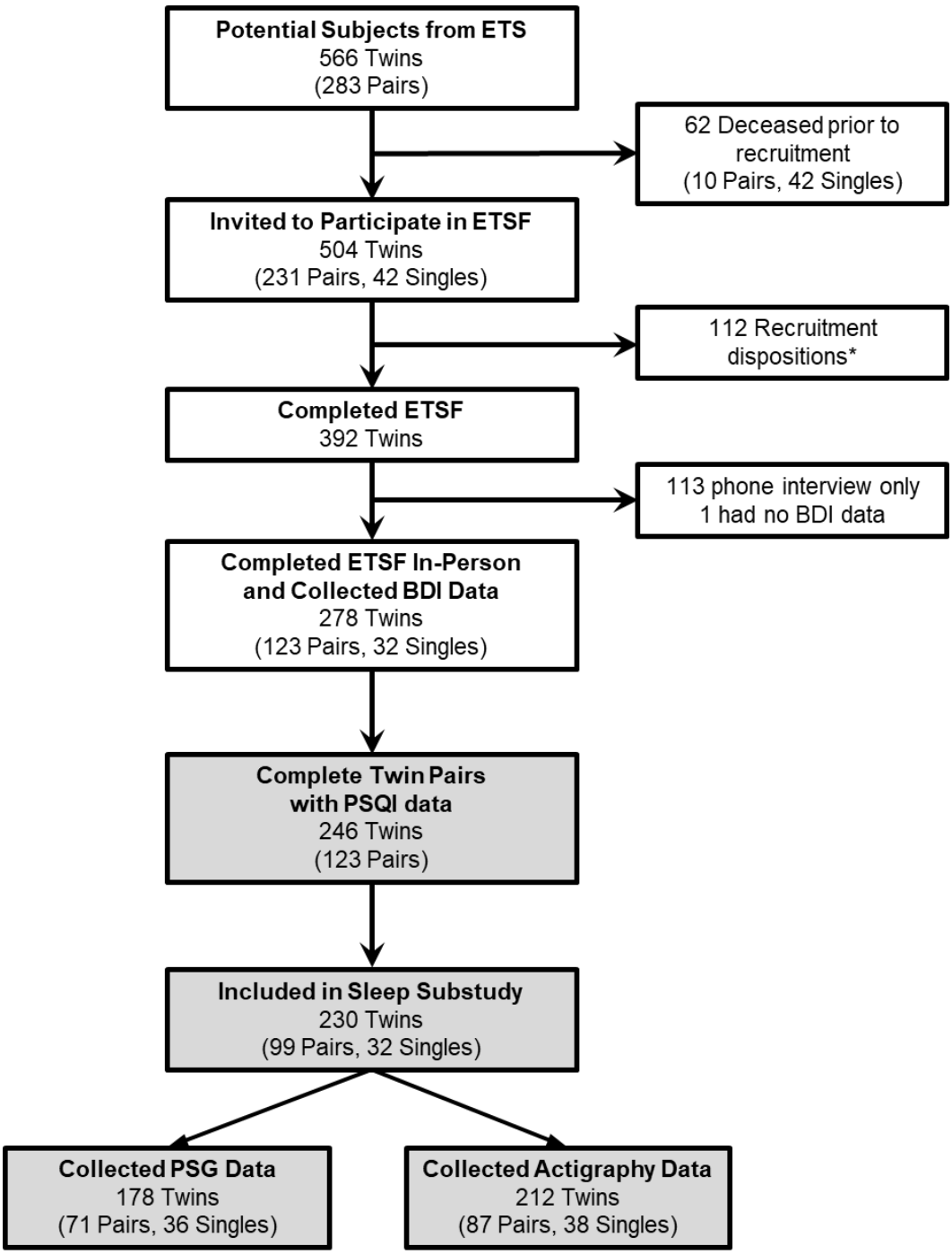
The strengths of this study include the use of multiple objective sleep measures including PSG and actigraphy, and the matched co-twin control study design. To our knowledge, this is the first twin study that evaluated the association of depressive symptoms with objectively measured sleep disturbance using both gold standard, in-lab PSG and in-home actigraphy. The co-twin study design increased precision by providing an internal control (the unaffected twin), and intrinsically controlled for shared genetic and early familial factors. In addition, as twin pairs were assessed together (in lab) and in the same week (at home), confounding from environmental or seasonal/temporal influences on sleep measurements was minimized. Our study also has limitations. First, our sample included mostly white, middle-aged men, thus the generalizability to women and other racial/ethnic groups is limited. However, although a homogenous sample reduces generalizability, it should increase validity, which was a major goal of our study. Second, because of the cross-sectional design, we were unable to assess the directionality of the association. Third, the sample size for MDD-discordant twin pairs was relatively small, limiting our analysis of MDD. However, our results for MDD overall showed a similar pattern as those for the BDI. Future epidemiologic studies with more diverse study populations, a longitudinal design, and a larger sample size are needed to confirm our findings.

4.6 Conclusions

Using a unique approach of objectively measuring multiple dimensions of sleep disturbance in the context of a controlled twin design, we demonstrate that depression is

associated with distinct features of altered sleep. While individuals with depression suffer from sleep disruption in the home environment, such as lower sleep efficiency, more fragmentation, and higher sleep duration variability, their depressive symptoms showed minimal association with SDB or PLMS. Individuals with depression are also vulnerable towards disruption of REM sleep, including longer REM latency and lower percentage of TST in REM. Our twin study shows that genetic and familial factors may not explain these associations. Our findings contribute to clarify the link between depression and sleep disturbance, but future research is needed to evaluate the directionality of this association. Our results help inform future research on prevention and treatment strategies to mitigate sleep disturbance among depressed individuals.

Figure 4.1 Participant Flow Diagram



* Recruitment dispositions: 95 never responded or refused to participate; 10 deceased during recruitment; 5 were too ill to participate; 1 withdrew; 1 incarcerated during recruitment.

Abbreviations: BDI: Beck Depression Inventory; ETS: Emory Twin Study; ETSF: Emory Twin Study Follow-up; PSG: polysomnography; PSQI: Pittsburgh Sleep Quality Index.

Table 4.1 Characteristics of 246 twins by tertiles of BDI score

Characteristics, mean (SD)	Total (N=246)	Tertile 1: BDI (0 – 2) (n=87)	Tertile 2: BDI (3 – 7) (n=89)	Tertile 3: BDI (8 – 44) (n=70)
BDI score	5.9 (6.3)	1.0 (0.8)	4.6 (1.4)	13.8 (6.6)
Sociodemographic factors				
Age, years	68 (2)	68 (2)	68 (3)	68 (3)
White, No. (%)	238 (97)	84 (97)	88 (99)	66 (94)
Education, No. (%)				
High school or less	54 (22)	16 (18)	20 (22)	18 (26)
Some college or associate	109 (44)	42 (48)	36 (40)	31 (44)
College degree	44 (18)	14 (16)	17 (19)	13 (19)
Graduate education or degree	39 (16)	15 (17)	16 (18)	8 (11)
Employed, No. (%)	84 (34)	33 (38)	27 (30)	24 (34)
Health factors				
BMI*	29 (4)	29 (4)	29 (4)	30 (4)
Ever smokers, No. (%)	157 (64)	56 (64)	55 (62)	46 (66)
Alcohol abuse, No. (%)*	56 (23)	14 (16)	17 (19)	25 (36)
Baecke score for physical activity*	7.9 (1.4)	8.2 (1.4)	8.0 (1.4)	7.5 (1.5)
Systolic blood pressure, mmHg	139 (18)	136 (19)	140 (19)	139 (17)
Diastolic blood pressure, mmHg	78 (11)	76 (11)	80 (10)	79 (12)
History of hypertension, No. (%)	146 (59)	45 (52)	54 (61)	47 (67)
History of diabetes, No. (%)*	53 (22)	18 (21)	13 (15)	22 (31)
Family history of CAD, No. (%)	41 (17)	11 (13)	17 (19)	13 (19)
PCL-4 score*	25 (11)	20 (4)	24 (9)	34 (13)
Lifetime history of PTSD, No. (%)*	59 (24)	9 (10)	21 (24)	29 (41)
Lifetime history of depression, No. (%)*	49 (20)	6 (7)	15 (17)	28 (40)
Current PTSD, No. (%)*	34 (14)	0 (0)	14 (16)	20 (29)
Current depression, No. (%)*	17 (7)	0 (0)	1 (1)	16 (23)

Medication use				
β-Blockers, No. (%)	58 (24)	16 (18)	22 (25)	20 (29)
Antidepressants, No. (%)*	37 (15)	3 (3)	9 (10)	25 (36)
Statin, No. (%)	126 (51)	42 (48)	48 (54)	36 (51)
ACE inhibitor, No. (%)	64 (26)	19 (22)	27 (30)	18 (26)
PSG metrics	(n=142)	(n=59)	(n=50)	(n=33)
Total sleep time, minutes	304 (64)	306 (60)	292 (68)	321 (61)
REM latency, minutes	145 (88)	131 (70)	150 (88)	161 (115)
Sleep efficiency, %	68 (14)	69 (13)	66 (16)	71 (14)
Percentage of total sleep time in N1, %	13 (8)	11 (6)	15 (9)	13 (10)
Percentage of total sleep time in N2, %	64 (11)	64 (11)	63 (11)	64 (11)
Percentage of total sleep time in N3, %	8 (10)	9 (10)	7 (9)	8 (11)
Percentage of total sleep time in REM, %	15 (8)	15 (7)	15 (7)	15 (8)
Total arousals index, per sleep hour	35 (22)	33 (22)	35 (23)	38 (21)
Periodic leg movement index, per sleep hour	24 (30)	26 (32)	24 (29)	22 (29)
Apnea/hypopnea index, per sleep hour	17 (19)	14 (16)	18 (19)	21 (23)
Respiratory disturbance index, per sleep hour	31 (23)	27 (23)	32 (22)	36 (25)
Oxygen desaturation index, per sleep hour	16 (18)	13 (15)	17 (18)	19 (21)
Percentage of sleep duration with SaO_2 <90%, %	9 (15)	5 (11)	11 (16)	11 (19)
Actigraphy metrics	(n=174)	(n=64)	(n=63)	(n=47)
Total sleep time, minutes	482 (64)	478 (52)	484 (62)	484 (81)
Sleep onset latency, minutes	8 (7)	7 (5)	8 (7)	8 (8)
Sleep efficiency, %*	88 (6)	90 (4)	88 (5)	88 (8)
Wake after sleep onset, minutes*	49 (25)	42 (17)	53 (25)	52 (32)
Fragmentation index, %*	21 (10)	18 (7)	22 (9)	23 (13)
SD of sleep duration, minute	66 (36)	65 (31)	62 (35)	75 (44)
SD of sleep onset timing, minute	46 (28)	46 (32)	44 (27)	51 (24)
Number of naps during 7 days*	1.2 (1.7)	0.7 (1.2)	1.6 (1.8)	1.5 (2.0)
Average nap duration, minute	34 (50)	26 (53)	36 (49)	41 (46)

PSQI metrics	(n=246)		(n=87)		(n=89)		(n=70)	
PSQI total score*	6.3	(3.6)	4.6	(2.8)	6.5	(3.6)	8.5	(3.5)
PSQI subscale: sleep quality*	1.0	(0.7)	0.7	(0.5)	1.1	(0.7)	1.3	(0.7)
PSQI subscale: sleep latency*	1.2	(1.0)	0.9	(0.9)	1.2	(1.0)	1.4	(1.0)
PSQI subscale: sleep duration*	0.7	(0.9)	0.5	(0.7)	0.7	(0.9)	1.0	(1.1)
PSQI subscale: sleep efficiency*	0.8	(1.1)	0.5	(0.9)	1.0	(1.1)	0.9	(1.1)
PSQI subscale: sleep disturbance*	1.5	(0.6)	1.2	(0.5)	1.5	(0.6)	1.7	(0.7)
PSQI subscale: need meds to sleep*	0.7	(1.2)	0.4	(1.0)	0.5	(1.1)	1.1	(1.4)
PSQI subscale: sleep issues cause dysfunction*	0.7	(0.6)	0.3	(0.5)	0.6	(0.6)	1.1	(0.6)

* Indicates significant difference in participants' characteristics between BDI tertiles at $P < 0.05$. P values were calculated using ANOVA tests (continuous variables) and chi-squared tests (categorical variables).

Abbreviations: ACE: angiotensin-converting enzyme; BDI: Beck Depression Inventory; BMI: body mass index; CAD: coronary artery disease; PCL-4: PTSD Checklist for Diagnostic and Statistical Manual of Mental Disorder, 4^{th} Edition; PSG: polysomnography; PSQI: Pittsburgh Sleep Quality Index; PTSD: posttraumatic stress disorder; REM: rapid eye movement; SaO_2: oxygen saturation; SD: standard deviation.

Table 4.2 Within-pair analysis of the association between BDI score and PSG metrics

PSG sleep metrics[a]	Model 1[b]		Model 2[c]		Model 3[d]	
Total sleep time, minutes	8.2	(-0.5, 16.8)	5.4	(-3.5, 14.3)	5.3	(-3.8, 14.4)
REM latency, minutes	12.8	(1.1, 24.4)*	18.2	(6.6, 29.9)*	19.7	(8.6, 30.8)*
Sleep efficiency, %	1.7	(-0.3, 3.6)	0.7	(-1.2, 2.7)	0.7	(-1.2, 2.7)
Percentage of total sleep time in N1, %	0.1	(-1.0, 1.1)	0.3	(-0.8, 1.3)	0.2	(-0.9, 1.3)
Percentage of total sleep time in N2, %	1.1	(-0.4, 2.6)	1.0	(-0.5, 2.6)	1.2	(-0.2, 2.7)
Percentage of total sleep time in N3, %	-0.7	(-2.0, 0.6)	-0.7	(-2.1, 0.7)	-1.0	(-2.4, 0.4)
Percentage of total sleep time in REM, %	-0.9	(-1.9, 0.1)	-1.2	(-2.2, -0.1)*	-1.1	(-2.1, -0.1)*
Total arousals index, per sleep hour	-0.4	(-2.9, 2.1)	-0.2	(-2.9, 2.4)	-0.5	(-3.1, 2.2)
Periodic leg movement index, per sleep hour	1.8	(-1.4, 5.0)	0.9	(-2.5, 4.3)	1.5	(-1.9, 4.9)
Apnea/hypopnea index, per sleep hour	0.9	(-1.2, 3.0)	0.8	(-1.5, 3.0)	0.9	(-1.2, 3.0)
Respiratory disturbance index, per sleep hour	0.8	(-2.0, 3.7)	1.4	(-1.6, 4.4)	1.4	(-1.5, 4.2)
Oxygen desaturation index, per sleep hour	0.6	(-1.4, 2.7)	0.6	(-1.5, 2.8)	0.7	(-1.3, 2.7)
Percentage of sleep duration with SaO_2 <90%, %	1.0	(-1.2, 3.1)	1.0	(-1.3, 3.2)	1.4	(-0.8, 3.5)

* Indicates significant association at P <0.05.

Abbreviations: BDI: Beck Depression Inventory; BMI: body mass index; PSG: polysomnography; REM: rapid eye movement; SaO_2: oxygen saturation.

[a] Results are shown as β coefficients in the mixed models, per 5-unit increase in BDI score. Sample size N=142 (or 71 pairs).

[b] Base model adjusted for within-pair difference of BDI.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, and alcohol abuse.

[d] Model 3 = Model 2 + BMI and history of hypertension.

Table 4.3 Within-pair analysis of the association between BDI score and actigraphy metrics over 7 days

Actigraphy sleep metrics[a]	Model 1[b]		Model 2[c]		Model 3[d]		Model 4[e]	
Total sleep time, minutes	-0.7	(-8.0, 6.6)	1.6	(-5.3, 8.6)	2.0	(-5.0, 9.0)	2.1	(-4.8, 9.1)
Sleep onset latency, minutes	0.2	(-0.5, 0.9)	0.2	(-0.5, 1.0)	0.2	(-0.5, 1.0)	0.2	(-0.5, 1.0)
Sleep efficiency, %	-0.4	(-1.0, 0.1)	-0.5	(-1.1, -0.1)*	-0.5	(-1.1, -0.1)*	-0.5	(-1.1, -0.1)*
Wake after sleep onset, minutes	1.2	(-1.0, 3.4)	2.0	(-0.2, 4.2)	2.0	(-0.2, 4.2)	1.9	(-0.3, 4.1)
Fragmentation index, %	1.3	(0.6, 2.0)*	1.3	(0.6, 2.0)*	1.2	(0.6, 1.9)*	1.2	(0.5, 1.9)*
SD of sleep duration, minute	5.1	(1.0, 9.2)*	5.4	(1.4, 9.5)*	5.8	(1.7, 9.9)*	6.1	(2.0, 10.1)*
SD of sleep onset timing, minute	-0.8	(-4.4, 2.9)	-0.1	(-3.2, 3.1)	0.1	(-3.1, 3.2)	0.1	(-3.1, 3.2)

* Indicates significant association at P <0.05.

Abbreviations: BDI: Beck Depression Inventory; BMI: body mass index; SD: standard deviation.

[a] Results are shown as β coefficients in the mixed models, per 5-unit increase in BDI score. Sample size N=174 (or 87 pairs).

[b] Base model adjusted for within-pair difference of BDI.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, and alcohol abuse.

[d] Model 3 = Model 2 + BMI and history of hypertension.

[e] Model 4 = Model 3 + number of naps and average nap duration.

Table 4.4 Within-pair analysis of the association between BDI score and PSQI metrics

PSQI sleep metrics[a]	Model 1[b]		Model 2[c]		Model 3[d]	
PSQI total score	1.10	(0.78, 1.42)*	1.16	(0.83, 1.50)*	1.16	(0.83, 1.49)*
PSQI subscale: sleep quality	0.20	(0.14, 0.27)*	0.23	(0.16, 0.30)*	0.23	(0.16, 0.30)*
PSQI subscale: sleep latency	0.09	(-0.01, 0.19)	0.09	(-0.01, 0.19)	0.09	(-0.01, 0.19)
PSQI subscale: sleep duration	0.13	(0.04, 0.23)*	0.16	(0.07, 0.25)*	0.16	(0.07, 0.25)*
PSQI subscale: sleep efficiency	0.08	(-0.02, 0.19)	0.11	(0.01, 0.22)*	0.11	(0.01, 0.22)*
PSQI subscale: sleep disturbance	0.16	(0.11, 0.22)*	0.16	(0.11, 0.22)*	0.16	(0.10, 0.22)*
PSQI subscale: need meds to sleep	0.17	(0.05, 0.29)*	0.14	(0.01, 0.27)*	0.13	(0.01, 0.26)*
PSQI subscale: sleep issues cause dysfunction	0.27	(0.22, 0.33)*	0.27	(0.22, 0.33)*	0.27	(0.22, 0.33)*

* Indicates significant association at $P < 0.05$.

Abbreviations: BDI: Beck Depression Inventory; BMI: body mass index; PSQI: Pittsburgh Sleep Quality Index.

[a] Results are shown as β coefficients in the mixed models, per 5-unit increase in BDI score. Sample size N=246 (or 123 pairs).

[b] Base model adjusted for within-pair difference of BDI.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, and alcohol abuse.

[d] Model 3 = Model 2 + BMI and history of hypertension.

Table S.4.1 Characteristics of 246 twins by lifetime history of MDD status

Characteristics, mean (SD)	Total (N=246)		MDD (n=49)		No MDD (n=197)	
Sociodemographic factors						
Age, years	68	(2)	67	(2)	68	(2)
White, No. (%)*	238	(97)	45	(92)	193	(98)
Education, No. (%)						
High school or less	54	(22)	11	(22)	43	(22)
Some college or associate	109	(44)	20	(41)	89	(45)
College degree	44	(18)	11	(22)	33	(17)
Graduate education or degree	39	(16)	7	(14)	32	(16)
Employed, No. (%)	84	(34)	15	(31)	69	(35)
Health factors						
BMI	29	(4)	29	(5)	29	(4)
Ever smokers, No. (%)	157	(64)	36	(73)	121	(61)
Alcohol abuse, No. (%)*	56	(23)	21	(43)	35	(18)
Baecke score for physical activity	7.9	(1.4)	7.8	(1.5)	7.9	(1.4)
Systolic blood pressure, mmHg	139	(18)	138	(19)	139	(18)
Diastolic blood pressure, mmHg	78	(11)	80	(14)	78	(10)
History of hypertension, No. (%)	146	(59)	33	(67)	113	(57)
History of diabetes, No. (%)	53	(22)	9	(18)	44	(22)
Family history of CAD, No. (%)	41	(17)	9	(18)	32	(16)
BDI score*	5.9	(0.5)	11.2	(1.3)	4.6	(0.3)
PCL-4 score*	25	(11)	38	(13)	23	(8)
Lifetime history of PTSD, No. (%)*	59	(24)	30	(61)	29	(15)
Current PTSD, No. (%)*	34	(14)	24	(49)	10	(5)
Current depression, No. (%)*	17	(7)	17	(35)	0	(0)
Medication use						
β-Blockers, No. (%)	58	(24)	10	(20)	48	(24)
Antidepressants, No. (%)*	37	(15)	23	(47)	14	(7)
Statin, No. (%)	126	(51)	19	(39)	107	(54)
ACE inhibitor, No. (%)	64	(26)	13	(27)	51	(26)
PSG metrics	(N=142)		(n=25)		(n=117)	
Total sleep time, minutes	304	(64)	317	(70)	302	(62)
REM latency, minutes*	145	(88)	186	(123)	137	(78)
Sleep efficiency, %	68	(14)	71	(16)	68	(14)
Percentage of total sleep time in N1, %	13	(8)	15	(11)	12	(7)
Percentage of total sleep time in N2, %	64	(11)	66	(11)	63	(11)
Percentage of total sleep time in N3, %	8	(10)	6	(9)	9	(10)
Percentage of total sleep time in REM, %	15	(8)	13	(9)	16	(7)
Total arousals index, per sleep hour	35	(22)	37	(25)	34	(22)
Periodic leg movement index, per sleep hour	24	(30)	29	(34)	23	(29)
Apnea/hypopnea index, per sleep hour	17	(19)	21	(26)	17	(17)
Respiratory disturbance index, per sleep hour	31	(23)	35	(28)	30	(22)
Oxygen desaturation index, per sleep hour	16	(18)	19	(24)	15	(16)
Percentage of sleep duration with SaO_2 <90%, %	9	(15)	12	(20)	8	(14)

Actigraphy metrics	(N=174)	(n=33)	(n=141)
Total sleep time, minutes	482 (64)	482 (91)	482 (57)
Sleep onset latency, minutes	8 (7)	8 (8)	8 (6)
Sleep efficiency, %	88 (6)	88 (7)	89 (5)
Wake after sleep onset, minutes	49 (25)	52 (29)	48 (24)
Fragmentation index, %	21 (10)	43 (17)	41 (15)
SD of sleep duration, minute*	66 (36)	79 (47)	63 (33)
SD of sleep onset timing, minute	46 (28)	52 (24)	45 (29)
Number of naps during 7 days	1.2 (1.7)	1.5 (2.0)	1.2 (1.7)
Average nap duration, minute	34 (50)	35 (42)	33 (52)
PSQI metrics	(N=246)	(n=49)	(n=197)
PSQI total score*	6.3 (3.6)	8.4 (3.7)	5.8 (3.5)
PSQI subscale: sleep quality*	1.0 (0.7)	1.3 (0.7)	0.9 (0.7)
PSQI subscale: sleep latency	1.2 (1.0)	1.4 (1.0)	1.1 (1.0)
PSQI subscale: sleep duration*	0.7 (0.9)	1.0 (1.1)	0.6 (0.9)
PSQI subscale: sleep efficiency	0.8 (1.1)	0.9 (1.1)	0.8 (1.1)
PSQI subscale: sleep disturbance*	1.5 (0.6)	1.7 (0.6)	1.4 (0.6)
PSQI subscale: need meds to sleep*	0.7 (1.2)	1.1 (1.4)	0.6 (1.1)
PSQI subscale: sleep issues cause dysfunction*	0.7 (0.6)	1.2 (0.7)	0.5 (0.6)

Abbreviations: ACE: angiotensin-converting enzyme; BDI: Beck Depression Inventory; BMI: body mass index; CAD: coronary artery disease; MDD: major depressive disorder; PCL-4: PTSD Checklist for Diagnostic and Statistical Manual of Mental Disorder, 4th Edition; PSG: polysomnography; PSQI: Pittsburgh Sleep Quality Index; PTSD: posttraumatic stress disorder; SaO_2: oxygen saturation; SD: standard deviation.

* Indicates significant difference between MDD status at $P < 0.05$. *P* values were calculated using two-sample *t* test (continuous variables) and chi-squared test (categorical variables).

Table S.4.2 Within-pair analysis of the association between MDD and PSG metrics

PSG sleep metrics[a]	Model 1[b]		Model 2[c]		Model 3[d]	
Total sleep time, minutes	11.1	(-15.0, 37.2)	9.1	(-17.6, 35.7)	9.6	(-17.1, 36.2)
REM latency, minutes	30.1	(-5.6, 65.9)	54.1	(18.4, 89.8)*	58.7	(23.8, 93.6)*
Sleep efficiency, %	2.4	(-3.4, 8.2)	1.0	(-4.9, 6.9)	1.2	(-4.7, 7.1)
Percentage of total sleep time in N1, %	1.2	(-1.9, 4.3)	0.3	(-2.9, 3.5)	0.1	(-3.0, 3.3)
Percentage of total sleep time in N2, %	0.5	(-4.3, 5.3)	1.8	(-3.2, 6.7)	1.9	(-2.9, 6.7)
Percentage of total sleep time in N3, %	-1.0	(-4.8, 2.8)	-1.4	(-5.4, 2.6)	-1.5	(-5.4, 2.5)
Percentage of total sleep time in REM, %	-3.6	(-6.6, -0.6)*	-4.4	(-7.5, -1.4)*	-4.7	(-7.7, -1.7)*
Total arousals index, per sleep hour	-2.8	(-10.3, 4.7)	-3.4	(-11.3, 4.5)	-2.9	(-10.6, 4.8)
Periodic leg movement index, per sleep hour	4.4	(-5.3, 14.0)	3.6	(-6.5, 13.8)	3.7	(-6.4, 13.7)
Apnea/hypopnea index, per sleep hour	3.9	(-2.6, 10.4)	4.1	(-2.7, 10.9)	4.7	(-1.7, 11.1)
Respiratory disturbance index, per sleep hour	0.6	(-8.0, 9.2)	1.0	(-8.0, 10.0)	1.9	(-6.6, 10.3)
Oxygen desaturation index, per sleep hour	3.0	(-3.2, 9.2)	3.1	(-3.4, 9.6)	3.6	(-2.5, 9.8)
Percentage of sleep duration with SaO_2 <90%, %	9.1	(2.7, 15.5)*	10.5	(4.0, 17.0)*	11.1	(5.1, 17.2)*

Abbreviations: BMI: body mass index; MDD: major depressive disorder; PSG: polysomnography; REM: rapid eye movement; SaO_2: oxygen saturation.

* Indicates significant association at P <0.05.

[a] Results are shown as β coefficients in the mixed models, comparing MDD to non-MDD. Sample size N=38 (or 19 pairs discordant for MDD).

[b] Base model adjusted for within-pair difference of MDD.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, and alcohol abuse.

[d] Model 3 = Model 2 + BMI and history of hypertension.

Table S.4.3 Within-pair analysis of the multivariable association between BDI score and AHI

Variables[a]		
BDI, per 5-unit increase	0.9	(-1.2, 3.0)
Education	-1.2	(-4.7, 2.2)
Employment	1.4	(-4.3, 7.1)
Ever smoking	1.0	(-5.2, 7.1)
Alcohol abuse	2.4	(-4.9, 9.7)
BMI	0.8	(0.1, 1.6)*
History of hypertension	7.8	(2.3, 13.2)*

Abbreviations: AHI: apnea/hypopnea index; BDI: beck depression inventory; BMI: body mass index.

* Indicates significant association at $P < 0.05$.

[a] Results are shown as β coefficients in the mixed models, per 5-unit increase in BDI score. Sample size N=142 (or 71 pairs).

Table S.4.4 Within-pair analysis of the association between MDD and AHI

Variables[a]		
MDD	4.7	(-1.7, 11.1)
Education	-1.5	(-4.9, 1.9)
Employment	2.3	(-3.4, 7.9)
Ever smoking	0.1	(-6.3, 6.4)
Alcohol abuse	2.6	(-4.4, 9.6)
BMI	0.8	(0.1, 1.5)*
History of hypertension	8.0	(2.6, 13.4)*

Abbreviations: AHI: apnea/hypopnea index; BMI: body mass index; MDD: major depressive disorder.

* Indicates significant association at $P < 0.05$.

[a] Results are shown as β coefficients in the mixed models, comparing MDD to non-MDD. Sample size N=38 (or 19 pairs discordant for MDD).

Table S.4.5 Within-pair analysis of the association between MDD and actigraphy metrics

Actigraphy sleep metrics[a]	Model 1[b]		Model 2[c]		Model 3[d]		Model 4[e]	
Total sleep time, minutes	-3.2	(-24.0, 17.6)	-9.4	(-28.6, 9.7)	-10.4	(-29.5, 8.8)	-10.8	(-29.8, 8.2)
Sleep onset latency, minutes	0.1	(-2.0, 2.2)	0.4	(-1.8, 2.5)	0.4	(-1.7, 2.6)	0.4	(-1.7, 2.6)
Sleep efficiency, %	-1.6	(-3.1, -0.1)*	-2.0	(-3.5, -0.5)*	-2.1	(-3.6, -0.6)*	-2.1	(-3.6, -0.6)*
Wake after sleep onset, minutes	4.8	(-1.5, 11.1)	5.4	(-0.9, 11.7)	5.6	(-0.7, 11.9)	5.5	(-0.8, 11.8)
Fragmentation index, %	2.5	(0.4, 4.7)*	2.6	(0.4, 4.8)*	2.7	(0.5, 4.8)*	2.6	(0.5, 4.8)*
SD of sleep duration, minute	15.3	(3.9, 26.6)*	17.4	(5.9, 28.9)*	17.9	(6.6, 29.2)*	17.9	(6.8, 28.9)*
SD of sleep onset timing, minute	2.5	(-8.1, 13.1)	6.1	(-3.8, 16.1)	6.8	(-3.3, 16.8)	6.7	(-3.3, 16.7)

Abbreviations: BMI: body mass index; MDD: major depressive disorder; SD: standard deviation.

* Indicates significant association at $P < 0.05$.

[a] Results are shown as β coefficients in the mixed models, comparing MDD to non-MDD. Sample size N=50 (or 25 pairs discordant for MDD).

[b] Base model adjusted for within-pair difference of MDD.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, and alcohol abuse.

[d] Model 3 = Model 2 + BMI and history of hypertension.

[e] Model 4 = Model 3 + number of naps and average nap duration.

Table S.4.6 Within-pair analysis of the association between MDD and PSQI metrics

PSQI sleep metrics[a]	Model 1[b]		Model 2[c]		Model 3[d]	
PSQI total score	1.18	(0.22, 2.14)*	1.17	(0.17, 2.16)*	1.20	(0.21, 2.19)*
PSQI subscale: sleep quality	0.26	(0.05, 0.47)*	0.27	(0.06, 0.49)*	0.27	(0.06, 0.49)*
PSQI subscale: sleep latency	-0.25	(-0.52, 0.02)	-0.26	(-0.54, 0.02)	-0.25	(-0.53, 0.03)
PSQI subscale: sleep duration	0.22	(-0.04, 0.48)	0.26	(-0.01, 0.52)	0.26	(-0.01, 0.52)
PSQI subscale: sleep efficiency	0.09	(-0.21, 0.39)	0.05	(-0.25, 0.36)	0.05	(-0.26, 0.35)
PSQI subscale: sleep disturbance	0.14	(-0.02, 0.31)	0.09	(-0.08, 0.26)	0.09	(-0.07, 0.26)
PSQI subscale: need meds to sleep	0.33	(-0.01, 0.68)	0.24	(-0.12, 0.59)	0.26	(-0.09, 0.61)
PSQI subscale: sleep issues cause dysfunction	0.52	(0.36, 0.69)*	0.56	(0.40, 0.73)*	0.56	(0.40, 0.73)*

Abbreviations: BMI: body mass index; MDD: major depressive disorder; PSQI: Pittsburgh Sleep Quality Index.

* Indicates significant association at $P < 0.05$.

[a] Results are shown as β coefficients in the mixed models, comparing MDD to non-MDD. Sample size N=74 (or 37 pairs discordant for MDD).

[b] Base model adjusted for within-pair difference of MDD.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, and alcohol abuse.

[d] Model 3 = Model 2 + BMI and history of hypertension.

Table S.4.7 Within-pair analysis of the association of BDI somatic and cognitive subscales with PSG metrics

PSG sleep metrics[a]	Model 1[b]		Model 2[c]		Model 3[d]	
BDI Somatic Subscale						
Total sleep time, minutes	0.15	(-0.02, 0.32)	0.09	(-0.08, 0.27)	0.09	(-0.08, 0.27)
REM latency, minutes	0.16	(-0.01, 0.33)	0.25	(0.09, 0.42)*	0.27	(0.11, 0.43)*
Sleep efficiency, %	0.15	(-0.01, 0.32)	0.07	(-0.10, 0.24)	0.07	(-0.10, 0.24)
Percentage of total sleep time in N1, %	-0.01	(-0.18, 0.17)	0.01	(-0.17, 0.19)	0.01	(-0.17, 0.19)
Percentage of total sleep time in N2, %	0.07	(-0.09, 0.23)	0.08	(-0.09, 0.24)	0.09	(-0.06, 0.25)
Percentage of total sleep time in N3, %	-0.01	(-0.18, 0.16)	0.01	(-0.18, 0.19)	-0.02	(-0.20, 0.16)
Percentage of total sleep time in REM, %	-0.15	(-0.32, 0.02)	-0.22	(-0.39, -0.04)*	-0.21	(-0.38, -0.04)*
Total arousals index, per sleep hour	0.01	(-0.16, 0.19)	0.02	(-0.16, 0.21)	0.02	(-0.16, 0.19)
Periodic leg movement index, per sleep hour	0.05	(-0.12, 0.22)	0.01	(-0.18, 0.18)	0.03	(-0.15, 0.21)
Apnea/hypopnea index, per sleep hour	0.11	(-0.06, 0.28)	0.09	(-0.08, 0.27)	0.11	(-0.06, 0.27)
Respiratory disturbance index, per sleep hour	0.09	(-0.08, 0.26)	0.11	(-0.07, 0.29)	0.11	(-0.05, 0.28)
Oxygen desaturation index, per sleep hour	0.08	(-0.08, 0.25)	0.07	(-0.10, 0.25)	0.09	(-0.08, 0.25)
Percentage of sleep duration with SaO_2 <90%, %	0.13	(-0.04, 0.30)	0.12	(-0.06, 0.29)	0.15	(-0.02, 0.31)
BDI Cognitive Subscale						
Total sleep time, minutes	0.15	(-0.02, 0.32)	0.10	(-0.07, 0.28)	0.10	(-0.08, 0.28)
REM latency, minutes	0.18	(0.02, 0.35)*	0.23	(0.06, 0.40)*	0.25	(0.09, 0.41)*
Sleep efficiency, %	0.11	(-0.06, 0.28)	0.04	(-0.13, 0.22)	0.04	(-0.13, 0.22)
Percentage of total sleep time in N1, %	0.03	(-0.14, 0.20)	0.08	(-0.10, 0.26)	0.07	(-0.11, 0.26)
Percentage of total sleep time in N2, %	0.15	(-0.01, 0.31)	0.14	(-0.02, 0.30)	0.16	(0.01, 0.32)*
Percentage of total sleep time in N3, %	-0.19	(-0.36, -0.02)*	-0.23	(-0.41, -0.05)*	-0.26	(-0.44, -0.08)*
Percentage of total sleep time in REM, %	-0.10	(-0.27, 0.06)	-0.13	(-0.30, 0.05)	-0.11	(-0.29, 0.06)
Total arousals index, per sleep hour	-0.09	(-0.26, 0.08)	-0.07	(-0.26, 0.11)	-0.10	(-0.28, 0.08)
Periodic leg movement index, per sleep hour	0.14	(-0.03, 0.31)	0.10	(-0.08, 0.28)	0.14	(-0.04, 0.32)
Apnea/hypopnea index, per sleep hour	0.01	(-0.16, 0.17)	0.01	(-0.18, 0.18)	0.01	(-0.17, 0.17)
Respiratory disturbance index, per sleep hour	-0.02	(-0.19, 0.15)	0.03	(-0.15, 0.21)	0.02	(-0.15, 0.19)
Oxygen desaturation index, per sleep hour	-0.01	(-0.17, 0.16)	0.01	(-0.17, 0.18)	0.01	(-0.16, 0.18)
Percentage of sleep duration with SaO_2 <90%, %	-0.02	(-0.19, 0.15)	-0.01	(-0.18, 0.17)	0.02	(-0.15, 0.19)

Abbreviations: BDI: Beck Depression Inventory; BMI: body mass index; PSG: polysomnography; REM: rapid eye movement; SaO_2: oxygen saturation.

* Indicates significant association at P <0.05.

[a] Results are shown as standardized β coefficients in the mixed models, per 1 SD increase in standardized BDI somatic or cognitive subscales. Sample size N=142 (or 71 pairs).

[b] Base model adjusted for standardized within-pair difference of BDI.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, and alcohol abuse.

[d] Model 3 = Model 2 + BMI and history of hypertension.

Table S.4.8 Within-pair analysis of the association of BDI somatic and cognitive subscales with actigraphy metrics

Actigraphy sleep metrics[a]	**Model 1**[b]		**Model 2**[c]		**Model 3**[d]		**Model 4**[e]	
BDI Somatic Subscale								
Total sleep time, minutes	-0.01	(-0.16, 0.14)	0.02	(-0.12, 0.16)	0.03	(-0.11, 0.17)	0.03	(-0.11, 0.17)
Sleep onset latency, minutes	0.06	(-0.09, 0.20)	0.07	(-0.08, 0.22)	0.07	(-0.08, 0.22)	0.07	(-0.08, 0.22)
Sleep efficiency, %	-0.15	(-0.30, -0.01)*	-0.18	(-0.32, -0.03)*	-0.17	(-0.32, -0.03)*	-0.17	(-0.32, -0.02)*
Wake after sleep onset, minutes	0.12	(-0.03, 0.26)	0.16	(0.02, 0.30)*	0.16	(0.01, 0.30)*	0.16	(0.01, 0.30)*
Fragmentation index, %	0.28	(0.15, 0.41)*	0.28	(0.15, 0.42)*	0.27	(0.14, 0.40)*	0.26	(0.14, 0.39)*
SD of sleep duration, minute	0.21	(0.06, 0.36)*	0.22	(0.08, 0.37)*	0.23	(0.08, 0.37)*	0.24	(0.10, 0.39)*
SD of sleep onset timing, minute	0.03	(-0.11, 0.17)	0.06	(-0.07, 0.18)	0.06	(-0.07, 0.18)	0.06	(-0.07, 0.18)
BDI Cognitive Subscale								
Total sleep time, minutes	-0.01	(-0.16, 0.14)	0.05	(-0.09, 0.19)	0.06	(-0.09, 0.20)	0.05	(-0.09, 0.20)
Sleep onset latency, minutes	-0.01	(-0.15, 0.14)	-0.01	(-0.16, 0.15)	0.01	(-0.15, 0.16)	-0.01	(-0.16, 0.15)
Sleep efficiency, %	-0.05	(-0.20, 0.09)	-0.08	(-0.23, 0.07)	-0.08	(-0.23, 0.07)	-0.09	(-0.24, 0.06)
Wake after sleep onset, minutes	--	--	0.06	(-0.08, 0.21)	0.06	(-0.09, 0.20)	0.06	(-0.09, 0.20)
Fragmentation index, %	0.13	(-0.01, 0.26)	0.14	(0.01, 0.27)*	0.13	(0.01, 0.26)*	0.13	(0.01, 0.26)*
SD of sleep duration, minute	0.12	(-0.03, 0.27)	0.12	(-0.04, 0.27)	0.14	(-0.01, 0.30)	0.14	(-0.02, 0.29)
SD of sleep onset timing, minute	-0.11	(-0.25, 0.03)	-0.09	(-0.21, 0.04)	-0.08	(-0.21, 0.05)	-0.09	(-0.21, 0.04)

Abbreviations: BDI: Beck Depression Inventory; BMI: body mass index.

* Indicates significant association at $P < 0.05$.

[a] Results are shown as standardized β coefficients in the mixed models, per 1 SD increase in BDI somatic or cognitive subscales. Sample size N=174 (or 87 pairs).

[b] Base model adjusted for standardized within-pair difference of BDI.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, and alcohol abuse.

[d] Model 3 = Model 2 + BMI and history of hypertension.

[e] Model 4 = Model 3 + number of naps and average nap duration.

Table S.4.9 Within-pair analysis of the association of BDI somatic and cognitive subscales with PSQI metrics

BDI Somatic Subscale						
PSQI sleep metrics[a]	**Model 1**[b]		**Model 2**[c]		**Model 3**[d]	
PSQI total score	0.44	(0.31, 0.56)*	0.46	(0.33, 0.58)*	0.46	(0.34, 0.58)*
PSQI subscale: sleep quality	0.36	(0.24, 0.48)*	0.39	(0.27, 0.51)*	0.39	(0.27, 0.51)*
PSQI subscale: sleep latency	0.12	(-0.01, 0.25)	0.12	(-0.01, 0.25)	0.12	(-0.01, 0.25)
PSQI subscale: sleep duration	0.23	(0.10, 0.35)*	0.26	(0.14, 0.39)*	0.26	(0.14, 0.39)*
PSQI subscale: sleep efficiency	0.14	(0.01, 0.27)*	0.16	(0.03, 0.29)*	0.16	(0.04, 0.29)*
PSQI subscale: sleep disturbance	0.34	(0.22, 0.46)*	0.33	(0.21, 0.45)*	0.33	(0.21, 0.45)*
PSQI subscale: need meds to sleep	0.17	(0.04, 0.29)*	0.13	(0.01, 0.26)*	0.13	(0.01, 0.26)*
PSQI subscale: sleep issues cause dysfunction	0.52	(0.41, 0.62)*	0.52	(0.41, 0.63)*	0.52	(0.41, 0.63)*
BDI Cognitive Subscale						
PSQI total score	0.29	(0.17, 0.41)*	0.31	(0.18, 0.44)*	0.31	(0.18, 0.43)*
PSQI subscale: sleep quality	0.26	(0.14, 0.38)*	0.32	(0.19, 0.44)*	0.31	(0.19, 0.44)*
PSQI subscale: sleep latency	0.10	(-0.03, 0.22)	0.09	(-0.04, 0.22)	0.09	(-0.04, 0.22)
PSQI subscale: sleep duration	0.08	(-0.05, 0.20)	0.11	(-0.02, 0.24)	0.11	(-0.02, 0.24)
PSQI subscale: sleep efficiency	0.02	(-0.11, 0.15)	0.06	(-0.07, 0.19)	0.05	(-0.08, 0.18)
PSQI subscale: sleep disturbance	0.30	(0.18, 0.42)*	0.31	(0.18, 0.43)*	0.30	(0.17, 0.42)*
PSQI subscale: need meds to sleep	0.15	(0.02, 0.27)*	0.12	(-0.01, 0.25)	0.10	(-0.02, 0.23)
PSQI subscale: sleep issues cause dysfunction	0.43	(0.32, 0.55)*	0.42	(0.30, 0.54)*	0.43	(0.31, 0.55)*

Abbreviations: BDI: Beck Depression Inventory; BMI: body mass index; PSQI: Pittsburgh Sleep Quality Index.

* Indicates significant association at $P < 0.05$.

[a] Results are shown as standardized β coefficients in the mixed models, per 1 SD increase in BDI somatic or cognitive subscales. Sample size N=246 (or 123 pairs).

[b] Base model adjusted for standardized within-pair difference of BDI.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, and alcohol abuse.

[d] Model 3 = Model 2 + BMI and history of hypertension.

Table S.4.10 Within-pair analysis of the association between BDI score and PSG metrics, with adjustment for additional covariates

PSG sleep metrics[a]	Model A[b]		Model B[c]		Model C[d]		Model D[e]	
Total sleep time, minutes	5.3	(-3.8, 14.4)	3.2	(-6.2, 12.7)	10.0	(-2.8, 22.8)	5.4	(-4.0, 14.7)
REM latency, minutes	19.7	(8.6, 30.8)*	16.1	(4.8, 27.5)*	21.3	(5.1, 37.5)*	17.0	(5.8, 28.1)*
Sleep efficiency, %	0.7	(-1.2, 2.7)	0.1	(-1.9, 2.1)	1.9	(-0.8, 4.7)	0.5	(-1.5, 2.6)
Percentage of total sleep time in N1, %	0.2	(-0.9, 1.3)	0.6	(-0.6, 1.7)	0.5	(-1.1, 2.1)	0.3	(-0.9, 1.4)
Percentage of total sleep time in N2, %	1.2	(-0.2, 2.7)	1.3	(-0.2, 2.8)	2.1	(-0.1, 4.3)	1.1	(-0.4, 2.6)
Percentage of total sleep time in N3, %	-1.0	(-2.4, 0.4)	-1.2	(-2.7, 0.2)	-2.2	(-4.1, -0.2)*	-1.1	(-2.5, 0.4)
Percentage of total sleep time in REM, %	-1.1	(-2.1, -0.1)*	-0.9	(-2.0, 0.2)	-0.3	(-1.7, 1.2)	-0.8	(-1.8, 0.2)
Total arousals index, per sleep hour	-0.5	(-3.1, 2.2)	0.3	(-2.5, 3.1)	-1.6	(-5.4, 2.2)	-0.5	(-3.2, 2.3)
Periodic leg movement index, per sleep hour	1.5	(-1.9, 4.9)	2.2	(-1.3, 5.8)	0.2	(-4.7, 5.1)	1.9	(-1.6, 5.5)
Apnea/hypopnea index, per sleep hour	0.9	(-1.2, 3.0)	1.1	(-1.1, 3.2)	-1.9	(-4.9, 1.0)	0.6	(-1.6, 2.7)
Respiratory disturbance index, per sleep hour	1.4	(-1.5, 4.2)	1.7	(-1.2, 4.7)	0.6	(-3.5, 4.7)	1.4	(-1.6, 4.3)
Oxygen desaturation index, per sleep hour	0.7	(-1.3, 2.7)	0.9	(-1.1, 3.0)	-2.8	(-5.6, 0.1)	0.4	(-1.7, 2.4)
Percentage of sleep duration with SaO_2 <90%, %	1.4	(-0.8, 3.5)	0.3	(-1.8, 2.5)	-1.3	(-4.3, 1.7)	0.6	(-1.6, 2.8)

Abbreviations: BDI: Beck Depression Inventory; BMI: body mass index; PSG: polysomnography; REM: rapid eye movement; SaO_2: oxygen saturation.

* Indicates significant association at P <0.05.

[a] Results are shown as β coefficients in the mixed models, per 5-unit increase in BDI score. Sample size N=142 (or 71 pairs).

[b] Model A represents the fully adjusted model in the primary analysis, with adjustment for within-pair difference of BDI, education, employment status, ever smoking status, alcohol abuse, BMI, and history of hypertension.

[c] Model B = Model A + antidepressant use.

[d] Model C = Model A + PCL-4.

[e] Model D = Model A + PTSD.

Table S.4.11 Within-pair analysis of the association between BDI score and actigraphy metrics over 7 days, with adjustment for additional covariates

Actigraphy sleep metrics[a]	Model A[b]		Model B[c]		Model C[d]		Model D[e]	
Total sleep time, minutes	2.1	(-4.8, 9.1)	1.9	(-5.5, 9.4)	2.3	(-7.9, 12.4)	2.2	(-4.9, 9.3)
Sleep onset latency, minutes	0.2	(-0.5, 1.0)	0.1	(-0.7, 0.9)	0.1	(-0.9, 1.1)	0.2	(-0.5, 1.0)
Sleep efficiency, %	-0.5	(-1.1, -0.1)*	-0.4	(-0.9, -0.1)*	-0.4	(-1.1, 0.4)	-0.5	(-1.0, 0.1)
Wake after sleep onset, minutes	1.9	(-0.3, 4.1)	1.4	(-0.9, 3.7)	1.0	(-2.2, 4.1)	1.6	(-0.7, 3.8)
Fragmentation index, %	1.2	(0.5, 1.9)*	0.8	(0.1, 1.5)*	1.2	(0.2, 2.2)*	1.1	(0.5, 1.8)*
SD of sleep duration, minute	6.1	(2.0, 10.1)*	5.4	(1.1, 9.6)*	3.9	(-1.6, 9.3)	4.6	(0.7, 8.5)*
SD of sleep onset timing, minute	0.1	(-3.1, 3.2)	-1.5	(-4.5, 1.5)	-1.1	(-6.1 3.8)	-0.4	(-3.5, 2.8)

Abbreviations: BDI: Beck Depression Inventory; BMI: body mass index; SD: standard deviation.

* Indicates significant association at $P < 0.05$.

[a] Results are shown as β coefficients in the mixed models, per 5-unit increase in BDI score. Sample size N=174 (or 87 pairs).

[b] Model A represents the fully adjusted model in the primary analysis, with adjustment for within-pair difference of BDI, education, employment status, ever smoking status, alcohol abuse, BMI, history of hypertension, number of naps, and average nap duration.

[c] Model B = Model A + antidepressant use.

[d] Model C = Model A + PCL-4.

[e] Model D = Model A + PTSD.

Table S.4.12 Within-pair analysis of the association between BDI score and PSQI metrics, with adjustment for additional covariates

PSQI sleep metrics[a]	Model A[b]		Model B[c]		Model C[d]		Model D[e]	
PSQI total score	1.10	(0.78, 1.42)*	1.01	(0.68, 1.34)*	0.81	(0.22, 1.39)*	1.05	(0.71, 1.38)*
PSQI subscale: sleep quality	0.20	(0.14, 0.27)*	0.22	(0.15, 0.29)*	0.01	(-0.11, 0.12)	0.20	(0.13, 0.26)*
PSQI subscale: sleep latency	0.09	(-0.01, 0.19)	0.08	(-0.02, 0.18)	-0.01	(-0.18, 0.15)	0.09	(-0.01, 0.19)
PSQI subscale: sleep duration	0.13	(0.04, 0.23)*	0.14	(0.04, 0.23)*	0.02	(-0.14, 0.18)	0.12	(0.03, 0.22)*
PSQI subscale: sleep efficiency	0.08	(-0.02, 0.19)	0.06	(-0.05, 0.17)	0.17	(-0.03, 0.38)	0.10	(-0.01, 0.21)
PSQI subscale: sleep disturbance	0.16	(0.11, 0.22)*	0.15	(0.09, 0.20)*	0.01	(-0.07, 0.10)	0.14	(0.08, 0.19)*
PSQI subscale: need meds to sleep	0.17	(0.05, 0.29)*	0.08	(-0.05, 0.21)	0.23	(0.02, 0.44)*	0.11	(-0.02, 0.24)
PSQI subscale: sleep issues cause dysfunction	0.27	(0.22, 0.33)*	0.26	(0.20, 0.31)*	0.20	(0.10, 0.29)*	0.26	(0.20, 0.32)*

Abbreviations: BDI: Beck Depression Inventory; BMI: body mass index; PSQI: Pittsburgh Sleep Quality Index.

* Indicates significant association at P <0.05.

[a] Results are shown as β coefficients in the mixed models, per 5-unit increase in BDI score. Sample size N=246 (or 123 pairs).

[b] Model A represents the fully adjusted model in the primary analysis, with adjustment for within-pair difference of BDI, education, employment status, ever smoking status, alcohol abuse, BMI, and history of hypertension.

[c] Model B = Model A + antidepressant use.

[d] Model C = Model A + PCL-4.

[e] Model D = Model A + PTSD.

CHAPTER 5: AIM 2: THE TEMPORAL RELATIONSHIPS BETWEEN SLEEP DISTURBANCE AND AUTONOMIC DYSREGULATION

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5.1 ABSTRACT

Introduction: Sleep disturbance is associated with autonomic dysregulation, but the temporal directionality of this relationship remains uncertain. The objective of this study was to evaluate the temporal relationships between objectively measured sleep disturbance and autonomic dysregulation in a co-twin control study.

Methods: A total of 122 members (61 pairs) of the Vietnam Era Twin Registry were studied. Twins underwent one-night in-lab polysomnography (PSG) (Study I) and 7-day in-home actigraphy (Study II) to derive objective measures of sleep disturbance. Autonomic function indexed by heart rate variability (HRV) was obtained using 24-hour Holter electrocardiography (ECG) (Study I) and 7-day ECG monitoring with a wearable patch (Study II). Multivariable mixed-effects regression models and vector autoregressive models with Granger causality tests were used to examine the temporal directionality of the association of HRV with sleep metrics, within twin pairs, using 24-hour and 7-day collected ECG data.

Results: Twins were all male, mostly white (96%), with mean (SD) age of 69 (2) years. For PSG (Study I), the associations between daytime HRV with sleep stages and cumulative hypoxic burden were bidirectional. For actigraphy (Study II), daytime HRV measures were bidirectionally and similarly associated with longer total sleep time and lower wake after sleep onset, and their temporal dynamics may be extended to a window of 48 hours.

Conclusions: Autonomic function indexed by daytime HRV has bidirectional associations with several sleep dimensions. Autonomic function during wakefulness and sleep disturbance are closely interrelated and their influence on each other may extend beyond 24 hours.

5.2 Introduction

Sleep is an essential component of physiological regulation and is critical for optimal brain and bodily functions. Sleep disturbance is associated with higher risk for many chronic conditions, especially cardiovascular disease (CVD).[20,247] However, the precise biological mechanisms linking sleep disturbance with CVD risk are only beginning to be understood. One of these potential mechanisms is dysregulation of the autonomic nervous system (ANS), which controls basic bodily functions such as heartbeat, digestion and respiration.[158,248]

ANS regulation can be assessed noninvasively using heart rate variability (HRV), which provides a measure of beat-to-beat heart rate fluctuations over time. Reduced HRV is indicative of an imbalance between sympathetic and parasympathetic modulation, i.e., heightened sympathetic activity and/or vagal withdrawal,[114] and is an independent predictor of CVD morbidity and mortality.[28,67,143]

At present, the pathways linking ANS function and sleep remain unclear. Some studies have suggested that sleep disturbance, including obstructive sleep apnea and measures of sleep quality, may cause autonomic imbalance by triggering a dominance of sympathetic over parasympathetic activity.[51-53] In contrast, other studies have proposed that ANS regulation, measured by HRV, is a predictor of subsequent sleep quality and sleep architecture.[54-56] However, no prior studies have comprehensively evaluated the temporal directionality of these associations using a full spectrum of objective sleep measures. More information is also needed on the temporal dynamics between HRV and sleep, i.e., the extent to which their influence is maintained over time, since prior studies assessed primarily short-term effects.[55,57,58]

In addition to limited information regarding the temporal directionality of the association between HRV and sleep, most existing data are based on patients with specific clinical problems,

such as chronic fatigue syndrome, narcolepsy, and obstructive sleep apnea.[53,54,59,60] Literature in healthy populations is limited and results have differed.[58,61,62] Furthermore, most prior studies used laboratory-based methods to measure sleep.[56,59,63,64] While this provides a controlled environment, it may not illuminate sleep problems in normal life.[65,66] Finally, no previous study has taken into account the potential influence of familial and genetic factors, which is an important consideration since autonomic regulation and sleep could share common pathophysiology.[75,76]

To address these limitations, we conducted a co-twin control study to evaluate the temporal relationships between autonomic dysregulation indexed by reduced HRV and objectively measured sleep disturbance, using both laboratory polysomnography (PSG) and actigraphy. We sought to evaluate the temporal dynamics and directionality of the association between HRV and sleep characteristics. We hypothesized that the association between HRV and sleep would be bidirectional and that the influence of these phenotypes on each other would be relatively brief, within 24 hours.

5.3 Methods and Materials

Study Population

The participants in this study are part of the Sleep Substudy of the Emory Twin Study Follow-up (ETSF). Twins were recruited from the Vietnam Era Twin (VET) Registry, a national registry of >7,000 male twins who served on active duty during the Vietnam war.[80] Details on the construction of the study sample are shown in **Figure 5.1**. As part of the ETSF we re-examined in person 279 twins (including 124 pairs and 31 singles) who had participated in the initial Emory Twin Study (ETS).[209,249] The ETS included twin pairs where at least one member

had PTSD or major depression, and control pairs free of these psychiatric conditions based on information from previous registry surveys. Twins who self-reported any history of cardiovascular diseases according to 1990-1991 registry data were excluded from ETS.[80] The Sleep Substudy of the ETSF collected objective sleep measures among 230 twins (99 pairs, 32 singles). Of these, a total of 122 paired twins (61 pairs) also had good quality autonomic function data obtained in-lab or at-home, thus they presented the analytical sample for this study.

All twin pairs were examined together at Emory University on the same day to match environmental exposures. Comprehensive medical history and behavioral data were obtained using standardized forms, and anthropometric measurements were taken. Zygosity information was collected and verified by DNA typing.[210] We obtained written informed consent from all participants, and the Institutional Review Board at Emory University approved this study.

Assessment of Sleep

We used a combination of both PSG (Study I) and actigraphy (Study II) to obtain a comprehensive and objective evaluation of sleep. We measured sleep architecture and sleep disorders (sleep disordered breathing [SDB] and periodic leg movements [PLMS]) using one-night PSG in a controlled lab at the Emory Sleep Center (Study I). We generated the following PSG measures: (1) sleep architecture variables: the proportions of total sleep time (TST) spent in N1, N2, N3, and REM sleep; (2) the PLMS index (PLMSI: defined as the total number of PLMS divided by TST x 60; expressed as movements per hour); and (3) SDB-related indices, including the apnea/hypopnea index (AHI) (expressed as breathing events per hour) and the percentage of TST with oxygen saturation <90%. Although PSG also generates data on sleep efficiency (SE), and wake after sleep onset (WASO), we did not use these data derived from PSG because of

their low short-term stability in the context of a single lab night. Instead, we relied on actigraphy (Study II; see below), derived from up to 7 nights of data, to provide more stable estimates for these parameters. A total of 179 twins, including 71 pairs had usable PSG data.

Following the one-night in-lab PSG, each participant returned home with a wrist-worn actigraph (Actiwatch SpectrumPro, Phillips Respironics) device to derive objective sleep metrics in a naturalistic environment (Study II). All twins were instructed to wear the device on their non-dominant wrist for up to 7 days. Wrist actigraphy has been recognized as a useful adjunctive tool in sleep medicine.[65,66] It measures body movement over 24-hours using a calibrated accelerometer that records physical movement in 1-minute epochs, which can be used to estimate various parameters of sleep.[218] Raw actigraphy data (including activity counts and event markers) were first adjudicated using a sleep diary kept by each participant, and then we applied a standardized scoring algorithm to the data,[219] using Actiwatch software (version 6.0). Various sleep metrics were obtained and summarized for each 24-hour day up to 7 days. Our primary actigraphy measures included: (1) TST, defined as the total number of minutes spent asleep during the night (not including daytime naps); (2) SE, the percentage of the nocturnal sleep period spent asleep; and (3) WASO, the total minutes of wakefulness during the sleep period after sleep onset. A total of 212 twins had usable actigraphy data, including 87 pairs (n=174) that were included in the within-pair analysis. All twins had at least 4 days of actigraphy data; almost all (n=170/174, 98%) had at least 6 days of data, and most (n=151/174, 87%) had 7 days of data. Thus, all twins were included in the analysis to maximize sample size.

Assessment of Autonomic Dysregulation

During the clinic visit (Study I), twins wore an ambulatory Holter electrocardiogram (ECG) monitor for 24 hours. We followed previously published procedures to maximize accuracy of recordings and minimize potential confounding.[222] Both twins in the same pair were evaluated at the same time, and their recording times, schedule, and activity level during recording were similar. We used manufacturer's custom-built validated software to extract the raw signal and convert it into WFDB format.[224] Then we extracted RR intervals and computed the frequency domains using a previously validated HRV toolbox from the Clifford lab.[225] Specifically, a signal to quality index (SQI) based on beat detection was computed for each ECG signal.[226] Non-sinus rhythm and beats with SQI lower than 90% were removed to obtain a normal to normal (NN) interval time series. The power spectra of the NN time series was generated using the Lomb periodogram, and frequency domain HRV metrics were calculated on 5 minutes 30 seconds sliding windows on the NN time series signal. Each tape of Holter recordings was digitally processed and analyzed, and was further segmented into sleep (nighttime) and wake (daytime) periods as determined by the beginning and end of the in-lab PSG recording (i.e. day 1, night, and day 2). We evaluated four discrete frequency domains, including ultra-low frequency (ULF, <0.0033 Hz), very low frequency (VLF, 0.0033-0.04 Hz), low frequency (LF, 0.04-0.15 Hz), and high frequency (HF, 0.15-0.40 Hz).[68,143] We also calculated deceleration capacity (DC), which provides an average speed of heart rate deceleration, and it is potentially more useful than other HRV metrics in evaluating parasympathetic nervous function and predicting adverse events.[70] A total of 151 twins had usable Holter HRV, including 53 pairs who were included in the within-pair analysis.

For HRV home monitoring (Study II), we used the CardeaSolo^{TM} patch, which is a non-invasive and wearable ambulatory ECG monitoring adhesive patch monitor. A study coordinator applied the device over the left pectoral region of each participant's chest, and instructed him to wear the patch for 7 days. ECG data were extracted and processed using the manufacturer's custom-built validated software in the methods that used to process the 24-hour Holter ECG data.[224,225] On each day, four frequency domains (i.e. ULF, VLF, LF, and HF) and DC were obtained, similar to 24-hour Holter recording. Data were also further separated into sleep (nighttime) and wake (daytime) periods as determined by the adjudicated actigraphy data for up to 7 days. Twins with low quality data (e.g. loss of electrode contact, movement artifacts, low SQI <90%, or twins with <75% data) were excluded, reducing the number of subjects with usable HRV home monitoring data to 115 twins, including 34 twin pairs (n=68) who were included in the within-pair analysis. There were no differences between twins who did (n=115) and did not have (n=97) complete HRV assessments in terms of sociodemographic and health-related characteristics (data not shown).

Other Measurements

At the ETSF clinic visit, a research nurse or physician assistant obtained medical history and medication use. Anthropometric data, blood pressure, fasting blood glucose, lipid profile, and health behaviors were measured as previously described.[11] Habitual physical activity was measured using the Baecke physical activity questionnaire.[227,228] This is a 16-question instrument recording physical activity levels at work, during sports and non-sports activities, rendering a global physical activity score. History of hypertension was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg, or use of anti-hypertensive medications,

following the Joint National Committee (JNC)-7 classification for Stage 1 hypertension which was the accepted staging at the time.[229] History of coronary artery disease that might have occurred from the time of the initial screen was also assessed. Diabetes mellitus was defined as having a measured fasting glucose level more than 126 mg/dL or any treatment with antidiabetic medications. A lifetime diagnosis of major depressive disorder, posttraumatic stress disorder (PTSD), and alcohol abuse disorder were obtained using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorder, 4th Edition (DSM-4), or SCID.

Statistical Analysis

Using PSG and Holter data collected during clinic visit day (Study I), we analyzed the temporal directionality of associations of the within-pair difference in PSG metrics with the within-pair difference in HRV metrics. In a study of twins, within-pair differences intrinsically control for potential confounding by shared genetic factors and early familial background, as well as environmental factors during ambulatory monitoring as twins were examined together. We first evaluated the association of the average within-pair difference in HRV during day 1 (i.e. from start of data collection to nighttime sleep onset) with PSG findings during nighttime. Second, we examined the reverse, i.e., the association of nighttime PSG findings with HRV during day 2, (i.e. from sleep offset to end of data collection), with adjustment for average nighttime HRV data. Illustration of this analysis is shown in **Figure 5.2**. For all analyses, we fitted multivariable mixed-effects regression models and accounted for twin pair as a random effect. All models were adjusted for potential confounding factors (smoking status, habitual physical activity, BMI, history of hypertension, and history of depression, PTSD and alcohol

abuse). As HRV data were skewed, logarithmic transformations were used to normalize the distributions.

Using actigraphy and ECG data collected during 7-day home monitoring (Study II), we further evaluated the temporal relationships between sleep measures and HRV in a naturalistic environment. The within-pair analysis included a total of 362 observations (days) reflecting data from 68 twins, and on average, each twin contributed 5.3 observations (days) of data. We fit bivariate vector autoregressive (VAR) models to analyze the longitudinal data.[230] We included each combination of HRV (ULF, VLF, LF, HF, and DC) and sleep metric (TST, SE, and WASO) in separate models. Then for each combination of HRV and sleep measures, we built bivariate VAR models that adjusted for the same potential confounders as in Study I.

In Study II, the recording of multiple 24-hour periods of simultaneous ECG and actigraphy allowed us to model potential temporal causality using time-lagged models. To determine the length of time the association between HRV and sleep was maintained, we built a series of models by adding lagged values of the dependent variables. The Bayesian Information Criterion (BIC) was used for order selection, with lower BIC values indicating better model fit. To formally test whether the associations between HRV and sleep persist beyond a single 24-hour period, we used likelihood ratio tests to compare VAR models with the lowest BIC with the first order models.

After determining the appropriate lag order, to evaluate the temporal directionality of the associations between HRV and sleep, we conducted *F* tests of Granger causality. In a Granger causality test, if the *F*-value is statistically significant, it means that the past values of predictor *X* contain information that helps predict outcome *Y* in addition to the information contained in the past values of *Y* alone, after controlling for other covariates; in other words, *X* "Granger-causes"

or predicts *Y*. We individually tested if each of the HRV metric predicted each of the sleep measure, and vice versa, and conducted VAR models separately for wake and sleep periods to evaluate the day and night difference in the relationship between HRV and sleep. We further conducted mixed-effects regression models to clarify the direction of significant effects after controlling for the same set of covariates as in the VAR models. In these models, predictor variables were expressed as daily variation from individual's averages.

To assess potential shared genetic influence on the association between HRV and sleep disturbance, we examined the associations separately in MZ and DZ twins to evaluate effect modification by zygosity. Because MZ twin pairs share 100% of their genes while DZ twin pairs only share 50% on average, if a larger effect of HRV on sleep or vice versa is observed within DZ pairs than in MZ pairs, then it may suggest that genetic factors play a role in this association.

A two-sided p-value less than 0.05 was used for statistical significance and 95% confidence intervals (CI) were calculated from mixed-effect model parameters. All statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC) and Stata 14.0 (StataCorp, College Stata, TX).

5.4 Results

Participants' Characteristics

A total of 122 paired twins (61 pairs) with data collected during clinic visit (i.e. PSG and Holter HRV) or during home monitoring (i.e. actigraphy and patch HRV) were included in the within-pair base sample for this study. Among these twins, 118 (97%) were white, with a mean age ± SD of 69 (2) years (**Table 5.1**). Of the 122 twins, 39 pairs (n=78, 64%) were MZ twins and 22 pairs (n=44) were DZ twins. Participants' HRV and sleep data are summarized in

Supplemental Table S.5.1. Overall, participants had similar HRV data collected during home monitoring compared to that obtained in clinic visit, but, as expected, on average they had longer TST at home (477 minutes) than in the sleep lab (305 minutes). On average, participants had 87% SE and 52 minutes of WASO during home monitoring.

Association of HRV with Sleep Measured by PSG in the Laboratory (Study I)

For the association between HRV and sleep architecture (**Table 5.2**), we observed a bidirectional association across multiple sleep stages. For example, a higher ULF and VLF HRV were associated with decreased N1 sleep, increased N2 sleep, and decreased REM sleep, in both directions. However, more N3 sleep time was significantly associated with higher HF HRV and DC values on the following day, but not vice versa. We also observed a bidirectional association of a higher ULF and VLF HRV with less cumulative hypoxic burden (TST% with SaO_2 <90%). But we did not observe any associations with the AHI or PLMSI in either direction.

Association of HRV with Sleep Measured by Actigraphy Over 7 Days (Study II)

Results of VAR models were examined to identify the most appropriate lag level and best model fit (from 1 to 7 days). The BIC for the 2-day VAR model was the smallest, indicating the best model fit, for nearly all models examining the association of sleep metrics with daytime or nighttime HRV. Likelihood ratio test results were consistent with BIC (**Tables S.5.2-S.5.3**). This indicates that 2-day models yielded significantly better model fit than the 1-day models for both daytime and nighttime HRV across almost all combinations of HRV and sleep metrics. There was no material difference in the results comparing 1-day VAR and 2-day VAR in the models that examined the association of HRV to TST. Therefore, for consistency, Granger causality tests

were conducted with the 2-day VAR across all models to examine the temporal directionality of association between HRV and sleep disturbance.

F test results of Granger causality showed that daytime HRV metrics during the previous days were significantly associated with two sleep measures in the subsequent night, including TST and WASO, after adjusting for smoking status, history of alcohol abuse, physical activity, BMI, history of hypertension, depression, and PTSD (**Table 5.3**). These two sleep measures also significantly predicted multiple daytime HRV metrics on the following day. Mixed-effects regression models provided the estimates for these effects (**Figure 5.3**). Specifically, higher daytime HF and DC HRV predicted subsequent longer TST; and higher ULF, LF, HF and DC HRV predicted decreased WASO. Looking at the opposite direction from sleep to subsequent daytime HRV, longer TST and lower WASO predicted higher HRV. In contrast, we did not find any significant associations involving nighttime HRV, i.e., between nighttime HRV and sleep measures in the subsequent nights, or between previous sleep measures from previous nights and subsequent nighttime HRV (**Table S.5.4**).

However, due to the small sample size especially for the Study II analysis, we were not able to generate reliable estimates separately in MZ and DZ twins in order compare the magnitude of associations. Thus, in the current analysis we were not able to assess the role of genetic factor on this association.

5.5 Discussion

In this co-twin control study, we aimed at characterizing the temporal relationships between autonomic dysregulation indexed by reduced HRV, and sleep disturbance objectively measured in multiple dimensions. We found that most of these relationships are bidirectional.

Higher values in several daytime HRV domains, denoting better ANS function, were associated with a number of PSG-derived sleep measures in Study I, including N1, N2 and REM sleep, and lower hypoxic burden, after adjusting for relevant sociodemographic, behavioral and health-related factors. In turn, lower N1 and REM sleep, higher N2 sleep, and less severe oxygen desaturation were associated with higher HRV in the day following the laboratory PSG. During a week of monitoring in the home environment (Study II), a higher daytime HRV was bidirectionally associated with better sleep duration and continuity measured by actigraphy, as indicated by longer TST and lower WASO. We also found that the relationships between daytime HRV and sleep duration and continuity measures generally persisted to 48 hours, but no longer. In contrast to daytime HRV, nighttime HRV (Study II) was not related to sleep duration or continuity longitudinally. Because our analysis examined differences within twin pairs, results are inherently independent of shared familial environment.

Our findings are consistent with previous research showing that autonomic function represented by HRV is closely related to sleep architecture.[55,250,251] However, most studies focused on a simultaneous monitoring of HRV and sleep, and therefore were unable to evaluated the temporal directionality of such association. Our Study I results suggest that, of all HRV metrics, higher ULF and VLF HRV during the day have the strongest and most consistent effects on sleep stages, including N1, N2, and REM sleep. ULF HRV reflects a circadian rhythm in the heart rate signal, and VLF HRV reflects a combination of overall sympathovagal balance and activity of the renin-angiotensin system (RAS).[155] Thus, our results suggest that circadian rhythms of sympathovagal balance and RAS activity may be interrelated to sleep architecture. Our findings agree with prior research showing that the autonomic function affects sleep architecture,[55] and also suggest that the association may be bidirectional. Our results confirm

previous findings that better daytime autonomic function indexed by higher HRV is related to decreased sleep time in N1 and increased sleep time in N2, indicating less fragmented sleep.[55,252] However, in our study higher HRV was also related to shorter sleep time in REM, which could impact the restorative quality of sleep.[253]

Our Study I results also demonstrates a bidirectional association between HRV and SDB measures such as hypoxic burden. Specifically, we observed that higher daytime ULF and VLF HRV predict lower hypoxic burden during sleep, which agrees with prior studies.[254-256] However, using another measure of sleep apnea, the AHI, we also observed relationships with nighttime measures predicting daytime autonomic function in that a higher AHI predicted lower ULF HRV and VLF HRV during the next day. This is consistent with prior observations that recurrent episodes of apneas and awakenings may have an adverse impact on autonomic regulation during the following day.[53,64] Contrary to prior studies,[59,257] however, we did not find significant associations of the periodic leg movement index with HRV metrics in either direction. These different results may be due to differences in study populations.

In Study II, our overall findings for actigraphy measures during home monitoring are consistent with prior literature showing that higher HRV predicts better subsequent sleep duration and continuity, although the HRV domains implicated differ across studies.[54-57,157] We observed that all HRV metrics predict indices of better sleep, including longer TST and lower WASO. Of note, DC HRV, which represents changes in PNS activity, demonstrated the most consistent association with both of these sleep measures. LF power captures baroreflex function, and is modulated by inputs from both SNS and PNS to the sino-atrial node; HF power is almost exclusively modulated by PNS function.[258] Thus, our findings suggest that both SNS and PNS activity during the day are involved in sleep duration and continuity. Contrary to previous

studies,[56,157,164] however, we did not find significant relationships between HRV and SE, likely because the measure of SE included sleep latency in its calculation. Our findings that daytime HRV rather than nighttime HRV is associated with selected sleep measures, agree with prior studies that assessed HRV both during wakefulness and sleep.[57,259] Using multiple HRV frequency domains and objective sleep duration and continuity measures, our findings suggest that ANS regulation during wakefulness, perhaps reflecting exposures to daily stressors, may alter the sympathovagal balance during the daytime and play a key role in sleep.[163,260,261]

The existing literature is inconsistent on whether poor sleep duration and continuity can adversely affect, be affected by, or otherwise be associated with HRV. For example, one study showed that subjects with insomnia compared to normal sleepers had decreased HRV,[262] while another study did not find such association.[263] Using a longitudinal, naturalistic design over consecutive 24-hour periods, our study extended previous understanding of the temporal relationships between sleep and HRV and showed that TST and WASO, can predict subsequent daytime HRV in multiple domains. It has been noted that low sleep duration and poor sleep continuity can increase sympathetic over parasympathetic dominance, which may be reflected in reduced HRV.[51,52,160] Sleep disturbance may also lead to decreased sensitivity of hormonal receptors such as corticotropin-releasing hormone and serotonin receptors, which may result in dysregulation of stress responses and autonomic function.[51]

Our findings may not be generalizable to women and other racial and age groups, as our sample included mostly white older men. Due to noise and nyquist frequency, the use of 5-minute windows to process HRV data may have not generated reliable estimates for lower frequency bands, such as ULF and VLF. In addition, due to the relatively small sample size, especially for the analysis using home monitoring data, our analysis may have been

underpowered to detect significant bidirectional associations across some sleep dimensions. The small sample size also does not allow a reliable evaluation of association separately in monozygotic and dizygotic twins in order to evaluate of role of genetic factors on the association. A reduction in sample size is inevitable in our design, given that within-pair analyses rely on complete pairs and consecutive data are necessary during home monitoring to properly calculate lagged values. However, our co-twin control study design should have improved internal validity and precision by intrinsically adjusting for unknown or unmeasured confounders. The small sample size also limited our ability to assess the role of genetic factors on the association between HRV and sleep. Future research with larger sample sizes is needed to evaluate the potential role of genetic predisposition on the association.

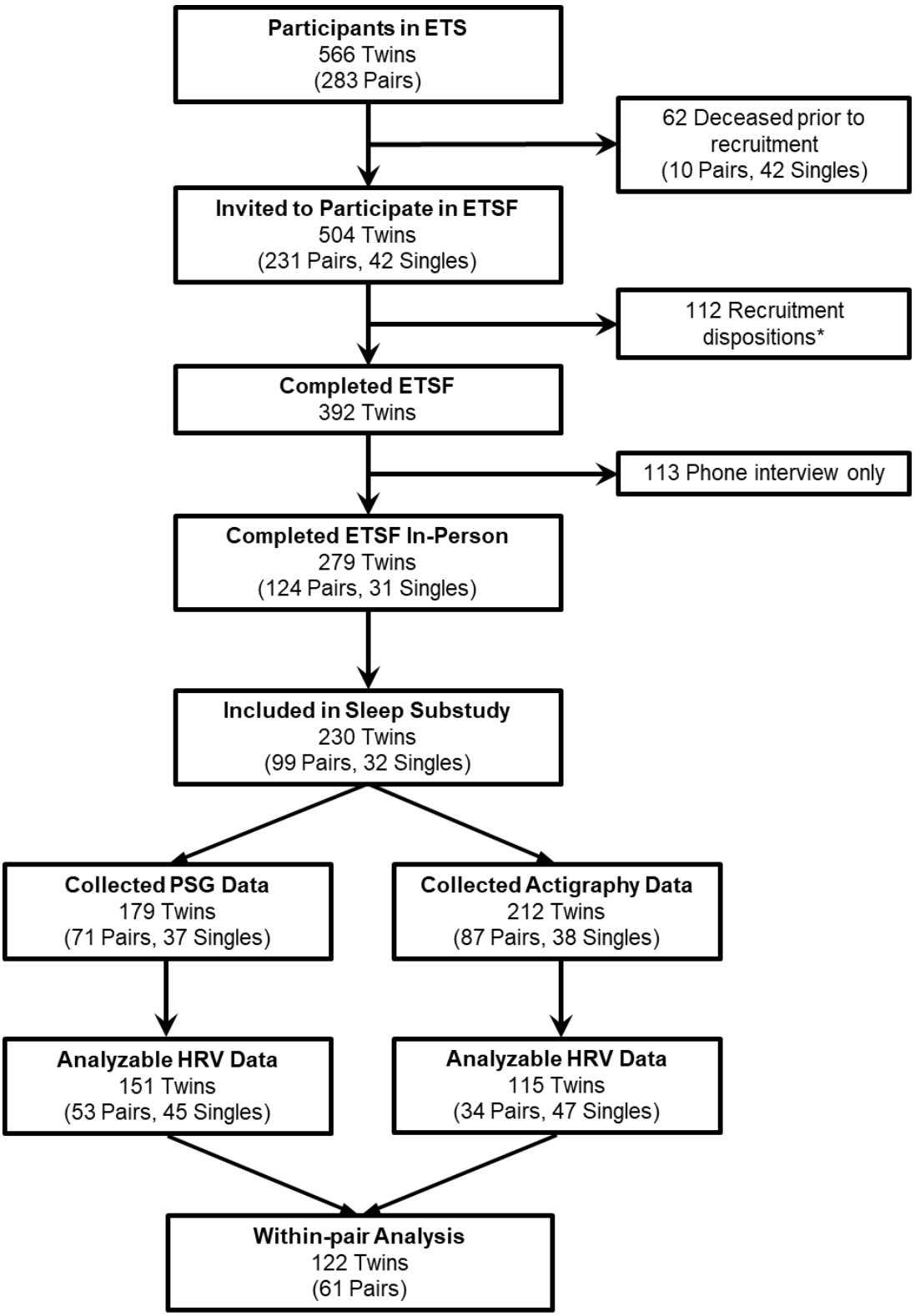
Despite these limitations, to our knowledge, this is the first study that evaluated the temporal dynamics and directionality of relationships between HRV and objective sleep measures over successive 24-hour periods and modeled day-night associations in a time-lagged model, allowing inferences via Granger causality. Our comprehensive approach incorporated objective evaluation of multiple sleep dimensions, measured both in the laboratory and at home. The VAR models fitting bivariate time series and associated Granger causality tests provided an informative method to assess the temporal directionality of associations, and it allowed us to not only evaluate temporal relationships between HRV and sleep, but also the length of time during which HRV exerted effects on sleep and vice versa.

5.6 Conclusions

In the context of a controlled twin design, the present study provides evidence of a significant bidirectional association between autonomic function and sleep measures. In the

home environment, the relationship between autonomic dysregulation during daytime and worse sleep duration and continuity persists beyond a 24-hour period. Autonomic function and sleep are closely inter-related, and highlights the importance of a healthy autonomic function in the regulation of sleep and vice versa. These results should inform sleep health promotion strategies, and underscore that targeting daytime autonomic function is a key factor. Furthermore, our results suggest that both autonomic function and sleep health should be targeted mutually in prevention strategies of chronic conditions linked to sleep disturbance, such as CVD.

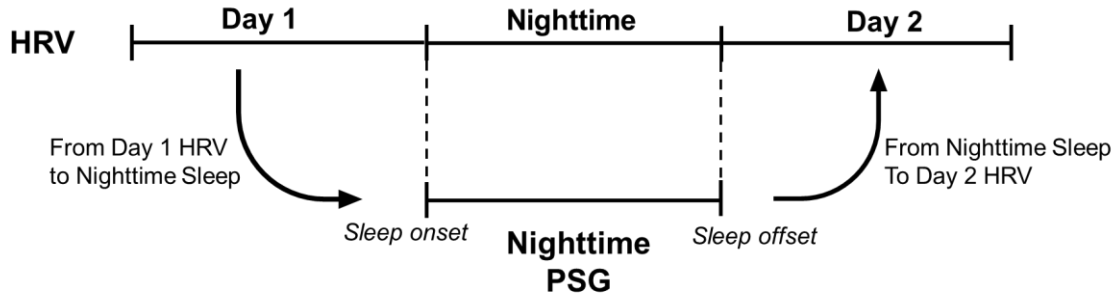
Figure 5.1 Participant Flow Diagram



Abbreviations: ETS: Emory Twin Study; ETSF: Emory Twin Study Follow-up; HRV: heart rate variability; PSG: polysomnography.

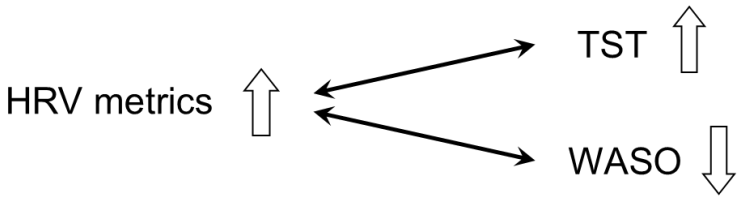
* Recruitment dispositions: 95 never responded or refused to participate; 10 deceased during recruitment; 5 were too ill to participate; 1 withdrew; 1 incarcerated during recruitment.

Figure 5.2 Analysis Diagram for the Association between PSG and Holter HRV Data (Study I)



Abbreviations: HRV: heart rate variability; PSG: polysomnography.

Figure 5.3 Directions of Significant Effects between Daytime Heart Rate Variability with Sleep Disturbance (Study II)



Abbreviations: HRV: heart rate variability; TST: total sleep time; WASO: wake after sleep onset.

Table 5.1 Characteristics of 122 Twins (61 pairs)

Characteristics, mean (SD), or No. (%)	Total (N=122)	
Sociodemographic factors		
Age, years	69	(2)
White, No. (%)	118	(97)
Education, No. (%)		
High school or less	19	(16)
Some college or associate	61	(50)
College degree	22	(18)
Graduate education or degree	20	(16)
Employed, No. (%)	34	(28)
Health factors		
BMI	30	(4)
Ever smokers, No. (%)	77	(63)
History of alcohol abuse, No. (%)	29	(24)
Baecke score for physical activity	7.9	(1.3)
Systolic blood pressure, mmHg	139	(19)
Diastolic blood pressure, mmHg	79	(12)
History of hypertension, No. (%)	68	(56)
History of diabetes, No. (%)	19	(16)
Family history of CAD, No. (%)	14	(11)
BDI score	5.7	(7.0)
PCL-4 score	25	(11)
Lifetime history of PTSD, No. (%)	32	(26)
Lifetime history of depression, No. (%)	21	(17)
Current PTSD, No. (%)	18	(15)
Current depression, No. (%)	11	(9)
Medication use		
β-Blockers, No. (%)	29	(24)
Antidepressants, No. (%)	18	(15)
Statin, No. (%)	67	(55)
ACE inhibitor, No. (%)	24	(20)

Abbreviations: ACE: angiotensin-converting enzyme; BDI: Beck Depression Inventory; BMI: body mass index; CAD: coronary artery disease; PCL-4: PTSD Checklist for DSM-4; PTSD: posttraumatic stress disorder; SD: standard deviation.

Table 5.2 Adjusted Within-pair Analysis of the Association between PSG and HRV Metrics (Study I)[a]

PSG metrics	ULF		VLF		LF		HF		DC	
Direction from day 1 HRV to nighttime PSG										
Percentage of TST in N1, %	-2.1	(-4.3, 0.1)	-3.1	(-5.4, -0.8)*	-0.6	(-2.6, 1.4)	0.4	(-1.0, 1.7)	0.3	(-2.9, 3.5)
Percentage of TST in N2, %	5.6	(1.9, 9.4)*	4.5	(0.4, 8.7)*	0.9	(-2.3, 4.0)	-1.3	(-3.5, 0.9)	0.6	(-4.8, 6.0)
Percentage of TST in N3, %	-1.5	(-4.8, 1.8)	0.4	(-3.1, 4.0)	0.6	(-2.2, 3.5)	0.8	(-1.2, 2.7)	-0.2	(-4.9, 4.4)
Percentage of TST in REM, %	-3.0	(-5.4, -0.6)*	-2.8	(-5.5, -0.2)*	-1.5	(-3.5, 0.5)	-0.3	(-1.8, 1.1)	-2.1	(-5.6, 1.3)
Periodic leg movement index	0.1	(-8.5, 8.8)	-0.9	(-10.3, 8.4)	5.1	(-2.2, 12.4)	3.7	(-1.3, 8.8)	7.1	(-5.0, 19.3)
Apnea/hypopnea index	-5.0	(-10.4, 0.5)	-1.5	(-7.5, 4.5)	0.1	(-4.6, 4.7)	-1.2	(-4.4, 2.0)	1.7	(-6.1, 9.5)
TST% with SaO2 <90%, %	-7.2	(-11.7, -2.8)*	-6.5	(-11.4, -1.5)*	-3.3	(-7.4, 0.8)	-1.7	(-4.4, 1.1)	-4.9	(-11.5, 1.6)
Direction from nighttime PSG to day 2 HRV										
Percentage of TST in N1, per 10%	-0.15	(-0.27, -0.03)*	-0.17	(-0.28, -0.06)*	-0.21	(-0.34, -0.08)*	-0.22	(-0.36, -0.08)*	-0.05	(-0.12, 0.01)
Percentage of TST in N2, per 10%	0.14	(0.07, 0.21)*	0.13	(0.06, 0.20)*	0.06	(-0.02, 0.14)	0.02	(-0.08, 0.12)	0.01	(-0.04, 0.05)
Percentage of TST in N3, per 10%	-0.02	(-0.11, 0.07)	0.02	(-0.06, 0.10)	0.09	(-0.01, 0.18)	0.16	(0.06, 0.27)*	0.06	(0.01, 0.10)*
Percentage of TST in REM, per 10%	-0.14	(-0.25, -0.02)*	-0.15	(-0.26, -0.04)*	-0.11	(-0.23, 0.01)	-0.03	(-0.18, 0.12)	-0.04	(-0.10, 0.02)
Periodic leg movement index, per 10	0.01	(-0.03, 0.04)	-0.01	(-0.03, 0.03)	-0.02	(-0.05, 0.02)	-0.03	(-0.08, 0.01)	-0.01	(-0.02, 0.02)
Apnea/hypopnea index, per 10	-0.06	(-0.12, -0.01)*	-0.05	(-0.10, 0.01)	-0.01	(-0.07, 0.04)	-0.03	(-0.10, 0.04)	-0.01	(-0.04, 0.02)
TST% with SaO2 <90%, per 10%	-0.08	(-0.14, -0.02)*	-0.10	(-0.15, -0.04)*	-0.07	(-0.13, -0.01)*	-0.06	(-0.13, 0.02)	-0.03	(-0.06, 0.01)

* Indicates statistically significant association ($p < 0.05$).

[a] Models were fully adjusted for potential confounding factors (smoking status, alcohol abuse, habitual physical activity, body mass index, history of hypertension, depression, and PTSD).

Abbreviations: DC: deceleration capacity; HF: high frequency; HRV: heart rate variability; LF: low frequency; PSG: polysomnography; REM: rapid eye movement; SaO_2: saturated oxygen; TST: total sleep time; ULF: ultra-low frequency; VLF: very low frequency.

Table 5.3 F-test Results of Granger Causality for the Within-Pair Association of Daytime Heart Rate Variability and Sleep Disturbance During 7-Day Monitoring Using 48-hour Lag (Study II)[a]

	TST	SE	WASO
Direction from Previous Daytime HRV to Sleep			
ULF	1.98	0.69	3.07*
VLF	1.71	0.26	2.48
LF	1.03	1.40	4.91*
HF	3.63*	1.82	4.45*
DC	5.21*	0.96	4.53*
Direction from Sleep to HRV in Following Day			
ULF	4.27*	2.71	3.30*
VLF	7.09*	2.13	2.07
LF	3.78*	0.57	3.80*
HF	0.77	0.09	0.61
DC	4.25*	0.88	4.34*

* Indicates that the F-value was statistically significant ($p < 0.05$).

[a] Models were fully adjusted for potential confounding factors (smoking status, alcohol abuse, habitual physical activity, body mass index, history of hypertension, depression, and PTSD).

Abbreviations: DC: deceleration capacity; HF: high frequency; HRV: heart rate variability; LF: low frequency; SE: sleep efficiency; TST: total sleep time; ULF: ultra-low frequency; VLF: very low frequency; WASO: wake after sleep onset.

Table S.5.1 HRV and Sleep Characteristics of 122 Twins (61 pairs)

Characteristics, mean (SD)	Total (N=122)	
HRV during clinic visit (n=106)*		
Ultra-low frequency	6.5	(0.5)
Very low frequency	7.5	(0.6)
Low frequency	6.4	(0.7)
High frequency	5.5	(1.0)
Deceleration capacity	2.2	(0.4)
HRV during home monitoring (n=68; 362 observations)*		
Ultra-low frequency	6.3	(0.6)
Very low frequency	7.4	(0.6)
Low frequency	6.2	(0.7)
High frequency	5.3	(0.8)
Deceleration capacity	2.0	(0.4)
PSG metrics (n=106)		
Total sleep time, minutes	305	(63)
Percentage of total sleep time in N1, %	12	(8)
Percentage of total sleep time in N2, %	63	(11)
Percentage of total sleep time in N3, %	10	(10)
Percentage of total sleep time in REM, %	16	(8)
Periodic leg movement index, per sleep hour	24	(29)
Apnea/hypopnea index, per sleep hour	17	(19)
Percentage of sleep duration with SaO2 <90%, %	8	(15)
Actigraphy metrics (n=68; 362 observations)		
Total sleep time, minutes	477	(79)
Sleep efficiency, %	87	(8)
Wake after sleep onset, minutes	52	(35)

Abbreviations: HRV: heart rate variability; PSG: polysomnography; REM: rapid eye movement; SaO_2: oxygen saturation; SD: standard deviation.

* HRV data were averaged over the monitoring period during clinic visit or during home monitoring, then log transformed.

Table S.5.2 Likelihood ratio tests evaluating superiority of 2-day (48-hour) vs. 1-day (24-hour) model fit for temporal relationships between daytime HRV and sleep disturbance (Study II)

	Outcomes							
Predictors	ULF	VLF	LF	HF	DC	TST	SE	WASO
ULF	--	--	--	--	--	3.7	8.5*	8.0*
VLF	--	--	--	--	--	2.9	8.1*	7.9*
LF	--	--	--	--	--	1.6	9.8*	7.0*
HF	--	--	--	--	--	3.3	8.3*	7.1*
DC	--	--	--	--	--	2.7	7.9*	6.4*
TST	12.8*	15.1*	18.6*	26.2*	27.4*	--	--	--
SE	15.3*	17.5*	18.7*	26.7*	26.5*	--	--	--
WASO	14.9*	16.3*	18.8*	26.2*	27.8*	--	--	--

Abbreviations: DC: deceleration capacity; HF: high frequency; LF: low frequency; SE: sleep efficiency; TST: total sleep time; ULF: ultra-low frequency; VLF: very low frequency; WASO: wake after sleep onset.

* indicates significant chi-square statistics ($p < 0.05$), suggesting that 2-day models of the association between HRV and sleep disturbance yield significantly better model fit than 1-day models.

Table S.5.3 Likelihood ratio tests evaluating superiority of 2-day (48-hour) vs. 1-day (24-hour) model fit for temporal relationships between nighttime HRV and sleep disturbance (Study II)

	Outcomes							
Predictor	ULF	VLF	LF	HF	DC	TST	SE	WASO
ULF	--	--	--	--	--	1.2	17.8*	17.6*
VLF	--	--	--	--	--	0.4	19.3*	16.0*
LF	--	--	--	--	--	0.5	18.5*	15.2*
HF	--	--	--	--	--	0.4	17.7*	15.6*
DC	--	--	--	--	--	4.2	21.0*	15.1*
TST	19.4*	18.0*	24.6*	22.9*	26.1*	--	--	--
SE	20.6*	25.3*	26.6*	23.6*	24.2*	--	--	--
WASO	20.2*	24.6*	28.1*	29.1*	23.0*	--	--	--

Abbreviations: DC: deceleration capacity; HF: high frequency; LF: low frequency; SE: sleep efficiency; TST: total sleep time; ULF: ultra-low frequency; VLF: very low frequency; WASO: wake after sleep onset.

* indicates significant chi-square statistics ($p < 0.05$), suggesting that 2-day models of the association between HRV and sleep disturbance yield significantly better model fit than 1-day models.

Table S.5.4 F-test Results of Granger Causality between Within-pair Difference in Nighttime Heart Rate Variability and Sleep Disturbance Using 48-hour Data[a] (Study II)

	TST	SE	WASO
Direction from Previous Nighttime HRV to Sleep			
ULF	0.29	0.21	0.82
VLF	0.30	0.65	0.34
LF	1.17	0.69	0.39
HF	0.50	1.15	1.37
DC	0.04	1.11	0.75
Direction from Previous Sleep to Nighttime HRV			
ULF	1.33	1.46	1.27
VLF	1.03	2.30	2.01
LF	1.93	2.16	0.99
HF	2.91	1.86	0.80
DC	1.19	2.28	0.86

* All F-values were not statistically significant ($p > 0.05$).

Abbreviations: DC: deceleration capacity; HF: high frequency; HRV: heart rate variability; LF: low frequency; SE: sleep efficiency; TST: total sleep time; ULF: ultra-low frequency; VLF: very low frequency; WASO: wake after sleep onset.

[a] Models were fully adjusted for potential confounding factors (smoking status, alcohol abuse, habitual physical activity, body mass index, history of hypertension, depression, and PTSD).

CHAPTER 6: AIM 3: DEPRESSION AND AUTONOMIC DYSREGULATION AND TIME TO ADVERSE HEALTH OUTCOMES

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6.1 ABSTRACT

Background: Depression and reduced heart rate variability (HRV) is associated with a higher risk of mortality and cardiovascular disease (CVD) in patients with known CVD. However, the prognostic implications of depression and alterations in HRV in populations without CVD is less clear. The objective of this study was to evaluate the prognostic values of baseline depression and HRV and their associations with risk of mortality and CVD during follow-up.

Methods: This study analyzed 450 members (225 pairs) from the Vietnam Era Twin Registry. At baseline assessments, depressive symptoms were measured using the Beck Depression Inventory-II (BDI), and major depression was assessed using structured clinical interview. HRV was measured through 24-hour electrocardiogram monitoring. During an average 12-year follow-up, mortality data were collected via National Death Index database, and within a subset of twins, CVD events data were obtained and verified via medical chart review. Kaplan-Meier analyses and multivariable frailty models with random effect for pairs were used to examine the hazard ratios for mortality and CVD events within pairs.

Results: Twins were all males, mostly white (97%), with mean (SD) age of 56 (3) years at baseline. BDI and major depression was not associated with risk of all-cause mortality, but one unit increase in BDI significantly predicted 12% increased risk of cancer mortality (95%CI: 1.02-1.25). Major depression was associated with increased risk for major CVD events. All log-transformed HRV frequency domains were associated with decreased hazard for all-cause mortality, with low frequency domain and deceleration capacity showing significantly decreased hazards of 22% and 27% per 1-SD increment, respectively, after multivariable adjustment.

Higher values of all HRV metrics were linked to decreased hazard for CVD, but only daytime ultra-low frequency domain showed significant association (51% decreased hazard, per 1-SD increment). Overall, daytime HRV showed similar but slightly stronger association with risk for mortality and CVD compared to nighttime HRV. There was no material difference in the associations by zygosity.

Conclusions: More depressive symptoms are associated with cancer-specific mortality, and depression predict incident major CVD events. Higher HRV is associated with decreased hazard for mortality and CVD events, and daytime HRV have may stronger predictive power than nighttime HRV.

6.2 Introduction

Depression is a prevalent psychiatric condition, with a lifetime prevalence of 16% in the United States, translating into 33 to 35 million adults with depression some time in their lives.[1-3] It remains a recognized risk factor for adverse health outcomes, such as all-cause mortality, as well as the development and progression of cardiovascular disease (CVD).[4-9]

Autonomic nervous system (ANS) controls basic bodily functions such as heartbeat, digestion, respiration and blood pressure regulation. The dysregulation of the ANS system can be a complication of many diseases, and is associated with various pathological conditions, such as higher blood pressure, incident cardiovascular disease (CVD), and mortality.[67,142-144] Autonomic dysregulation can be measured noninvasively using heart rate variability (HRV), which provides a measure of beat-to-beat heart rate fluctuations over time. Reduced HRV is indicative of an imbalance between sympathetic and parasympathetic modulation, i.e. heightened sympathetic activity and/or vagal withdrawal,[114] and is suggestive of increased morbidity and mortality.

Numerous studies have been conducted on the association of depression with increased risk of all-cause mortality in general population as well as various patient groups. As for autonomic dysregulation indexed by reduced HRV, although it is associated with mortality and cardiovascular events in individuals with known CVD,[143,184,199,264] few studies have evaluated the association between HRV and the risk of mortality and CVD in a population without known CVD, and none of them evaluated a full spectrum of frequency domains of HRV. In addition, no prior study has evaluated and compared the prognostic implications of both daytime and nighttime HRV in predicting mortality and CVD events. As HRV can be easily influenced by physical activity and mental stress levels, the use of nighttime HRV measures may avoid incidental influences and have a better predictive value than 24-hour average HRV measures.

Prior studies suggest that inter-individual differences in depression and HRV may be largely explained by genetic factors and familial predispositions, which are linked to higher risk of mortality and cardiac events.[10,11,77-79] However, currently it is unclear how much shared genes and familial factors can explain the association of depression and HRV with adverse health outcomes, such as mortality and CVD events. A co-twin control study design provides a natural "counterfactual" design to examine phenotypic associations, as twins are matched for similar genetic and early familial factors.[83] it allows us to assess the genetic influence on the association of interest by evaluating the associations among monozygotic (MZ) and dizygotic (DZ) twin pairs.

In a sample of middle-aged veteran twins without CVD, we sought to investigate the prognostic implications of depression and a full spectrum of HRV frequency domains, during both daytime and nighttime, in predicting future risk of mortality and CVD events, using a co-twin control design. We also evaluated whether genetic and familial factors play a role in the association. Findings from this study can help quantify the association between depression and HRV and the risk of mortality and cardiovascular events among individuals without known CVD. We hypothesized that more depressive symptoms and higher values of both daytime and nighttime HRV were associated with decreased risk for mortality and CVD, and genetic and familial factors play a role in this association.

6.3 Methods and Materials

Study Population

The participants in this study were recruited form the Vietnam Era Twin (VET) Registry, which is one of the largest national samples of adult male monozygotic (MZ) and dizygotic (DZ)

twins who served on active duty during the Vietnam war (1964-1975).[80] The present study is based on the 566 twins (283 pairs) recruited from VET Registry and participated in the Emory Twin Study (ETS).[81] The objective of the ETS was to evaluate the role of biological, psychological, and behavioral risk factors in the development of subclinical CVD.[81,82] We included twin pairs who were born between 1946 and 1956, and excluded twin pairs if either member of the twin pair self-reported history of CVD based on previous survey data obtained by the Registry in 1990.[207,209] The twin pairs were discordant for depression or posttraumatic stress disorder (PTSD), or free of these psychiatric conditions as control pairs. Of the 566 ETS twins, we collected HRV data in 501 twins (225 pairs and 51 singles), and among them, 351 twins (132 pairs and 87 singles) completed the Emory Twin Study Follow-up (ETSF) visit, either in-person or by phone, with an average follow-up period of 12 years after the initial ETS. Thus, the 225 pairs (n=450) with available baseline HRV data and known vital status during follow-up represented the analytical sample for this study. **Figure 6.1** shows the construction of the study population.

At ETS visit, twin pairs were examined together at Emory University on the same day using identical assessment protocols to minimize measurement error. We obtained twins' comprehensive medical history data during a two-day admission under controlled conditions, and collected blood samples, autonomic function data, anthropometric measurements, behavioral and psychosocial assessments using identical protocols and similar schedule for the two twins. Zygosity was obtained and verified by DNA typing.[210] We obtained written informed consent from all twins, and the Emory University institutional review board approved this research.

Measurements of Depression

At ETS visit, the Beck Depression Inventory-II (BDI-II) was administered to assess the severity of depressive symptoms. The BDI is a validated scale providing a continuous measure of depressive symptoms, including 21 items each scored from 0 to 3, with a total score ranging from 0 to 63.[211-213] A higher BDI indicates more depressive symptoms. In the analysis related to Specific Aim #1, we removed the sleep item in BDI to eliminate the potential influence it may have on the association of BDI with sleep disturbance. At both visit, we also administered the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorder, 4th Edition (DSM-4), or SCID, to obtain a clinical diagnosis of major depressive disorder (MDD).

Measurements of Heart Rate Variability

At ETS visit, twin wore an ambulatory electrocardiogram monitor for 24 hours. We followed previously published procedures to maximize accuracy of recordings and minimize potential confounding.[222] Both twins in the same pair were evaluated at the same time, and their recording times, schedule, and activity level during each recording were similar. Twins were refrained from smoking, drinking alcohol, and having coffee during measurements. We used frequency-domain methods to analyze the HRV data, utilizing customized software to assign bands of frequency and then count the number of beat-to-beat intervals that match each band.[11,223] Each tape of Holter recordings was digitally processed and analyzed using methods as previously described in the literature,[11,223] and was further segmented into daytime (6am to 10pm) and nighttime (10pm to 6am) periods as determined by time stamps on Holter recording. The HRV spectrum was computed using a fast Fourier transform with a Parzen window on the 24-hour R-R interval file. We evaluated 24-hour average, as well as daytime and nighttime

average values for four discrete frequency bands, including ultra-low frequency (ULF, <0.003 Hz), very low frequency (VLF, 0.0033-0.04 Hz), low frequency (LF, 0.04-0.15 Hz), and high frequency (HF, 0.15-0.40 Hz).[68,143] We also calculated deceleration capacity (DC), which provides an average speed of heart rate deceleration, which is a potentially more useful indicator than other HRV metrics in evaluating parasympathetic nervous function and predicting adverse health outcomes.[70] The HRV data processing was performed blindly to twins' characteristics.

Measurements of Mortality and Cardiovascular Events

Vital status data during follow-up, including mortality dates and causes of deaths (e.g. cancer, CVD, etc.), were collected and verified by National Death Index database through December 31st, 2017. All-cause mortality was the primary outcome of this study. Comprehensive medical history data, including all cardiovascular events and hospitalization dates, were obtained among twins who completed ETSF, either in-person or over the phone. Data on CVD outcomes, including dates and reasons for hospitalizations, were objectively measured and adjudicated by a thorough medical chart review. As a secondary outcome, we evaluated a composite measure of major CVD events, including myocardial infarction (MI), congestive heart failure (CHF), and stroke.

Other Measurements

At the baseline visit (2002-2010), a thorough assessment including medical history and physical examination were obtained by a research nurse or physician assistant. Sociodemographic and anthropometric data, health behaviors, fasting blood glucose and lipid profile were measured as previously described.[81,207] Habitual physical activity was measured

using the Baecke Questionnaire of Habitual Physical Activity. This is a 16-question instrument recording physical activity levels at work, during sports and non-sports activities, rendering a global physical activity score.[227,228] History of hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or self-reported use of anti-hypertensive medications, following the Joint National Committee (JNC)-7 classification for Stage 1 hypertension which was the accepted staging at the time.[229] History of coronary artery disease that might have occurred from the time of the initial screen was also assessed. Diabetes mellitus was defined as having a measured fasting glucose of more than 126 mg/dL or any current treatment with antidiabetic medications. Current use of beta-blockers, antidepressants, statins, and angiotensin-converting enzyme inhibitors were also recorded. A clinical diagnosis of PTSD (lifetime and current), as well as alcohol abuse disorder, were obtained using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorder, 4th Edition (DSM-IV), or SCID.

Statistical Analysis

We conducted descriptive analyses by summarizing participants' characteristics at the baseline visit, sociodemographic factors, health-related factors, medication use, depression status, and 24-hour average HRV. Continuous variables were described as mean and standard deviation (SD), and categorical variables as frequencies (percentage). The HRV data were log-transformed owing to non-normality. We also compared the characteristics among twins who deceased during 12-year follow-up to those who survived, using two sample t-test (for continuous variables) and chi-squared test (for categorical variable). Kaplan-Meier curves for all-cause mortality and a composite measure of major CVD events were computed by within-pair

difference of depression (i.e. within-pair difference of BDI or MDD >0 vs. <0) or log-HRV (i.e. within-pair difference of log-HRV >0 vs. <0), and log rank tests were used to compare the survival curves. To assess potential selection bias, we also compared the characteristics among twins with and without the follow-up assessment.

Our primary analysis focused on the associations between the within-pair difference in depression and log-HRV with time to all-cause mortality. The end of follow-up time was the last contact date, the date of death, or the last day of available NDI data (12/31/2017), whichever was the latest. For all analyses, BDI, MDD and 24-hour average HRV metrics were used as primary predictors of interest, and we also examined daytime and nighttime average HRV metrics. As a secondary analysis, we also evaluated the associations between depression and log-HRV and time to first major CVD events, including MI, CHF, and stroke. In a study of twins, within-pair differences intrinsically control for potential confounding by shared genetic and early familial confounding, as well as environmental factors (e.g. physical activity, diet) during ambulatory monitoring as twins were examined together. For all analyses, we fitted multivariable frailty models and accounted for twin pair as random effect. The frailty models are the extensions of the Cox proportional hazard models, with random effect to account for heterogeneity in clustered data (such as in twins dataset).[231] To allow comparisons between different HRV metrics, the HRV metrics were standardized so that the β coefficients can be interpreted as hazard ratios for all-cause mortality or CVD events, per 1-SD increment in log-HRV metrics.

To avoid model overfitting, we constructed a series of models to examine the impact of sets of a priori selected variables on the association of interest. The base model, or model 1, was unadjusted, and only included between-pair difference of HRV metrics. We then progressively adjusted for sociodemographic and behavioral variables (education, employment status, ever

smoking status, alcohol abuse, and physical activity) in model 2, and further adjusted for CVD risk factors that are likely related to depression, HRV and adverse health outcomes (BMI, history of hypertension, history of coronary artery disease, and diabetes) in model 3.[163] In model 4, we additionally adjusted for medication use, including beta-blockers and antidepressants.

To assess potential shared genetic influence on the HRV and adverse health outcome, we examined the associations separately in MZ and DZ twins to evaluate effect modification by zygosity. Because MZ twin pairs share 100% of their genes while DZ twin pairs only share 50% on average, if a larger effect of HRV on adverse health outcomes is observed within DZ pairs than in MZ pairs, then it may suggest that genetic factors play a role in this association.

We also conducted a series of sensitivity analyses to expand our primary analytic approach. First, we examined the association between HRV and cause-specific mortality including cancer and CVD, and further accounted for competing risk due to non-cancer or non-CVD mortality, respectively, using Fine & Gray subhazard models.[232] Second, we repeated all analyses by examining twins as individuals instead of within-pair, to allow an evaluation of potential familial and environmental influence on the associations of interest. Third, we examined whether the results remained robust after additionally adjusting for depression and PTSD diagnosis or symptoms, as well as adjusting for 24-hour average heart rate, as prior research pointed out that the correlation between HRV and mortality could be partially attributable to concurrent change in HR.[233] We also tested the effect modification between depression and HRV by including an interaction term between the two variables in all models.

Missing data were rare (<5%) for all variables, thus we used all available data without imputation. We checked linearity assumptions of all continuous variables, as well as potential multicollinearity by variance inflation factors. A two-sided p-value of less than 0.05 was used to

indicate statistical significance, and hazard ratios and associated 95% confidence intervals (CI) were calculated for model parameters. All statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC) and Stata 14.0 (StataCorp, College Station, TX).

6.4 Results

Participants' Characteristics

Of the 566 twins participated at the baseline visit, 501 individuals had any analyzable HRV data, including 225 twin pairs (142 MZ pairs and 83 DZ pairs), and 51 single twins. The 225 paired twins (n=450) represented our analytical sample for the within-pair analysis for mortality (**Figure 6.1**). Of these twins, 436 (97%) were white, with a mean age (SD) of 56 (3) years (**Table 6.1**). A total of 126 (28%) twins met criteria for lifetime history of MDD, and the mean (SD) BDI score was 6.1 (7.9). During a mean (SD) follow-up of 12 (3) years, a total of 53 (12%) out of 450 twins deceased. Comparing deceased twins with those who survived follow-up (n=397), twins who deceased had significantly lower BMI, less physical activity, and consistently lower HRV in almost all domains, except for HF HRV (**Table 6.1**). Overall, the characteristics were similar comparing twins with and without follow-up assessment, except that twins who did not attend follow-up visit had more smokers and significantly higher BDI score compared to twins who completed follow-up (**Table S.6.1**).

Depression, Heart Rate Variability and All-cause Mortality

During the 12-year follow-up, 53 (112%) out of 450 twins died and were verified by NDI, with a mean (SD) time to mortality of 6.7 (4.4) years. Among the twins who died, 22 (42%) and 14 (26%) were due to cancer or CVD causes, specifically. Other causes of death

included natural causes, motor vehicle accident, suicide, endocrine, lung, or gastrointestinal disorders.

Kaplan-Meier survival curves suggested that twins with a within-pair difference of >0 in VLF, LF or DC domain, compared to their co-twins with within-pair difference <0 in the same domain, had better survival rate in terms of all-cause mortality (**Figure 2**). Log rank tests indicated that the differences in survival curves by LF and DC HRV domains were statistically significant (p=0.048 and p=0.047, respectively). Kaplan-Meier curves showed that twins with higher ULF and HF HRV had similar survival compared to their co-twins with lower HRV. Kaplan-Meier curves suggested that twins with higher BDI or MDD within-pair did not have significantly increased risk for all-cause mortality.

For the within-pair analysis of the association between baseline depression and HRV and all-cause mortality during follow-up, higher BDI and MDD within-pairs were not significantly associated with increased risk for all-cause mortality, but higher values in all domains of 24-hour average HRV demonstrated decreased hazard for mortality across all models, after adjusting for sociodemographic and behavioral factors, CVD risk factors, and medication use (**Table 2**). The hazard ratios (HR) for HRV ranged from 0.73 to 0.96 after multivariable adjustment, suggesting a 4% to 27% decreased hazard per 1-SD increment in log-HRV domains. Of note, LF and DC HRV showed the strongest and most consistent association with decreased risk for all-cause mortality, with HRs (95% CI) of 0.78 (0.62, 0.98) and 0.73 (0.56, 0.95), respectively. Compared to nighttime data, daytime average HRV showed similar associations with all-cause mortality in ULF, VLF and LF domains, but showed slightly stronger association in HF and DC domains. Specifically, a 1-SD increment in daytime average log-LF and log-DC HRV showed significantly decreased hazard for all-cause mortality, with HRs (95% CI) of 0.77 (0.61, 0.98)

and 0.71 (0.55, 0.92); while for nighttime average HRV, log-DC showed significantly decreased hazard (HR: 0.76, 95% CI: 0.58-0.99), and log-LF HRV showed marginally significant result (HR: 0.77, 95% CI: 0.59-1.00).

Depression, Heart Rate Variability and CVD Events

Kaplan-Meier survival curves did not show any material difference in survival probabilities for major CVD events by within-pair difference in depression or any HRV domains, and the log-rank tests showed non-significant results in all comparisons (**Figure S.6.1**). Among twin pairs who survived and participated in ETSF (132 pairs), a total of 18 (7%) twins reported any major CVD events during follow-up, including MI, CHF, and stroke, thus the 132 pairs represented our analytical sample for the within-pair analysis for CVD events. The mean (SD) time from baseline to first major CVD event was 9.1 (3.8) years. Higher BDI within-pair were not associated with increased risk for mortality. MDD is associated with a 5.5-fold increased risk (HR=5.51, 95% CI: 0.49-61.91) of major CVD events, however this association did not reach statistical significant, likely due to small number of CVD event especially among discordant MDD pairs. A 1-SD increment in 24-hour average log-HRV values in all domains were associated with 29% to 45% decreased hazard for major CVD events, with HRs ranging from 0.55 to 0.71, however none of the HRs was statistically significant (**Table 6.3**). Daytime average HRV consistently had stronger association with CVD events compared to nighttime HRV data across all HRV domains and models, with HRs ranging from 0.49 to 0.65 for daytime HRV and 0.66 to 0.81 for nighttime HRV. Specifically, after multivariable adjustment, a 1-SD increment in log-HRV was associated with 35% to 51% decreased hazard for CVD events, with log-ULF HRV showed significantly decreased risk (HR=0.49, 95% CI: 0.26-0.95) and log-LF HRV

showed marginally significant association (HR=0.63, 95% CI: 0.39-1.00). In contrast, nighttime average HRV domains were associated with 19% to 34% decreased hazard for CVD, and none of the associations was statistically significant.

Additional Analyses

The stratified analysis by zygosity was only completed for all-cause mortality, as the sample sizes for other outcomes (i.e. cause-specific mortality and CVD events) were too small to yield reliable estimates of effects. Overall, the associations of depression and HRV with all-cause mortality were slightly stronger in DZ twins compared with MZ twins across almost all models and HRV domains (**Table S.6.2**), however the interaction term with zygosity was consistently not significant in any of the models.

In the analysis evaluating within-pair difference of baseline depression and HRV with cancer-specific mortality, MDD predicted increased hazard, and 1-unit increase in BDI was significantly associated with increased hazard for cancer mortality (HR=1.12, 95% CI: 1.01-1.25). Higher values in all HRV metrics showed decreased hazard for cancer mortality, with DC showed significant and strongest association (HR=0.62 per 1-SD increment in log-DC HRV, 95% CI: 0.40-0.96) (**Table S.6.3**). The associations for nighttime HRV were slightly stronger compared to daytime HRV, specifically for VLF, LF and DC domains. As for CVD-specific mortality, BDI did not show significant association with increased hazard, while MDD was associated with two-fold increased hazard of CVD mortality (HR=2.16, 95% CI: 0.39-11.92) (**Table S.6.4**). None of the baseline HRV metrics was significantly associated with hazard for CVD-specific mortality across all HRV domains and models. There was no material difference

in the results with or without accounting for competing risk due to non-cancer or non-CVD mortality, respectively, using Fine & Gray models (results not shown).

Consistently across all models, the additional adjustment for depression on the association of HRV with outcome variables, or adjustment for HRV on the association of depression with outcome, did not materially changes the results. In all models that we tested, the interaction term between depression and HRV was consistently not significant, suggesting a lack of effect modification of the two phenotypes. The results of analysis evaluating twins as individuals were similar compared to within-pair analysis (**Tables S.6.5-S.6.6**). For example, a 1-SD increment in log-LF and log-DC HV was significantly associated with 21% and 30% decreased hazard for all-cause mortality, respectively. None of the HRV domains was significantly associated with risk for major CVD events, and associations were consistently weaker compared to within-pair analysis. Additional adjustment for depression, PTSD, or 24-hour average heart rate did not materially change the associations (results not shown).

6.5 Discussion

In this co-twin control study, higher values of all HRV frequency domains, denoting better autonomic function, were associated with 4%-27% decreased hazard for all-cause mortality and 29%-45% decreased hazard for major CVD events during an average of 12-year follow-up. A 1-unit increase in BDI was significantly associated with 12% increased hazard for cancer mortality, and MDD was associated with increased hazard for mortality and major CVD events. The associations remained robust after adjusting for relevant sociodemographic, behavioral and health-related factors, and medication use. Both LF and DC HRV domains showed the strongest and most consistent associations with all-cause mortality, while ULF HRV

showed the strongest predictive values on major CVD events. Overall, daytime HRV metrics showed similar but slightly stronger associations with all-cause mortality and CVD events compared to nighttime HRV. Higher values of all HRV metrics were associated with decreased hazard for cancer-specific mortality, but were not associated with CVD mortality. The genetic factors may not play a major role in the associations of HRV with all-cause mortality, as the differences of the associations were not statistically significant between MZ and DZ twins.

Consistent with previous findings, we found major depression was associated with an increased risk of cancer mortality and major CVD events, even though such associations were not statistically significant.[90,94,97,98,101] Our results did not show a substantial increased risk of mortality among participants with major depression or more depressive symptoms, either within-pair or as individuals, as previous research suggested.[7] The different results in our investigation compared to others may be due to the small numbers of participants with MDD and adverse health outcomes, presence of unmeasured or unknown confounders, or differences in the study population and participants' characteristics. In addition, twins with MDD or more depressive symptoms were more likely to drop out during follow-up, thus participants in ETSF who had available cardiovascular outcome data had better mental health status, which may have biased the association between depression and CVD events towards the null. Thus, a lack of statistically significant results does not rule out an association between depression and adverse health outcomes, including mortality and CVD.

Our findings are consistent with a meta-analysis of 28 cohort studies in patients with known CVD, showing that individuals with a lower HRV had 112% and 46% higher risk of all-cause death and cardiovascular events, respectively.[184] Our results also agree with prior community-based research in middle-aged to elderly participants,[28,188-190] including individuals

without known CVD,[67] which suggest that reduced HRV is associated with an adverse cardiovascular risk profile and an elevated risk of mortality and CVD events, and the elevated risk of mortality could not be attributable to a specific cause.

In line with prior investigations,[145,188] our study showed that, among all HRV frequency bands, LF power is the strongest HRV predictor with regard to mortality, and the prognostic implications of baseline HRV still remain after 5 years. The power in the LF HRV domain is modulated mainly through sympathetic nervous system (SNS) as a response to oscillations in blood pressure.[191] The reduction in the parasympathetic function may be an early sign of autonomic dysregulation, but it has been hypothesized that impaired sympathetic modulation indexed by reduced LF power may imply a more severe involvement of autonomic nervous system.[145,192-194] Contrary to previous studies, we did not find any significant prognostic values in VLF and HF domains in predicting all-cause mortality.[188] Our study also extended findings from previous investigations that evaluated predictive values of DC among patients with CVD, and demonstrated that DC at baseline significantly predicted all-cause mortality among individuals without known CVD.[70,195]

As for cause-specific mortality, our findings agreed with prior research showing that the association between reduced HRV and increased risk of mortality was not specifically due to CVD causes, and can be largely explained by non-CVD causes, such as cancer.[67,189] It has been reported that sympathetic activation, linked with reduced HRV, may have direct effects on the number, function, and subset distribution of circulating lymphocytes, which play a major role in the immune function and cancer risk.[196,197] Contrary to other studies,[198,199] we did not find significant predictive values of HRV in CVD mortality. One explanation for the discrepancy is that, in our study, we did not obtain cardiovascular or cancer disease status in deceased

participants, or collect data on comorbidity of both CVD and cancer. It is likely that participants die of cancer also had CVD, and they may have died of CVD instead if they did not also have cancer. In addition, the numbers of participants died of different causes are too small for a more comprehensive evaluation of cause-specific mortality. Thus, our study cannot rule out significant prognostic values of HRV in predicting CVD mortality.

Our study supports findings from previous literature that lower HRV in multiple domains is linked to increased risk for incident cardiovascular events, in patients with or without known CVD at baseline, and the elevated risk could not be attributable to other risk factors.[28,67,184] Of note, one study has shown that lower HRV significantly predicted a higher risk in patients with MI but not in patients with CHF.[184] However, due to the small number of twins in the within-pair analysis that had any major CVD events, we were not able to confirm this finding by conducting subgroup analysis and separately evaluating the predictive values of HRV in different types of events, such as MI, CHF and stroke.

Physical activity and mental stressors can influence the measurement of HRV, thus nighttime HRV measuring during sleep is not comparable with HRV assessment based on 24-hour Holter monitoring.[201,202] However, to date no prior study has evaluated and compared the prognostic values of daytime and nighttime HRV frequency domains in predicting mortality and CVD events. In our study, we found that both daytime and nighttime HRV are associated with similarly decreased risk for all-cause mortality, except that daytime HF and DC HRV showed stronger associations with mortality compared to nighttime HRV data. Both HF and DC are influenced by modulation of PNS activities, which suggests that alterations in daytime PNS functions may have stronger prognostic implications in mortality risk. We found that daytime HRV consistently had stronger effects on risk of major CVD events compared to nighttime

HRV, which contradicts with a previous study that found nighttime HRV instead of 24-hour HRV to be associated with stroke events.[202] The discrepancy may result from different study design, timing of HRV measurement, and different HRV metrics that were evaluated.

The pathophysiological mechanisms linking depression, reduced HRV and risk for mortality and CVD events still remain unclear. It has been proposed that higher mortality found among depressed individuals might be attributable to the mechanisms specific to the existing diseases, behavioral pathways such as treatment adherence and health behaviors, and biological pathways (e.g. neuroendocrine and neuro-immunological systems, and the circadian rhythm).[7,105] For cancer-specific mortality, one of the suggested mechanisms is stress affecting the development and progression of cancer by impacting the repair of damaged DNA and accelerating tumor cell growth, which may contribute to a shorter time to cancer death.[111,112] As for mechanisms linking depression to CVD, studies have suggested multifactorial pathophysiological pathways through which depression can increase CVD risk, including neuroendocrine dysregulation, metabolic and immune-inflammatory disturbance, and unhealthy lifestyle behaviors (e.g. smoking, alcohol abuse, physical inactivity, and unhealthy diet).[19,20,32,100,113-115]

HRV represents the adaptive responses in heart rate caused by fluctuations of both SNS and PNS activities of the autonomic nervous system. The dysfunction of the autonomic nervous system, indexed by reduced HRV, reflects the sympathovagal imbalance.[203] It has been hypothesized that the sympathovagal imbalance or an overshooting sympathetic activation may be linked to higher risk of mortality and cardiovascular events.[195,204] In our study, the association of HRV with risk of mortality and CVD was present in participants without known CVD, which suggests that low HRV precedes manifest diseases. Alternatively, the higher mortality and CVD

incidence associated with lower HRV may also be due to subclinical coronary artery disease. It has also been hypothesized that lower HRV is an indicator of unfavorable general health, such as immune function, which plays a major role in tumor formation and progression.[189] This may explain the association of HRV with increased risk of cancer-specific mortality in our study. DC indicates a measure of cardiac vagal modulation, and prior literature have shown a cardioprotective role of vagal activity,[205,206] which is supported by our findings.

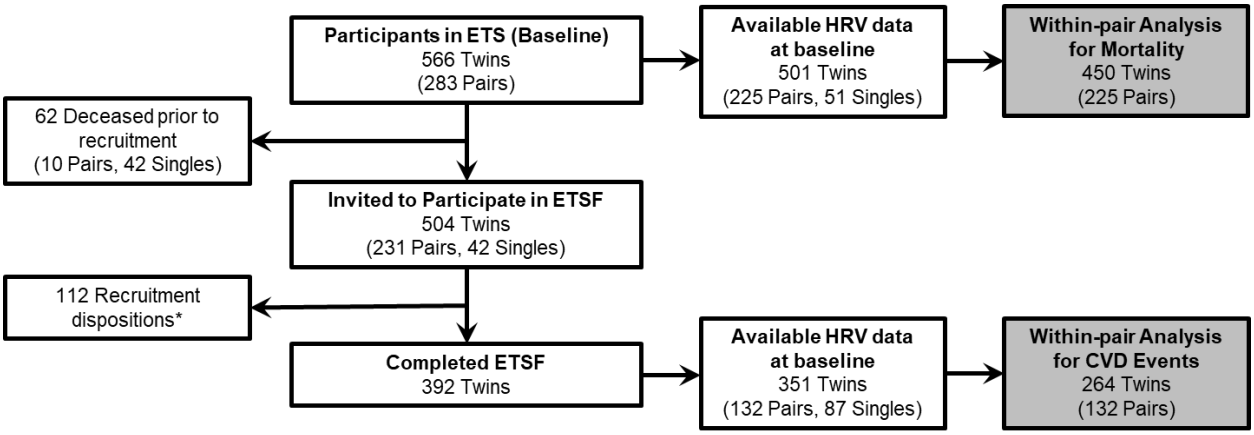
A limitation of our study is that the National Death Index has limited refresh frequency (annual) and has a 2-year reporting delay, although it is the current US gold standard for mortality data. Thus, we were not able to verify mortality data after December 31st, 2017, which may slightly overestimate the overall survival in our sample and biased the associations between HRV and mortality towards the null. Second, due to the small number of participants with CVD events (n=18) that were included in the within-pair analysis (n=264), we may have had limited power to detect any statistically significant effects. This may explain why the effect sizes were large (i.e. HRs from 0.55 to 0.71 per 1-SD increment in log-HRV) but none of the associations was statistically significant. Third, for the twins who deceased during follow-up, they were not included in the ETSF thus their cardiovascular outcomes were not obtained and verified, which may lead to an underestimation of CVD events in our sample. In addition, our study also has limited generalizability, as our sample included all males and mostly white middle-aged twins. However, our co-twin control study design should have improved internal validity and precision by intrinsically adjusting for unknown or unmeasured confounders. Using a co-twin control study design, our study is the first investigation that longitudinally examined the prognostic implications of a full spectrum of HRV frequency domains, during both daytime and nighttime, in predicting mortality and cardiovascular outcomes. In addition, our study is further

strengthened by a relatively long follow-up of 12 years, and a thorough medical chart review process to adjudicate all cardiovascular events that minimized potential recall bias and measurement error.

6.6 Conclusions

In the context of a controlled twin design, the present study provides evidence of strong prognostic values of higher HRV metrics, denoting better autonomic function, in predicting decreased risk for both mortality and cardiovascular events. Compared to nighttime HRV, daytime HRV may have similar predictive values in all-cause mortality but slightly stronger protective effects on CVD events. More depressive symptoms indexed by higher BDI significantly predicted mortality due to cancer. These associations are not explained by sociodemographic, behavioral and health-related factors, and medication use, and shared genetic factors may not play a major role in these associations. Our study demonstrates that reduced HRV is an indicator of compromised health and can be used as independent predictors for adverse health outcomes, such as mortality and CVD. Major depression and depressive symptoms are linked with higher risk of cancer mortality and CVD events. Furthermore, our findings suggest that HRV evaluation can be incorporated into the monitoring of autonomic function as a prevention strategy of adverse health outcomes, including mortality and CVD. Larger studies are needed to evaluate prognostic values of depression and HRV in cause-specific mortality as well as different types of cardiovascular events, such as MI, CHF and stroke.

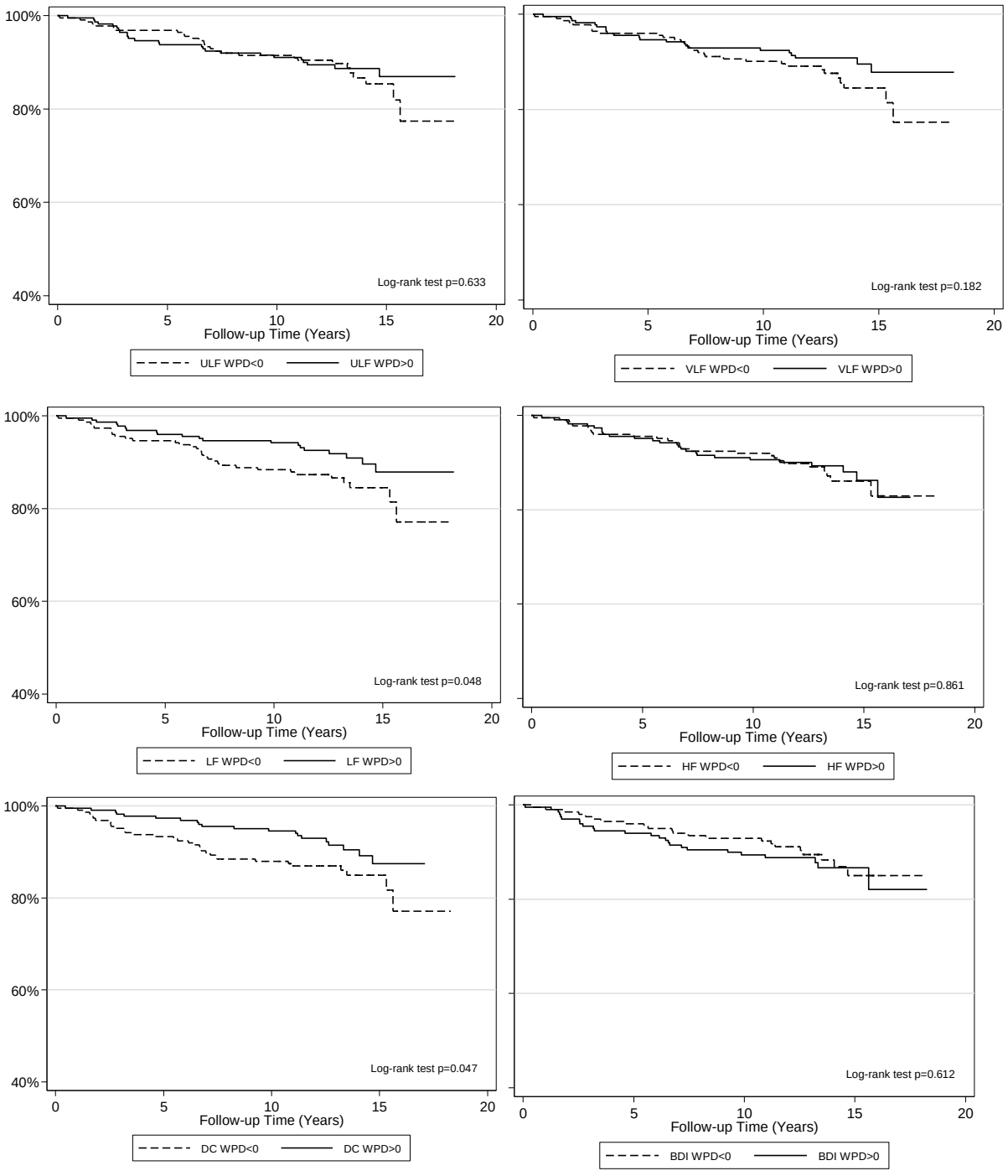
Figure 6.1 Participant Flow Diagram

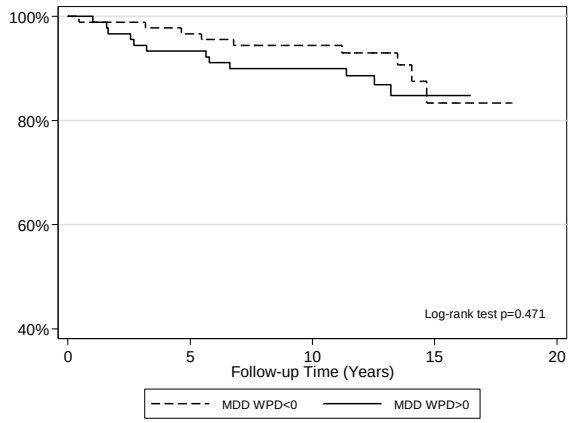


Abbreviations: CVD: cardiovascular disease; ETS: Emory Twin Study; ETSF: Emory Twin Study Follow-up; HRV: heart rate variability.

* Recruitment dispositions: 95 never responded or refused to participate; 10 deceased during recruitment; 5 were too ill to participate; 1 withdrew; 1 incarcerated during recruitment.

Figure 6.2 Kaplan-Meier survival probabilities for all-cause mortality by within-pair difference of heart rate variability domains or depression (n=450)





Abbreviations: BDI: beck depression inventory; DC: deceleration capacity; HF: high frequency; LF: low frequency; MDD: major depressive disorder; ULF: ultra-low frequency; VLF: very low frequency; WPD: within-pair difference.

Table 6.1 Characteristics of 450 twins (225 pairs) with available HRV data in ETS

Characteristics, mean (SD)	Total (N=450)		Deceased (N=53)		Survived (N=397)	
Sociodemographic factors						
Age, years	56	(3)	55	(3)	56	(3)
White, No. (%)	436	(97)	49	(92)	387	(97)
Year of education	15	(2)	15	(2)	15	(2)
Employed, No. (%)	361	(80)	39	(74)	322	(81)
Health factors						
BMI*	30	(5)	28	(6)	30	(5)
Ever smokers, No. (%)	289	(64)	40	(75)	249	(63)
Alcohol abuse, No. (%)	208	(46)	30	(57)	178	(45)
Baecke score for physical activity*	7.3	(1.8)	6.8	(2.1)	7.3	(1.8)
Systolic blood pressure, mmHg	124	(10)	121	(10)	124	(10)
Diastolic blood pressure, mmHg	74	(9)	72	(10)	74	(8)
History of hypertension, No. (%)	168	(37)	16	(30)	152	(38)
History of diabetes, No. (%)	54	(12)	7	(13)	47	(12)
Prior history of CAD, No. (%)	47	(10)	8	(15)	39	(10)
Lifetime history of PTSD, No. (%)	68	(15)	7	(13)	61	(15)
Lifetime history of depression, No. (%)	126	(28)	18	(34)	108	(27)
Current PTSD, No. (%)	30	(7)	5	(9)	25	(6)
Current depression, No. (%)	16	(4)	3	(6)	13	(3)
BDI-II score	6.1	(7.9)	7.0	(7.6)	6.0	(7.9)
Medication use						
β-Blockers, No. (%)	36	(8)	6	(11)	30	(8)
Antidepressants, No. (%)	72	(16)	10	(19)	62	(16)
Statin, No. (%)	113	(25)	8	(15)	105	(26)
ACE inhibitor, No. (%)	72	(16)	8	(15)	64	(16)
Heart rate variability						
24-hour average						
ln ULF*	6.7	(0.7)	6.5	(0.9)	6.7	(0.7)
ln VLF*	7.7	(0.7)	7.4	(0.9)	7.7	(0.7)
ln LF*	6.7	(0.8)	6.4	(1.0)	6.7	(0.7)
ln HF	5.5	(0.8)	5.5	(0.9)	5.5	(0.8)
ln DC*	2.3	(0.4)	2.2	(0.5)	2.4	(0.3)
Daytime average						
ln ULF*	6.8	(0.7)	6.5	(0.9)	6.8	(0.7)
ln VLF*	7.7	(0.7)	7.3	(0.9)	7.7	(0.7)
ln LF*	6.6	(0.8)	6.3	(1.0)	6.7	(0.7)
ln HF	5.4	(0.8)	5.4	(0.9)	5.4	(0.8)
ln DC*	2.3	(0.4)	2.2	(0.5)	2.3	(0.4)
Nighttime average						
ln ULF	6.3	(1.1)	6.1	(1.1)	6.3	(1.1)
ln VLF*	7.7	(1.0)	7.4	(1.0)	7.7	(1.0)
ln LF*	6.7	(0.9)	6.4	(1.1)	6.7	(0.9)
ln HF	5.6	(0.9)	5.7	(1.0)	5.6	(0.9)
ln DC*	2.4	(0.4)	2.3	(0.5)	2.5	(0.4)

* Indicates statistically significant ($p < 0.05$) difference between two groups, using two-sample t-tests for continuous variables, and chi-squared tests for categorical variables.
Abbreviations: ACE: angiotensin-converting enzyme; BDI: Beck Depression Inventory; BMI: body mass index; CAD: coronary artery disease; DC: deceleration capacity; ETS: Emory Twin Study; HF: high frequency; HRV: heart rate variability; LF: low frequency; PTSD: posttraumatic stress disorder; SD: standard deviation; ULF: ultra-low frequency; VLF: very low frequency.

Table 6.2 Within-pair analysis of the association between baseline HRV and depression and time to all-cause mortality during follow-up[a]

	Model 1[b]	Model 2[c]	Model 3[d]	Model 4[e]
BDI	1.02 (0.97, 1.08)	1.00 (0.94, 1.06)	1.00 (0.94, 1.06)	1.00 (0.94, 1.07)
MDD	1.35 (0.59, 3.05)	1.03 (0.44, 2.41)	1.02 (0.43, 2.40)	1.02 (0.43, 2.42)
24-hour average HRV (n=450)				
ln ULF	0.90 (0.72, 1.11)	0.89 (0.70, 1.14)	0.90 (0.71, 1.14)	0.90 (0.71, 1.14)
ln VLF	0.85 (0.68, 1.05)	0.84 (0.66, 1.06)	0.85 (0.68, 1.07)	0.85 (0.67, 1.07)
ln LF	0.79 (0.63, 0.98)*	0.79 (0.62, 0.99)*	0.79 (0.63, 0.98)*	0.78 (0.62, 0.98)*
ln HF	0.95 (0.72, 1.26)	0.96 (0.72, 1.28)	0.96 (0.72, 1.28)	0.96 (0.72, 1.28)
ln DC	0.73 (0.56, 0.94)*	0.75 (0.58, 0.97)*	0.74 (0.57, 0.96)*	0.73 (0.56, 0.95)*
Daytime average HRV (n=444)				
ln ULF	0.90 (0.73, 1.10)	0.89 (0.71, 1.13)	0.90 (0.72, 1.13)	0.90 (0.71, 1.13)
ln VLF	0.86 (0.70, 1.06)	0.85 (0.68, 1.06)	0.86 (0.68, 1.07)	0.85 (0.68, 1.07)
ln LF	0.79 (0.63, 0.98)*	0.78 (0.62, 0.99)*	0.78 (0.62, 0.98)*	0.77 (0.61, 0.98)*
ln HF	0.89 (0.67, 1.17)	0.89 (0.67, 1.18)	0.89 (0.67, 1.18)	0.88 (0.66, 1.17)
ln DC	0.72 (0.56, 0.94)*	0.74 (0.57, 0.96)*	0.73 (0.57, 0.94)*	0.71 (0.55, 0.92)*
Nighttime average HRV (n=418)				
ln ULF	0.95 (0.77, 1.18)	0.94 (0.74, 1.20)	0.93 (0.73, 1.17)	0.91 (0.72, 1.16)
ln VLF	0.89 (0.71, 1.10)	0.87 (0.68, 1.11)	0.85 (0.67, 1.09)	0.84 (0.65, 1.08)
ln LF	0.82 (0.64, 1.04)	0.81 (0.63, 1.04)	0.79 (0.61, 1.02)	0.77 (0.59, 1.00)
ln HF	1.05 (0.79, 1.40)	1.09 (0.81, 1.47)	1.08 (0.80, 1.46)	1.06 (0.78, 1.44)
ln DC	0.75 (0.57, 0.98)*	0.79 (0.61, 1.04)	0.79 (0.61, 1.02)	0.76 (0.58, 0.99)*

Abbreviations: BDI: beck depression inventory; BMI: body mass index; DC: deceleration capacity; HF: high frequency; HRV: heart rate variability; LF: low frequency; MDD: major depressive disorder; SD: standard deviation; ULF: ultra-low frequency; VLF: very low frequency.

* Indicates significant association at $P < 0.05$.

[a] Results are shown as standardized hazard ratios in the multivariable Cox frailty models, per 1-SD within-pair difference in log-HRV.

[b] Base model was unadjusted.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, alcohol abuse, and physical activity.

[d] Model 3 = Model 2 + BMI, history of hypertension, history of coronary artery disease, and diabetes mellitus.

[e] Model 4 = Model 3 + beta-blockers and antidepressants.

Table 6.3 Within-pair analysis of the association between baseline HRV and depression and time to major cardiovascular events during follow-up[a]

	Model 1[b]	Model 2[c]	Model 3[d]	Model 4[e]
BDI	0.92 (0.77, 1.11)	0.90 (0.73, 1.11)	0.91 (0.73, 1.13)	0.91 (0.73, 1.13)
MDD	5.99 (0.57, 62.75)	5.74 (0.55, 59.84)	5.50 (0.49, 61.80)	5.51 (0.49, 61.91)
24-hour average HRV (n=264)				
ln ULF	0.64 (0.36, 1.15)	0.62 (0.34, 1.14)	0.53 (0.28, 1.01)	0.55 (0.29, 1.07)
ln VLF	0.84 (0.50, 1.39)	0.82 (0.49, 1.40)	0.69 (0.39, 1.21)	0.71 (0.40, 1.27)
ln LF	0.92 (0.60, 1.41)	0.86 (0.55, 1.33)	0.70 (0.44, 1.11)	0.70 (0.44, 1.12)
ln HF	0.84 (0.52, 1.36)	0.77 (0.47, 1.26)	0.68 (0.41, 1.13)	0.68 (0.42, 1.12)
ln DC	0.92 (0.60, 1.41)	0.80 (0.49, 1.30)	0.68 (0.40, 1.16)	0.68 (0.40, 1.16)
Daytime average HRV (n=259)				
ln ULF	0.64 (0.35, 1.14)	0.62 (0.34, 1.12)	0.50 (0.27, 0.93)*	0.49 (0.26, 0.95)*
ln VLF	0.79 (0.48, 1.31)	0.76 (0.45, 1.28)	0.61 (0.35, 1.06)	0.61 (0.35, 1.09)
ln LF	0.88 (0.56, 1.38)	0.81 (0.51, 1.28)	0.63 (0.40, 1.00)	0.63 (0.39, 1.00)
ln HF	0.83 (0.51, 1.36)	0.76 (0.46, 1.24)	0.66 (0.40, 1.10)	0.64 (0.39, 1.07)
ln DC	0.92 (0.59, 1.44)	0.79 (0.48, 1.29)	0.66 (0.39, 1.12)	0.65 (0.38, 1.10)
Nighttime average HRV (n=245)				
ln ULF	0.87 (0.67, 1.15)	0.86 (0.65, 1.13)	0.79 (0.59, 1.06)	0.79 (0.59, 1.06)
ln VLF	0.91 (0.70, 1.20)	0.88 (0.67, 1.17)	0.81 (0.60, 1.09)	0.81 (0.60, 1.09)
ln LF	0.92 (0.68, 1.25)	0.86 (0.63, 1.16)	0.76 (0.54, 1.05)	0.75 (0.54, 1.04)
ln HF	0.88 (0.59, 1.32)	0.83 (0.55, 1.25)	0.73 (0.48, 1.10)	0.73 (0.49, 1.09)
ln DC	0.93 (0.59, 1.45)	0.83 (0.51, 1.35)	0.69 (0.40, 1.17)	0.66 (0.38, 1.14)

Abbreviations: BDI: beck depression inventory; BMI: body mass index; DC: deceleration capacity; HF: high frequency; HRV: heart rate variability; LF: low frequency; MDD: major depressive disorder; SD: standard deviation; ULF: ultra-low frequency; VLF: very low frequency.

* Indicates significant association at $P < 0.05$.

[a] Results are shown as standardized hazard ratios in the multivariable Cox frailty models, per 1-SD within-pair difference in log-HRV. Major cardiovascular events include myocardial infarction, congestive heart failure, or stroke.

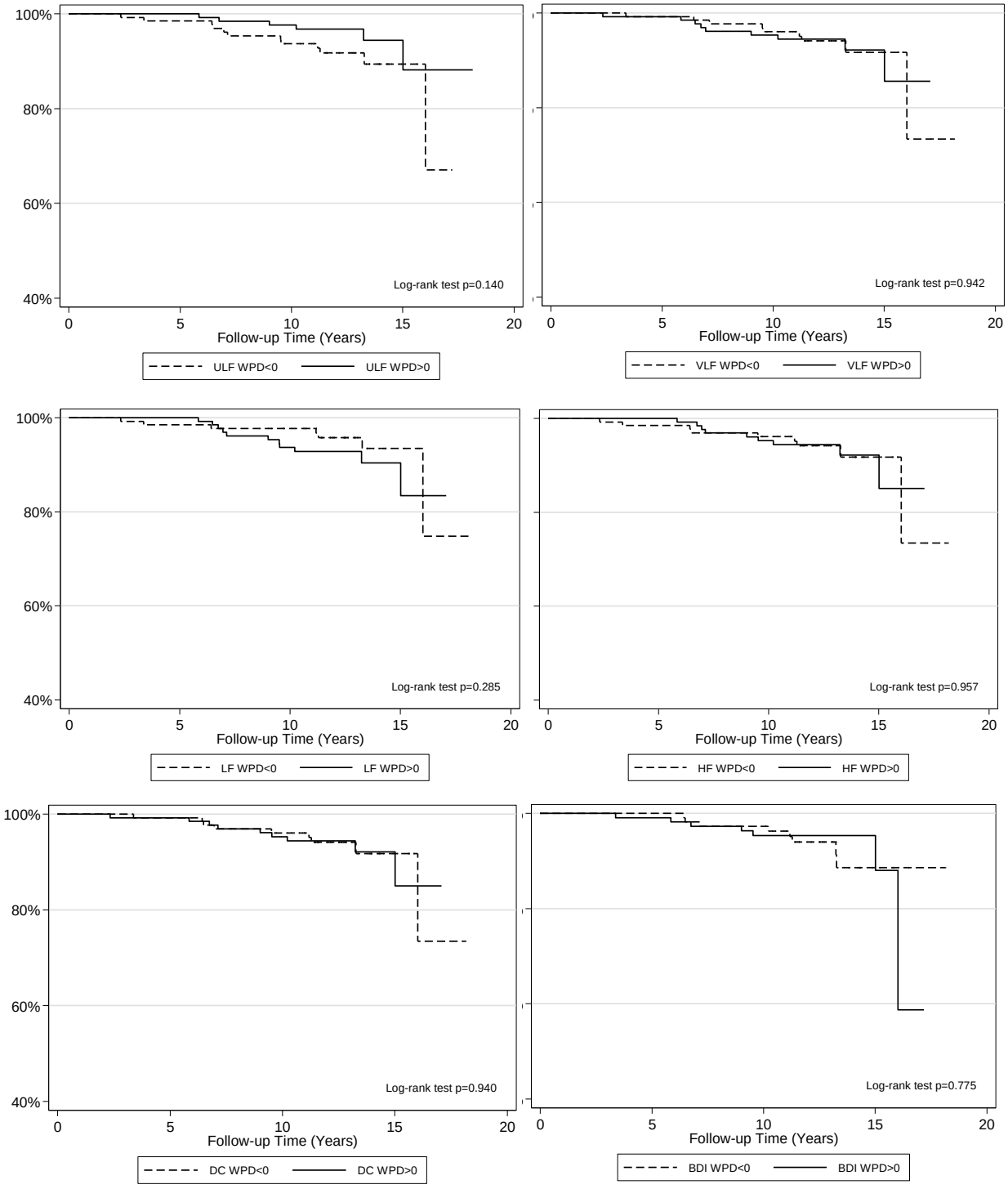
[b] Base model was unadjusted.

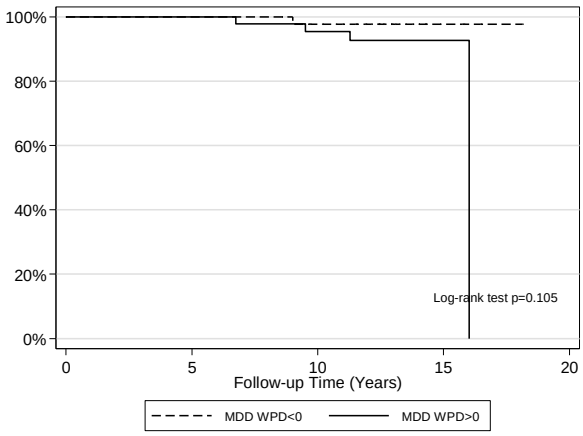
[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, alcohol abuse, and physical activity.

[d] Model 3 = Model 2 + BMI, history of hypertension, history of coronary artery disease, and diabetes mellitus.

[e] Model 4 = Model 3 + beta-blockers and antidepressants.

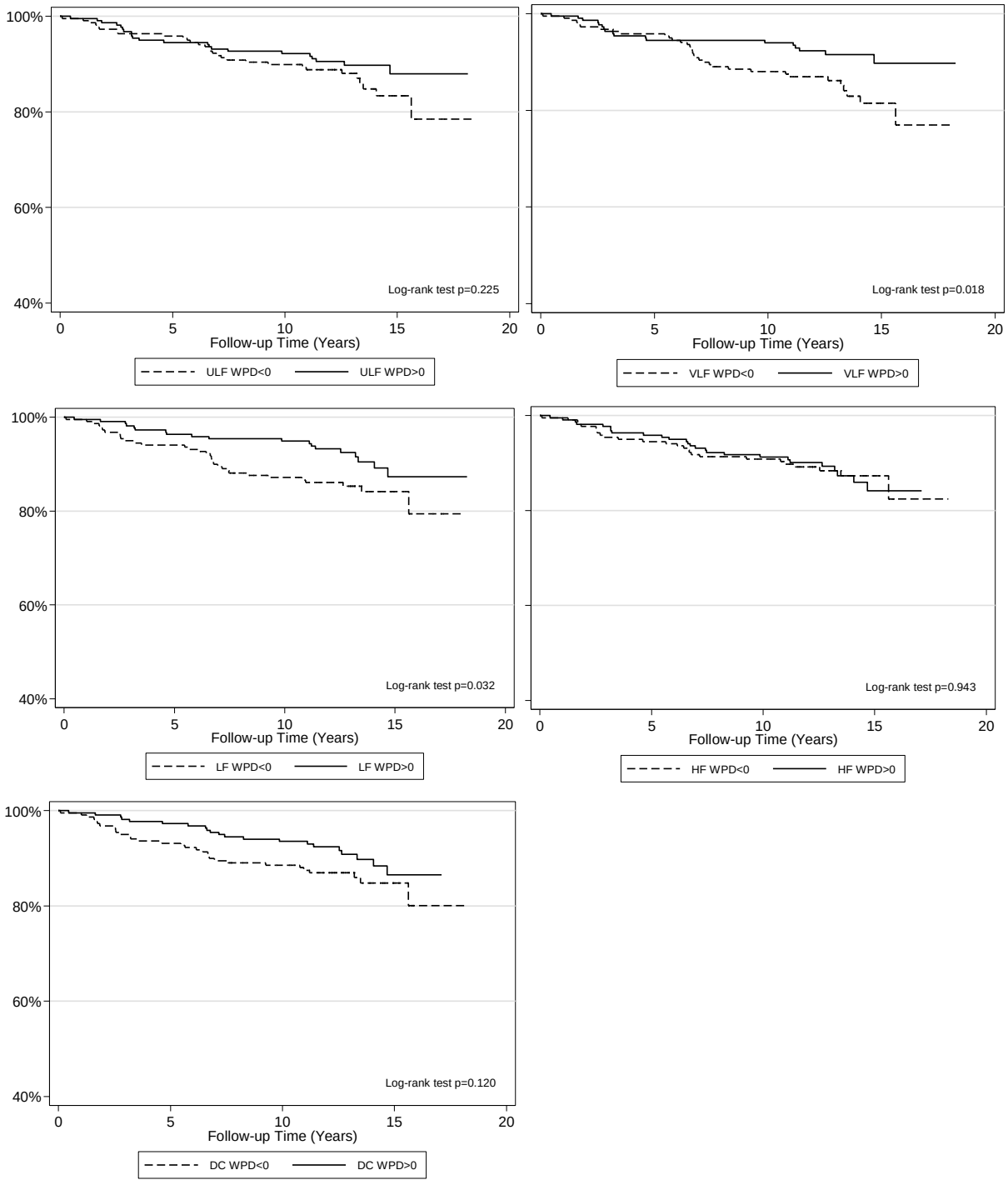
Figure S.6.1 Kaplan-Meier survival probabilities for major cardiovascular events by within-pair difference of heart rate variability domains and depression (n=264)





Abbreviations: BDI: beck depression inventory; DC: deceleration capacity; HF: high frequency; LF: low frequency; MDD: major depressive disorder; ULF: ultra-low frequency; VLF: very low frequency; WPD: within-pair difference.

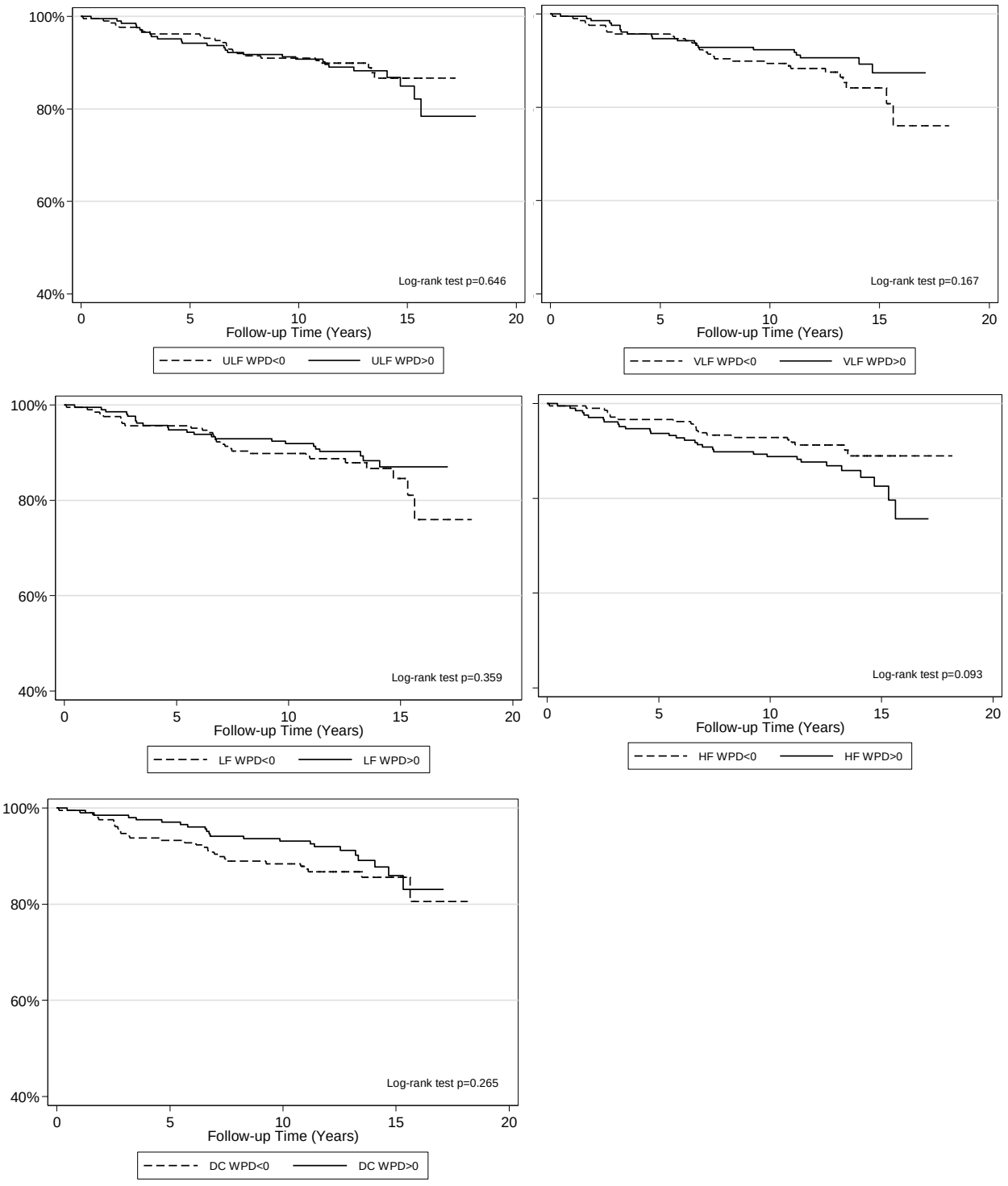
Figure S.6.2 Kaplan-Meier survival probabilities for all-cause mortality by within-pair difference of daytime heart rate variability domains (n=450)



Twins with a higher daytime HRV value than their brothers (WPD>0) were compared with their co-twins (WPD <0).

Abbreviations: DC: deceleration capacity; HF: high frequency; LF: low frequency; ULF: ultra-low frequency; VLF: very low frequency; WPD: within-pair difference.

Figure S.6.3 Kaplan-Meier survival probabilities for all-cause mortality by within-pair difference of nighttime heart rate variability domains (n=450)



Twins with a higher nighttime HRV value than their brothers (WPD>0) were compared with their co-twins (WPD <0).

Abbreviations: DC: deceleration capacity; HF: high frequency; LF: low frequency; ULF: ultra-low frequency; VLF: very low frequency; WPD: within-pair difference.

Table S.6.1 Comparison of characteristics of twins with and without follow-up assessment

Characteristics, mean (SD)	Total (N=450)		With Follow-up (N=308)		Without Follow-up (N=142)		P-value
Sociodemographic factors							
Age, years	56	(3)	56	(3)	55	(4)	0.146
White, No. (%)	436	(97)	300	(97)	136	(96)	0.355
Year of education	15	(2)	15	(2)	15	(2)	0.425
Employed, No. (%)	361	(80)	252	(82)	109	(77)	0.211
Health factors							
BMI	30	(5)	30	(4)	30	(6)	0.915
Ever smokers, No. (%)	289	(64)	185	(60)	104	(73)	0.007
Alcohol abuse, No. (%)	208	(46)	142	(46)	66	(46)	0.941
Baecke score for physical activity	7.3	(1.8)	7.4	(1.7)	7.0	(2.0)	0.042
Systolic blood pressure, mmHg	124	(10)	124	(10)	123	(11)	0.674
Diastolic blood pressure, mmHg	74	(9)	74	(9)	73	(9)	0.092
History of hypertension, No. (%)	168	(37)	120	(39)	48	(34)	0.317
History of diabetes, No. (%)	54	(12)	31	(10)	23	(16)	0.063
Prior history of CAD, No. (%)	47	(10)	28	(9)	19	(13)	0.167
Lifetime history of PTSD, No. (%)	68	(15)	44	(14)	24	(17)	0.472
Lifetime history of depression, No. (%)	126	(28)	85	(28)	41	(29)	0.780
Current PTSD, No. (%)	30	(7)	18	(6)	12	(8)	0.303
Current depression, No. (%)	16	(4)	10	(3)	6	(4)	0.602
BDI-II score	6.1	(7.9)	5.4	(7.5)	7.6	(8.5)	0.008
Medication use							
β-Blockers, No. (%)	36	(8)	23	(7)	13	(9)	0.540
Antidepressants, No. (%)	72	(16)	42	(14)	30	(21)	0.044
Statin, No. (%)	113	(25)	77	(25)	36	(25)	0.936
ACE inhibitor, No. (%)	72	(16)	45	(15)	27	(19)	0.236
Heart rate variability							
24-hour average							
ln ULF	6.7	(0.7)	6.7	(0.8)	6.6	(0.7)	0.080
ln VLF	7.7	(0.7)	7.7	(0.7)	7.6	(0.7)	0.041
ln LF	6.7	(0.8)	6.7	(0.7)	6.6	(0.8)	0.094
ln HF	5.5	(0.8)	5.5	(0.8)	5.5	(0.8)	0.739
ln DC	2.3	(0.4)	2.4	(0.3)	2.3	(0.4)	0.078
Daytime average							
ln ULF	6.8	(0.7)	6.8	(0.8)	6.7	(0.7)	0.110
ln VLF	7.7	(0.7)	7.7	(0.7)	7.6	(0.7)	0.051
ln LF	6.6	(0.8)	6.7	(0.7)	6.6	(0.8)	0.196
ln HF	5.4	(0.8)	5.4	(0.8)	5.4	(0.8)	0.717
ln DC	2.3	(0.4)	2.3	(0.4)	2.3	(0.4)	0.107
Nighttime average							
ln ULF	6.3	(1.1)	6.4	(1.2)	6.2	(1.0)	0.054
ln VLF	7.7	(1.0)	7.7	(1.0)	7.5	(0.9)	0.063
ln LF	6.7	(0.9)	6.7	(0.9)	6.6	(0.9)	0.138
ln HF	5.6	(0.9)	5.6	(0.9)	5.7	(0.9)	0.607
ln DC	2.4	(0.4)	2.5	(0.4)	2.4	(0.4)	0.145

Abbreviations: ACE: angiotensin-converting enzyme; BDI: Beck Depression Inventory; BMI: body mass index; CAD: coronary artery disease; DC: deceleration capacity; ETS: Emory Twin Study; HF: high frequency; HRV: heart rate variability; LF: low frequency; PTSD: posttraumatic stress disorder; SD: standard deviation; ULF: ultra-low frequency; VLF: very low frequency.

Table S.6.2 Within-pair analysis of the association between baseline HRV and depression and time to all-cause mortality during follow-up, stratified by zygosity

	Model 1[b]	Model 2[c]	Model 3[d]	Model 4[e]
Monozygotic twins (n=284)				
BDI	1.00 (0.93, 1.09)	0.99 (0.91, 1.07)	0.99 (0.91, 1.07)	0.99 (0.91, 1.08)
MDD	1.21 (0.43, 3.47)	0.93 (0.31, 2.81)	0.96 (0.32, 2.92)	0.93 (0.30, 2.84)
ln ULF	0.89 (0.69, 1.15)	0.89 (0.66, 1.20)	0.89 (0.66, 1.18)	0.89 (0.67, 1.19)
ln VLF	0.86 (0.68, 1.10)	0.85 (0.65, 1.13)	0.86 (0.66, 1.12)	0.86 (0.66, 1.13)
ln LF	0.81 (0.62, 1.05)	0.81 (0.61, 1.07)	0.81 (0.62, 1.06)	0.81 (0.62, 1.06)
ln HF	0.99 (0.68, 1.45)	1.01 (0.69, 1.48)	0.99 (0.68, 1.45)	1.00 (0.68, 1.46)
ln DC	0.74 (0.52, 1.06)	0.79 (0.55, 1.12)	0.77 (0.54, 1.10)	0.76 (0.54, 1.07)
Dizygotic twins (n=166)				
BDI	1.04 (0.96, 1.13)	1.02 (0.93, 1.12)	1.02 (0.92, 1.13)	1.00 (0.90, 1.11)
MDD	1.61 (0.42, 6.18)	1.30 (0.31, 5.42)	1.46 (0.32, 6.70)	1.46 (0.32, 6.72)
ln ULF	0.82 (0.47, 1.45)	0.82 (0.45, 1.49)	0.83 (0.45, 1.53)	1.03 (0.51, 2.08)
ln VLF	0.72 (0.43, 1.22)	0.69 (0.39, 1.21)	0.72 (0.40, 1.27)	0.87 (0.43, 1.78)
ln LF	0.70 (0.45, 1.11)	0.68 (0.42, 1.11)	0.66 (0.40, 1.09)	0.78 (0.38, 1.60)
ln HF	0.89 (0.57, 1.39)	0.78 (0.47, 1.28)	0.80 (0.46, 1.40)	0.82 (0.41, 1.62)
ln DC	0.71 (0.49, 1.03)	0.66 (0.43, 1.00)	0.63 (0.39, 1.01)	0.67 (0.36, 1.25)

Abbreviations: BDI: beck depression inventory; BMI: body mass index; DC: deceleration capacity; HF: high frequency; HRV: heart rate variability; LF: low frequency; MDD: major depressive disorder; SD: standard deviation; ULF: ultra-low frequency; VLF: very low frequency.

* Indicates significant association at $P < 0.05$.

[a] Results are shown as standardized hazard ratios in the multivariable Cox frailty models, per 1-SD within-pair difference in log-HRV.

[b] Base model was unadjusted.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, alcohol abuse, and physical activity.

[d] Model 3 = Model 2 + BMI, history of hypertension, history of coronary artery disease, and diabetes mellitus.

[e] Model 4 = Model 3 + beta-blockers and antidepressants.

Table S.6.3 Within-pair analysis of the association between baseline HRV and depression and time to cancer mortality during follow-up[a]

	Model 1[b]	Model 2[c]	Model 3[d]	Model 4[e]
BDI	1.11 (1.01, 1.22)*	1.10 (0.99, 1.22)	1.12 (1.00, 1.25)*	1.12 (1.01, 1.25)*
MDD	2.37 (0.65, 8.63)	2.23 (0.59, 8.43)	2.13 (0.56, 8.09)	2.17 (0.57, 8.25)
24-hour average HRV (n=450)				
ln ULF	0.82 (0.53, 1.28)	0.83 (0.52, 1.34)	0.82 (0.52, 1.31)	0.79 (0.48, 1.28)
ln VLF	0.74 (0.49, 1.11)	0.73 (0.47, 1.14)	0.73 (0.48, 1.13)	0.70 (0.45, 1.09)
ln LF	0.73 (0.50, 1.07)	0.75 (0.49, 1.13)	0.71 (0.48, 1.06)	0.69 (0.46, 1.05)
ln HF	0.71 (0.47, 1.07)	0.74 (0.48, 1.15)	0.72 (0.46, 1.13)	0.70 (0.44, 1.12)
ln DC	0.64 (0.43, 0.96)*	0.70 (0.47, 1.04)	0.64 (0.41, 0.99)*	0.62 (0.40, 0.96)*
Daytime average HRV (n=444)				
ln ULF	0.85 (0.57, 1.29)	0.86 (0.55, 1.35)	0.86 (0.56, 1.33)	0.83 (0.53, 1.31)
ln VLF	0.81 (0.55, 1.19)	0.80 (0.52, 1.21)	0.81 (0.54, 1.22)	0.77 (0.51, 1.19)
ln LF	0.79 (0.53, 1.17)	0.81 (0.54, 1.22)	0.78 (0.53, 1.16)	0.76 (0.51, 1.14)
ln HF	0.70 (0.45, 1.07)	0.73 (0.47, 1.14)	0.73 (0.47, 1.14)	0.71 (0.45, 1.13)
ln DC	0.71 (0.47, 1.07)	0.77 (0.52, 1.14)	0.72 (0.48, 1.09)	0.70 (0.46, 1.06)
Nighttime average HRV (n=418)				
ln ULF	0.87 (0.55, 1.37)	0.83 (0.49, 1.40)	0.87 (0.55, 1.36)	0.87 (0.54, 1.39)
ln VLF	0.69 (0.40, 1.18)	0.61 (0.34, 1.12)	0.64 (0.35, 1.16)	0.64 (0.35, 1.17)
ln LF	0.67 (0.42, 1.07)	0.64 (0.39, 1.07)	0.64 (0.39, 1.07)	0.65 (0.38, 1.09)
ln HF	0.79 (0.50, 1.22)	0.80 (0.49, 1.29)	0.79 (0.49, 1.28)	0.80 (0.49, 1.30)
ln DC	0.60 (0.40, 0.91)*	0.65 (0.43, 0.98)*	0.64 (0.42, 0.99)*	0.65 (0.42, 0.99)*

Abbreviations: BDI: beck depression inventory; BMI: body mass index; DC: deceleration capacity; HF: high frequency; HRV: heart rate variability; LF: low frequency; MDD: major depressive disorder; SD: standard deviation; ULF: ultra-low frequency; VLF: very low frequency.

[a] Results are shown as standardized hazard ratios in the multivariable Cox frailty models, per 1-SD within-pair difference in log-HRV.

[b] Base model was unadjusted.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, alcohol abuse, and physical activity.

[d] Model 3 = Model 2 + BMI, history of hypertension, history of coronary artery disease, and diabetes mellitus.

[e] Model 4 = Model 3 + beta-blockers and antidepressants.

Table S.6.4 Within-pair analysis of the association between baseline HRV and depression and time to cardiovascular mortality during follow-up[a]

	Model 1[b]	Model 2[c]	Model 3[d]	Model 4[e]
BDI	0.94 (0.84, 1.05)	0.94 (0.83, 1.05)	0.93 (0.83, 1.06)	0.93 (0.83, 1.06)
MDD	2.09 (0.40, 11.09)	2.08 (0.39, 11.19)	2.15 (0.39, 11.84)	2.16 (0.39, 11.92)
24-hour average HRV (n=450)				
ln ULF	1.06 (0.72, 1.56)	1.12 (0.71, 1.74)	1.10 (0.72, 1.69)	1.10 (0.72, 1.69)
ln VLF	1.01 (0.66, 1.55)	1.04 (0.66, 1.63)	1.03 (0.68, 1.58)	1.03 (0.67, 1.56)
ln LF	0.93 (0.58, 1.51)	0.94 (0.58, 1.53)	0.94 (0.59, 1.50)	0.93 (0.58, 1.48)
ln HF	1.29 (0.73, 2.29)	1.32 (0.72, 2.42)	1.33 (0.73, 2.45)	1.29 (0.70, 2.39)
ln DC	0.82 (0.48, 1.40)	0.79 (0.44, 1.41)	0.79 (0.45, 1.38)	0.75 (0.42, 1.34)
Daytime average HRV (n=444)				
ln ULF	1.05 (0.72, 1.52)	1.10 (0.71, 1.70)	1.09 (0.72, 1.64)	1.09 (0.71, 1.65)
ln VLF	0.98 (0.65, 1.48)	1.01 (0.65, 1.56)	1.00 (0.66, 1.52)	1.00 (0.66, 1.52)
ln LF	0.87 (0.53, 1.40)	0.88 (0.54, 1.43)	0.88 (0.55, 1.41)	0.87 (0.53, 1.40)
ln HF	1.18 (0.67, 2.08)	1.21 (0.66, 2.22)	1.21 (0.66, 2.23)	1.19 (0.64, 2.19)
ln DC	0.74 (0.43, 1.26)	0.72 (0.41, 1.28)	0.71 (0.41, 1.24)	0.68 (0.38, 1.21)
Nighttime average HRV (n=418)				
ln ULF	1.08 (0.73, 1.61)	1.11 (0.73, 1.69)	1.10 (0.72, 1.70)	1.08 (0.71, 1.66)
ln VLF	1.05 (0.69, 1.59)	1.07 (0.69, 1.65)	1.07 (0.68, 1.67)	1.04 (0.67, 1.62)
ln LF	1.02 (0.64, 1.64)	1.03 (0.64, 1.67)	1.03 (0.62, 1.69)	0.99 (0.60, 1.64)
ln HF	1.39 (0.77, 2.51)	1.41 (0.77, 2.59)	1.44 (0.78, 2.68)	1.43 (0.76, 2.70)
ln DC	0.88 (0.50, 1.52)	0.87 (0.49, 1.54)	0.86 (0.49, 1.51)	0.82 (0.45, 1.49)

Abbreviations: BDI: beck depression inventory; BMI: body mass index; DC: deceleration capacity; HF: high frequency; HRV: heart rate variability; LF: low frequency; MDD: major depressive disorder; SD: standard deviation; ULF: ultra-low frequency; VLF: very low frequency.

[a] Results are shown as standardized hazard ratios in the multivariable Cox frailty models, per 1-SD within-pair difference in log-HRV.

[b] Base model was unadjusted.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, alcohol abuse, and physical activity.

[d] Model 3 = Model 2 + BMI, history of hypertension, history of coronary artery disease, and diabetes mellitus.

[e] Model 4 = Model 3 + beta-blockers and antidepressants.

Table S.6.5 Analysis of the association between baseline HRV and depression and time to all-cause mortality during follow-up, twins as individuals

	Model 1[b]	Model 2[c]	Model 3[d]	Model 4[e]
BDI	1.02 (0.99, 1.06)	1.01 (0.97, 1.04)	1.01 (0.97, 1.04)	1.01 (0.97, 1.04)
MDD	1.38 (0.79, 2.43)	1.03 (0.56, 1.90)	1.03 (0.56, 1.91)	1.03 (0.56, 1.91)
ln ULF	0.88 (0.73, 1.04)	0.91 (0.74, 1.11)	0.91 (0.75, 1.10)	0.91 (0.75, 1.10)
ln VLF	0.82 (0.70, 0.97)*	0.86 (0.71, 1.03)	0.86 (0.72, 1.03)	0.86 (0.71, 1.03)
ln LF	0.77 (0.65, 0.92)*	0.81 (0.67, 0.98)*	0.79 (0.66, 0.96)*	0.79 (0.65, 0.95)*
ln HF	1.01 (0.77, 1.33)	1.09 (0.81, 1.46)	1.06 (0.80, 1.40)	1.06 (0.80, 1.40)
ln DC	0.69 (0.54, 0.88)*	0.76 (0.58, 0.99)*	0.70 (0.54, 0.92)*	0.70 (0.53, 0.92)*

Abbreviations: BDI: beck depression inventory; BMI: body mass index; DC: deceleration capacity; HF: high frequency; HRV: heart rate variability; LF: low frequency; MDD: major depressive disorder; SD: standard deviation; ULF: ultra-low frequency; VLF: very low frequency.

* Indicates significant association at P <0.05.

[a] Results are shown as standardized hazard ratios in the Cox proportional hazard models, per 1-SD difference in log-HRV.

[b] Base model was unadjusted.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, alcohol abuse, and physical activity.

[d] Model 3 = Model 2 + BMI, history of hypertension, history of coronary artery disease, and diabetes mellitus.

[e] Model 4 = Model 3 + beta-blockers and antidepressants.

Table S.6.6 Analysis of the association between baseline HRV and depression and time to major cardiovascular events during follow-up, twins as individuals

	Model 1[b]	Model 2[c]	Model 3[d]	Model 4[e]
BDI	0.89 (0.77, 1.03)	0.88 (0.75, 1.05)	0.88 (0.73, 1.05)	0.88 (0.73, 1.05)
MDD	0.87 (0.27, 2.75)	1.07 (0.32, 3.60)	1.12 (0.32, 3.88)	1.12 (0.32, 3.92)
ln ULF	0.86 (0.55, 1.35)	0.79 (0.52, 1.22)	0.80 (0.49, 1.28)	0.96 (0.78, 1.19)
ln VLF	0.87 (0.56, 1.34)	0.79 (0.52, 1.20)	0.78 (0.50, 1.22)	0.96 (0.78, 1.18)
ln LF	0.83 (0.56, 1.25)	0.72 (0.47, 1.09)	0.72 (0.46, 1.11)	0.93 (0.75, 1.16)
ln HF	0.95 (0.59, 1.53)	0.87 (0.53, 1.42)	0.90 (0.54, 1.50)	0.98 (0.78, 1.23)
ln DC	0.83 (0.54, 1.29)	0.70 (0.42, 1.15)	0.73 (0.43, 1.23)	1.02 (0.79, 1.32)

Abbreviations: BDI: beck depression inventory; BMI: body mass index; DC: deceleration capacity; HF: high frequency; HRV: heart rate variability; LF: low frequency; MDD: major depressive disorder; SD: standard deviation; ULF: ultra-low frequency; VLF: very low frequency.

* Indicates significant association at P <0.05.

[a] Results are shown as standardized hazard ratios in the Cox proportional hazards models, per 1-SD difference in log-HRV.

[b] Base model was unadjusted.

[c] Model 2 = Model 1 + sociodemographic and behavioral factors, including education, employment status, ever smoking status, alcohol abuse, and physical activity.

[d] Model 3 = Model 2 + BMI, history of hypertension, history of coronary artery disease, and diabetes mellitus.

[e] Model 4 = Model 3 + beta-blockers and antidepressants.

CHAPTER 7: SUMMARY AND FUTURE DIRECTIONS

7.1 Summary

Overall, this dissertation project evaluated the complex pathophysiology of depression, sleep disturbance, autonomic dysregulation, with the risk of adverse health outcomes, including mortality and CVD, using a co-twin control study design. We found that major depression and depressive symptoms are associated with distinct features of sleep disturbance such as REM disruption, lower sleep efficiency, more fragmentation, and higher sleep duration variability. We found that there is a significant bidirectional association between autonomic function and sleep measures, and their associations evaluated at home may extend beyond a 24-hour period. We also found that autonomic dysregulation indexed by lower HRV predicts increased risk of mortality and CVD, and depression is associated with more incident CVD events. In the context of a controlled twin design, we discovered that genetic factors may not play a major role in most of the associations among depression, sleep disturbance, autonomic dysregulation, and risk of mortality and CVD outcomes.

This dissertation project showed that depression, sleep disturbance, and autonomic dysregulation are all closely interrelated, and they together contribute to a higher risk of adverse health outcomes, such as mortality and CVD events. Sleep disturbance and autonomic dysregulation are among the potential pathophysiological pathways linking depression to adverse outcomes. Prevention and treatment strategies targeting sleep disturbance and autonomic dysregulation, such as behavioral interventions and medication use to improve sleep quality and autonomic function, may help aide in lowering the risk of mortality and cardiovascular events, especially among patients with depression.

Specific Aim #1

In **Aim 1** (Chapter 4; manuscript in preparation), in a co-twin control study, depressive symptoms and MDD were associated with a number of objectively measured indices of sleep disturbance. With PSG, twins with higher levels of depressive symptoms indexed by higher BDI score, or a history of MDD showed more REM sleep abnormalities than their brothers with fewer depressive symptoms or without MDD, including longer REM latency and lower percentage of TST in REM. Specifically, each 5-unit higher BDI, within-pair, was significantly associated with 19.7 minutes longer rapid eye movement (REM) sleep latency, and 1.1% shorter REM sleep after multivariable adjustment. Depression measures were also associated with more actigraphy-measured irregular and fragmented sleep, such as lower SE, more fragmentation and higher day-to-day variability of sleep duration. These associations persisted with adjustment for sociodemographic, behavioral and CVD risk factors, and were independent of presence of comorbid posttraumatic stress disorder and antidepressant use. In contrast, depression measures were not associated with several other sleep abnormalities, including TST, indices of SDB, PLMS, sleep architecture, and WASO. The results were similar in both MZ and DZ twins, and the interaction with zygosity was not significant, suggesting the absence of shared genetic and familial influence on the association of depression with sleep abnormalities.

We concluded that, using a comprehensive approach of objectively measuring multiple dimensions of sleep disturbance in the context of a controlled twin design, depression is associated with distinct features of altered sleep. While individuals with depression suffer from sleep disruption in the natural environment, such as lower sleep efficiency, more fragmentation, and higher sleep duration variability, their depressive symptoms showed minimal association with sleep dimensions measured in a controlled environment, such as SDB or PLMS. Individuals

with depression are also vulnerable towards disruption of REM sleep, including longer REM latency and lower percentage of TST in REM. Our twin study shows that genetic and familial factors may not explain these associations.

Specific Aim #2

In **Aim 2** (Chapter 5; manuscript in preparation), using a co-twin control study, we investigated the temporal relationships between autonomic dysregulation indexed by reduced HRV, and objectively measured sleep disturbance. We found that most of these relationships are bidirectional. Higher values in several daytime HRV domains, denoting better ANS function, were associated with a number of PSG-derived sleep measures, including N1, N2 and REM sleep, and lower hypoxic burden, after adjusting for relevant sociodemographic, behavioral and health-related factors. In turn, lower N1 and REM sleep, higher N2 sleep, and less severe oxygen desaturation were associated with higher HRV in the day following the laboratory PSG. During a week of monitoring in the natural (non-controlled) environment, a higher daytime HRV was bidirectionally associated with better sleep duration and continuity measured by actigraphy, as indicated by longer TST and lower WASO. We also found that the relationships between daytime HRV and sleep duration and continuity measures generally persisted to 48 hours, but no longer. In contrast to daytime HRV, nighttime HRV was not related to sleep duration or continuity longitudinally. Because our analysis examined differences within twin pairs, results are inherently independent of shared familial environment.

In the context of a controlled twin design, we concluded that there is a significant bidirectional association between autonomic function and sleep measures. In the home environment, the relationship between autonomic dysregulation during daytime and worse sleep

duration and continuity persists beyond a 24-hour period. Autonomic function and sleep are closely inter-related, and highlights the importance of a healthy autonomic function in the regulation of sleep and vice versa.

Specific Aim #3

In **Aim 3** (Chapter 7; manuscript in preparation), using a co-twin control study design, we found that higher values of all HRV frequency domains, denoting better autonomic function, were associated with 4%-27% decreased hazard for all-cause mortality and 29%-45% decreased hazard for major CVD events during an average of 12-year follow-up. A 1-unit increase in BDI was significantly associated with 12% increased hazard for cancer mortality, and MDD was associated with increased hazard for mortality and major CVD events. The associations remained robust after adjusting for relevant sociodemographic, behavioral and health-related factors, and medication use. Both LF and DC HRV domains showed the strongest and most consistent associations with all-cause mortality, while ULF HRV showed the strongest predictive values on major CVD events. Overall, daytime HRV metrics showed similar but slightly stronger associations with all-cause mortality and CVD events compared to nighttime HRV. Higher values of all HRV metrics were associated with decreased hazard for cancer-specific mortality, but were not associated with CVD mortality. The genetic factors may not play a major role in the associations of HRV with all-cause mortality, as the differences of the associations were not statistically significant between MZ and DZ twins.

We concluded that, in the context of a controlled twin design, high HRV metrics, denoting better autonomic function, have strong prognostic implications in predicting decreased risk for both mortality and cardiovascular events. Compared to nighttime HRV, daytime HRV

may have similar predictive values in all-cause mortality but slightly stronger protective effects on CVD events. More depressive symptoms indexed by higher BDI significantly predicted mortality due to cancer. These associations are not explained by sociodemographic, behavioral and health-related factors, and medication use, and shared genetic factors may not play a major role in these associations. Our study demonstrates that reduced HRV is an indicator of compromised health and can be used as independent predictors for adverse health outcomes, such as mortality and CVD. Major depression and depressive symptoms are linked with higher risk of cancer mortality and CVD events.

7.2 Strengths

We examined for the first time the pathophysiological pathways between depressive symptoms, sleep disturbances, autonomic dysregulation, and CVD outcomes, in a context of co-twin control study. This is the first twin study that evaluated the association of depression with sleep disturbances, using multiple objective sleep measures such as gold stand, in-lab PSG and at-home actigraphy. No previous study has evaluated the temporal dynamics and directionality of association between HRV metrics and objective sleep disturbance over successive 24-hour periods and modeled day-night associations in a time-lagged model, allowing inferences via Granger causality. Few previous studies have assessed the prognostic implications of a full spectrum of ECG-derived HRV frequency domains, separately in daytime and nighttime, in predicting adverse health outcomes including mortality and CVD events.

Our study is strengthened by a comprehensive measure of sleep, including both objective and subjective measures. Similarly, we assessed depression using self-rated questionnaire, as well as a clinical diagnosis which is the gold standard for measuring major depression. The

autonomic function indexed by HRV was also measured using validated tools, such as the gold standard, 24-hour Holtor recording, and ECG patch monitoring. These measures along with the standard measurement protocols should have minimized measurement error and improved the internal validity of the study. In addition, our study is further strengthened by a relatively long follow-up of 12 years, use of NDI to verify mortality which is the current gold standard in the US, and a thorough medical chart review process to adjudicate all cardiovascular events that minimized potential recall bias and measurement error.

Our matched twin study design enabled us to control for potential genetic and early familial confounding. The study of MZ and DZ twins provides information on common etiological pathways linking phenotypes of interest. The co-twin study design increased precision by providing an internal control (the unaffected twin), and intrinsically controlled for shared genetic and early familial factors. In addition, as twin pairs were assessed together (in lab) and in the same week (at home), confounding from environmental or seasonal/temporal influences on sleep measurements was minimized.

Throughout this dissertation project, we applied mixed-effect regression models with random effect, which allowed us to account for the heterogeneity of effect among different twin pairs. In all the Aims, the primary analyses were supported and extended by a series of additional sensitivity analysis, in order to confirm and improve the consistency and validity of results. In all the Aims, we constructed a series of models that progressively adjusted for variables that are potential confounders, in order to avoid model overfitting. In the Aim 2 analysis, the VAR models fitting bivariate time series and associated Granger causality tests provided an informative method to assess the temporal directionality of associations, and it allowed us to not

only evaluate temporal relationships between HRV and sleep, but also the length of time during which HRV exerted effects on sleep and vice versa.

7.3 Limitations

This dissertation project also has limitations. First, our sample included mostly white, middle-aged men, thus the generalizability to women and other racial/ethnic groups is limited. However, although a homogenous sample reduces generalizability, it should increase validity, which was a major goal of our study. Second, because of the cross-sectional design in Aim 1 analysis, we were unable to assess the directionality of the association between depression and sleep disturbance. For the HRV data processing in Aim 2 analysis, due to noise and nyquist frequency, the use of 5-minute windows to process HRV data may have not generated reliable estimates for lower frequency bands, such as ULF and VLF. For sleep data in Aim 1 and Aim 2 analyses, as participants traveled to Emory University from different locations in the US, different time zones may have influenced the sleep data collection in the lab, such as sleep duration and sleep latency. A further evaluation of the impact of different time zones on sleep disturbance data is needed in future studies. In Aim 3 analysis, another limitation of our study is that the National Death Index has limited refresh frequency (annual) and has a 2-year reporting delay, although it is the current US gold standard for mortality data. Thus, we were not able to verify mortality data after December 31st, 2017, which may slightly overestimate the overall survival in our sample and biased the associations of HRV and depression with mortality towards the null. In addition, for the twins who deceased during follow-up, they were not included in the ETSF thus their cardiovascular outcomes were not obtained and verified, which may lead to an underestimation of CVD events in our sample.

Overall, our study has relatively small sample size in all the Aims. Specifically, in Aim 1, the sample size for MDD-discordant twin pairs was relatively small, limiting our analysis of MDD. In Aim 2, due to the relatively small sample size for both Study I and Study II analyses, especially for the analysis using home monitoring data, our analysis may have been underpowered to detect significant bidirectional associations across some sleep dimensions. In Aim 2, the small sample size also does not allow a reliable evaluation of association separately in monozygotic and dizygotic twins in order to evaluate of role of genetic factors on the association. A reduction in sample size is inevitable in our design, given that within-pair analyses rely on complete pairs and consecutive data are necessary during home monitoring to properly calculate lagged values. However, our co-twin control study design should have improved internal validity and precision by intrinsically adjusting for unknown or unmeasured confounders. In Aim 3 analysis, due to the small number of participants with CVD events (n=18) that were included in the within-pair analysis (n=264), we may have had limited power to detect any statistically significant effects. This may explain why the effect sizes were large (i.e. HRs from 0.55 to 0.71 per 1-SD increment in log-HRV) but none of the associations was statistically significant. The small sample for both mortality and CVD outcomes in Aim 3 may cause the final models (i.e. fully adjusted models) to be overfitted, which may have potentially increased size of random error. Third, for the twins who deceased during follow-up, they were not included in the ETSF thus their cardiovascular outcomes were not obtained and verified, which may lead to an underestimation of CVD events in our sample. Future epidemiologic studies with larger sample size and more diverse study population, are needed to confirm the findings of this dissertation research.

7.4 Public Health Impact

This dissertation project evaluated the complex pathophysiology of depression, sleep disturbance, autonomic dysregulation, with the risk of adverse health outcomes, including mortality and CVD, using a co-twin control study design. This study pointed out that sleep disturbance and autonomic dysregulation are closely interrelated, and they consist of important pathways in which depression can lead to increased risk of mortality and CVD. Our findings contribute to a better understanding of the multifactorial mechanisms linking depression and its adverse health consequences, and shed light on potential prevention and treatment strategies of mortality and CVD events in patients with depression.

Specifically, this research project has provided a comprehensive evaluation on the association of depression with both objective and subjective sleep disturbance, in a full spectrum of sleep dimensions. It contributes to clarify the link between depression and sleep disturbance, and their roles in the pathophysiology of adverse health events. Our results help inform future research on prevention and treatment strategies to mitigate sleep disturbance, such as pharmacological treatment or behavioral interventions, among depressed individuals.

This study elucidates the temporal dynamics and directionality of association between sleep and HRV, and shows that autonomic function and sleep are closely inter-related. It highlights the importance of a healthy autonomic function in the regulation of sleep and vice versa. It also suggests that the changes in some sleep dimensions, potentially resulting from pharmacological treatments, may also have an impact on autonomic function; and pharmacological or non-pharmacological therapies to restore autonomic balance, such as exercise training, vagal nerve and carotid baroreceptor stimulation, may also have an effect on sleep disturbance.[265,266] Furthermore, our results suggest that both autonomic function and sleep

health should be targeted mutually in prevention strategies of chronic conditions linked to sleep disturbance, such as CVD.

In addition, this dissertation project sheds light on the prognostic implications of alternations in HRV and depressive symptoms in predicting mortality and CVD, which suggest the use of HRV monitoring in preventing and treating adverse health consequences, especially among individuals with depression. Our findings suggest that HRV evaluation can be incorporated into the monitoring of autonomic function as a prevention strategy of adverse health outcomes. Furthermore, capitalizing on the twin sample, this study helps evaluate the role of genetic predisposition in the underlying pathways from depression to mortality and CVD.

7.5 Future Directions

This dissertation project evaluated the complex pathophysiology of depression, sleep disturbance, autonomic dysregulation, with the risk of adverse health outcomes, but future research is needed further improve our study. First, because our study is limited by the small sample size and lack of generalizability due to mostly middle-aged white participants, future epidemiologic studies with more diverse study populations and a larger sample size are needed to confirm our findings. Second, because of the cross-sectional study design in Aim 1 analysis, we were unable to assess the directionality of association between depression and sleep disturbance. Future studies with a longitudinal design, such as a cross-lagged analysis, are necessary to evaluate the temporal relationships between depression and sleep. Third, as our sample size in MZ and DZ twins was too small in Aim 2 analysis to make a reliable comparison between the association by zygosity, more twin studies with larger sample size are needed to further evaluate the role of genetic factors on the temporal relationships between sleep disturbance and

autonomic dysregulation. Future research may evaluate a range of different cutpoints for depression to identify clinically useful cutpoints with implications for mortality and CVD risk. In addition, as the associations between depression, HRV and sleep are all likely to be bidirectional, future studies may be necessary to evaluate a hypothetical construct such as “biological depression”, encapsulating depressed mood, autonomic dysregulation and sleep disturbance as a single syndrome, and its association with adverse health outcomes. Last, larger longitudinal studies are needed to fully evaluate the prognostic values of depression and HRV in cause-specific mortality as well as different types of cardiovascular events, such as MI, CHF and stroke.

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