Stress Reactivity

Disturbances of the Neurocardiac Axis

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A thesis submitted for the degree of

Master of Science in Clinical Research

Laney Graduate School

Emory University

2021-03-17

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Acknowledgements

Foremost, I would like to thank my mentors and mentoring team. Dr. Shah has been a tremendous supporter of my academic growth, and has challenged me to continue to explore and learn, and create an area of academic excellence for myself. Dr. Alonso and Dr. Thames have all supported my efforts through direct mentorship and academic guidance. Dr. Quyyumi and Dr. Vaccarino have created a research environment that has laid the groundwork for these projects.

Second, I would like to thank the support staff that has allowed this research to unfold, from the EPICORE group with Nancy Murrah and Lucy Shallenberger, to the MSCR program with Cheryl Sroka and the outstanding MSCR instructors.

Finally, I would like to thank my family for their support, including Michelle for her patience, and of course Ripley and Harper for the always welcome distractions.

Abbreviations

There are several key abbreviations that will be used throughout. They have been outlined here for reference.

Term	Abbreviation
ANS	Autonomic Nervous System
Biobank	Emory Cardiovascular Biobank
CAD	Coronary Artery Disease
CFR	Coronary Flow Reserve
HRV	Heart Rate Variability
IHD	Ischemic Heart Disease
MACE	Major Adverse Cardiovascular Events
MI	Myocardial Infarction
MIMS	Myocardial Infarction and Mental Stress
MIPS	Mental Stress Ischemia Prognosis Study
MPI	Myocardial Perfusion Imaging
MSIMI	Mental Stress-Induced Myocardial Ischemia
PSIMI	Physical Stress-Induced Myocardial Ischemia
PTSD	Post-Traumatic Stress Disorder
Twins	Emory Twins Study

INTRODUCTION

1 The Problem

> "Why did he die on Tuesday and not on Monday?"
>
> — Douglas Zipes

The underlying premise in this question posed by Dr. Zipes is that the cardiac substrate can be *triggered* into an catastrophic maelstrom (1). That trigger is oftentimes psychological in nature, and in the right setting, such as a scarred myocardium, can lead to electrical instability and ventricular arrhythmias (2). The reaction of the body to such stresses is thus a critical step in the pathogenesis of major adverse cardiovascular events.

Patients with psychiatric disease have an increased of cardiovascular disease and overall mortality (3,4). Depression, for example, is the leading cause of disabilty in the world (5), and coronary artery disease (CAD) is the leading cause of death (6). The prevalence of depression is 20% in patients with CAD, and in those with comorbid depression and CAD, there is a 3-fold increase in cardiovascular mortality (7,8). Thus, understanding how the body reacts to stress can have implications in the the pathogenesis of the increased cardiovascular risk seen in psychological disease.

The purpose of this dissertation is to assess how physiological and psychological stress can be measured, and whether or not these measures of stress are clinically pertinent. Measuring stress and stress reactivity requires a method to quantify disturbances of the neurocardiac axis, and assessing changes from both a neuropsychological and cardiac perspective (9). Leveraging different types of stress along the neurocardiac axis is important, such as the effect of myocardial ischemia, acute mental stress, chronic psychological stress, and systemic diurnal changes.

2 The Approach

The suspected mechanism of behind stress reactivity is through dysfunction of the autonomic nervous system (ANS), particularly as there is an increased mortality with known ANS dysfunction (10,11). Both psychological stress and myocardial ischemia are known to have a relationship with ANS dysfunction (12), and the interaction between sympathetic and parasympathetic nervous activity is thus consequential.

To measure ANS dysfunction, a common surrogate is through electrocardiographic (ECG) changes based on sympathetic and vagal effects upon the sinoatrial node, or pacemaker, of the heart. The corresponding heart rate variability (HRV), and its mathematical derivatives, can thus inform us of the dynamic changes to autonomic tone (13). By measuring autonomic tone during different stress, we can quantify the stress reactivity of the phenomenon and model its impact on clinically relevant factors, such as depression, cardiovascular disease, and major adverse cardiovascular events.

BACKGROUND

3 Clinical Importance

Psychological stress is increasingly recognized as an important potentially modifiable risk factor for cardiovascular disease (14). Psychiatric disease leads to an increase cardiovascular disease and overall mortality. PTSD, for example, has twice the hazard ratio for not only progressive coronary artery disease, but also for overall mortality (4). Depression and loneliness have a distinct increase in all-cause mortality (15), and almost twice twice the risk of coronary artery disease (16). Specifically, depression is the leading cause of disability worldwide, estimated to be prevalent in over 300 million people, which accounts for roughly 4% of the global population (5). In the United States, the estimated economic burden of depression is $210 billion dollars as of 2010, doubling in cost from 2000 (17), suggesting a worsening, growing burden. CAD on the other hand remains the leading cause of death (6). This corresponds with approximately $204 billion dollars of estimated direct and indirect costs, with both continuing to climb (18).

In individuals with CAD, the prevalence of depression is estimated to be 20%, and in those with comorbid depression and CAD, there is over a 3-fold increase in cardiovascular mortality (7,8). This increased risk of mortality in CAD with depression has remained unchanged over the past 35 years (19), as only in recent years has depression been considered an additional prognostic marker of mortality in CAD (20).

Although interventions such as cognitive behavioral therapy and antidepressants are well-proven in reducing symptoms of psychological distress, their impacts are only modest in patients with CAD, and they do not impact event-free survival (21). Although the American College of Cardiology recommends depression should be routinely screened for in patients with cardiovascular disease, there is limited evidence that this leads to an improvement in overall mortality (22). More research

is needed to understand the therapeutic mechanism underlying psychological distress and CAD (19), as there are no effective therapies for these high-risk individuals in comorbid patients.

The causal mechanism is suspected to be through ANS dysfunction, as this has been found to occur in both diseases of the brain and heart (9). Not only are somatic symptoms of psychological stress predictive of cardiovascular mortality, but they may be explained by changes seen in HRV (23,24). Similarly, abnormalities in myocardial perfusion are related to and may be predated by abnormalities in HRV (26). Thus, investigating the role of autonomic dysfunction in neurocardiac disorders is both mechanistically plausible and may allow for the development of efficacious therapeutic interventions.

4 Relevant Literature

The heart itself harbors an intrinsic cardiac nervous system that responds to changes in autonomic tone, such as in myocardial ischemia and infarction, by changing chronotropic, inotropic, and dromotropic responses (27,28). Autonomic dysfunction occurs at multiple levels, from central neurological processes to cardiovascular reflexes (29). This includes vagal withdrawal seen in depression and heigtened sympathetic tone seen in PTSD or cardiovascular disease. Depression, for example, has been linked to dysregulation of the ANS through changes in levels of catecholamines (30), increased cardiovascular reactivity to stress (31), and decreased baroreflex sensitivity (32).

ANS-related mechanisms are important as they are related to both cardiac and neuropsychological disorders. The autonomic innervation of the heart has been show repeatedly to play a critical role in myocardial ischemia (33–35). Historically stellectomy was found to reduce angina pectoris and ventricular dysrhythmias (36). Modern interventions have shown to improve symptom burden not only in CAD, but also in depression (37). Vagal nerve stimulation has been shown to relieve cardiac arrhythmias, and has been effective against treatment-resistant depression (38). In PTSD, non-invasive vagal nerve stimulation has also been found to blunt the sympathetic response to stress (39). These relationships between chronic psychological stress, cardiac perfusion and arrhythmias, and the ANS help to establish the important influences on the neurocardiac axis.

HRV has been shown to be an effective ECG-based biomarker for ANS dysfunction. HRV is an accepted measure of autonomic activity, as it is an integration of sympathetic and parasympathetic efferent input at the level of the sinoatrial node (40). For example, the non-linear HRV marker of *Dyx* has been shown to predict ventricular dysrhythmia and cardiovascular mortality after myocardial infarction,

with a hazard ratio of 2.4 (95% CI 1.5 — 3.8) (41). In addition, individuals with chest pain and abnormal *Dyx* had an odds ratio of 8 (95% CI 3.1 — 23.99) for abnormal exercise stress test (42). Not only does HRV correlate well with cardiovascular disease, but is strongly correlated with depression (11). This relationship between psychological stress, cardiovascular disease, and the autonomic nervous system is thus an appropriate candidate for further investigation, particularly in justifying the clinical utility in measuing autonomic dysfunction (12). In addition to chronic mental stress, acute mental stress has become a more common way of assessing physiological reactivity. Mental stress has been shown to associate with chronic psychological stressors (43), and can also lead to the development of myocardial ischemia (44). This phenomenon, known as mental stress-induced myocardial ischemia (MSIMI), is an important reaction to stress that has been shown to predict long-term clinical outcomes (45). HRV has been shown that to associate with acute mental stress (46), but the relationship between MSIMI has not yet been determined.

The circadian rhythm in autonomic dysfunction is also relevant. The response to the stress of changing from sleep to wake has been seen to associate with increased cardiovascular mortality. There is an increased frequency of sudden cardiac death based on time of day, usually peaking between 6 AM and 10 AM (47,48), with a secondary peak between 6 PM and 8 PM (49). There is a circadian pattern to autonomic outflow, melatonin, cortisol, and circulating catecholamines which could increase vulnerability to ischemia and cardiac death during morning hours [(48); Scheer2010]. The relationship of circadian changes in HRV and myocardial perfusion has been shown to be related (26), but how circadian changes may be important in psychological stress and overall mortality is not yet known. Thus, studying circadian autonomic variability may reveal insights into the diurnal pattern of clinical disease.

METHODS

5 Specific Aims

The response to both physiological and psychological stress can be markers of overall cardiovascular adaptability. The following aims help to assess the clinical importance of stress reactivity as measured by disturbances to the neurocardiac axis.

AIM I — Autonomic mechanisms underlying psychological stress.

1. Explore the effect of acute mental stress on autonomic function. *We hypothesize that short-term autonomic metrics will be abnormal during both stress and recovery phases of acute mental stress challenge as compared to the rest phase.*
2. Explore the relationship of autonomic dysfunction at time of acute mental stress with chronic mental stress. *We hypothesize that abnormalities in short-term autonomic metrics will have an increased risk for depression and PTSD.*
3. Determine the relationship between autonomic dysfunction and chronic mental stress. *We hypothesize that abnormalities in morning autonomic metrics will have an increased risk for depression and PTSD. We hypothesize that circadian autonomic variability will have an increased risk for depression and PTSD.*

AIM II — Autonomic mechanisms underlying ischemic heart disease.

1. Determine the relationship of autonomic dysfunction with obstructive coronary artery disease (>70% stenosis) as measured by coronary angiography. *We hypothesize that autonomic metrics will be in abnormal in subjects with obstructive, epicardial disease.*
2. Explore the effect of revacularization of obstructive coronary artery disease with changes in autonomic function. *We hypothesize that in subjects undergoing revascularization of the coronary arteries, autonomic metrics will be different after revascularization as compared to before revascularization.*
3. Determine the relationship between autonomic dysfunction and perfusion

abnormalities in response to physical stress. *We hypothesize that abnormalities in autonomic metrics will associate with abnormal myocardial perfusion from either exercise or pharmacological stress. We hypothesize that circadian autonomic variability will be associated with abnormal myocardial perfusion.*

4. Determine the relationship between autonomic dysfunction and myocardial blood flow in response to physical stress. *We hypothesize that abnormalities in autonomic dysfunction will be associated with abnormalities in myocardial blood flow, as measured by coronary flow reserve, after pharmacological stress. We hypothesize that circadian autonomic variability will be associated with abnormalities in coronary flow reserve.*
5. Determine the relationship between autonomic dysfunction and perfusion abnormalities in response to acute and chronic mental stress. *We hypothesize that abnormalities in autonomic metrics will be associated with abnormalities in myocardial perfusion in response to acute mental stress.*

   We hypothesize that this relationship will be unaffected by the chronic mental stress of depression and PTSD.

AIM III — Autonomic mechanisms underlying the risk of major adverse cardiovascular events.

1. Determine the effect of autonomic function on the risk of major adverse cardiovascular events. *We hypothesize that abnormalities in autonomic metrics will be associated with increased overall and cardiovascular mortality. We hypothesize that circadian autonomic variability will be an associated with increased overall and cardiovascular mortality.*
2. Explore the relationship of stress-induced autonomic dysfunction on the risk of major adverse cardiovascular events. *We hypothesize that abnormal autonomic metrics in response to acute mental stress will be associated with an increased risk of cardiovascular mortality and recurrent cardiovascular events. We hypothesize that this risk will not be explained by mental stress-induced myocardial ischemia.*

To achieve these aims, we will leverage the several data sets, including the Emory Cardiovascular Biobank (*Biobank*), the Myocardial Infarction and Mental Stress (*MIMS*) and Mental Stress Ischemia Prognosis Study (*MIPS*), and the Emory Twins Study (*Twins*). Each of these data sets contribute variations of coronary artery physiology, acute and chronic mental stressors, and electrocardiographic data of varying recording lengths.

6 Study Design

6.1 Population Characteristics and Study Overview

6.1.1 Emory Cardiovascular Biobank

The *Biobank* studies major cardiovascular events, and also evaluates additional biomarkers for inflammation, cardiac injury, and genetics, with the goal of predicting CVD outcomes (50). All patients aged 18 years and older undergoing cardiac catherization were included. During the index cardiac catheterization, additional measures including lifestyle factors, psychological status, medical comorbidities, revascularization and previous procedures were ascertained via patient interview and chart review. Additionally, ambulatory ECG was collected with the VivaLNK patch, which was placed on the morning of cardiac catheterization and removed after catheterization for up to 24 hour of data recording. Patients were excluded if they have congenital heart disease, severe valvular heart disease, severe anemia, a recent blood transfusion, myocarditis, history of active inflammatory disease, cancer or are unable or not willing to provide consent (approximately 5%). Those that are found to have atrial fibrillation or have >20% ectopic beat burden or noise, as well as those that are pacer dependent were excluded. Those with known CAD were also excluded.

6.1.2 Emory Twins Study

The *Twins* is a cross-sectional study was designed to evaluate the relationship of abnormal stress myocardial perfusion with autonomic function, measured hourly over the course of 24 h, in individuals without known ischemic heart disease. Subjects were drawn from the Emory Twin Study, which recruited middle-aged male twin pairs from the Vietnam Era Twin Registry (51–53). Pairs of twins were examined

at the Emory University General Clinical Research Center, and all data collection occurred during a 24-hour admission under controlled conditions. The twins in each pair maintained a nearly identical schedule, with all data collection beginning and ending at the same time. The twins arrived at 11 AM, with ECG recording started at approximately 1 PM, questionnaires and exam performed between 2 and 4 PM, dinner at 5 PM, bedtime at 10 PM, wake-up time at 6:30 AM, and PET scans performed between 8 and 10 AM the following morning. The twins were followed longitudinally for follow-up events, including review of national registries, which were adjudicated. Subjects were excluded from analysis if they were unable to complete pharmacological stress testing.

6.1.3 Mental Stress Ischemia Mechanisms and Prognosis, Myocardial Infarction and Mental Stress

The study design has been described prior, and is the same between the two cohorts (54). The *MIMS* cohorts had recent myocardial ischemia within the 8 months prior to enrollment and were younger than 61 years of age at time of screening. The *MIPS* cohort included patients with stable CAD diagnosed via coronary angiogram, documented MI, or positive nuclear stress test. All patients underwent mental stress test and physical stress test using either treadmill or regadenosine, and were randomly assigned to complete one and then the other in two separate visits within a week. During the initial visit, medical history and psychological assessments were performed as well. During the mental stress testing, all patients had ECG recordings made of variable duration. Patients were followed longitudinally for 3-5 years for follow-up events, which were adjudicated. Patients were excluded for having acute coronary syndrome or decompensated heart failure, severe psychiatric conditions other than depression, pregnancy, uncontrolled high blood pressure, or contraindications to pharmacological stress testing.

6.2 Measurements

6.2.1 Electrocardiography Measures

In all three cohorts, ECG data was collected and analyzed using similar techniques. As described, in the *Biobank*, ECG was collected through a single, bipolar lead using the VivaLNK patch, with data being recorded for up to 24 h. In the *Twins*, ambulatory ECG was collected through Holter monitor (GE Marquette SEER digital system; GE Medical Systems, Waukesha, Wisconsin) for 24 h. Holter monitor was also used in the *MIMS/MIPS*, however the recording time was only for several hours.

Variations in heart rate can be assessed by a number of mathematical measures, usually divided into the time and frequency domains (55). HRV was calculated through the Physionet Cardiovascular Signal Toolbox (56), which is an open-source MATLAB software for analyzing ECG signal. Time domain measures we used include the RR interval duration (converted to heart rate in beats-per-minute), the standard deviation of normally conducted RR intervals (SDNN), the root mean square of successive differences in normally conducted RR intervals (RMSSD), and the proportion of normally conducted RR intervals that differ by more than 50 ms divided by the total number of normally conducted RR intervals (PNN50). Frequency-domain measures computed through power spectral analysis categorize variability as very low frequency (VLF, 0.0033 to <0.04 Hz), low frequency (LF, 0.04 to <0.15 Hz) or high frequency (HF, 0.15 to 0.40 Hz) (57). These frequency categories reflect autonomically mediated heart rate responses to physiologic stimuli, including influences of the renin-angiotensin-aldosterone system, baroreceptor activity, and respiration (57). The sympathetic and parasympathetic nervous systems influence them to different degrees. HF reflects primarily parasympathetic nervous system activity, while LF reflects both sympathetic and parasympathetic activity (58). Total power HRV is a nonspecific global measure. RMSSD is an approximate correlate of HF, and SDNN is an approximate correlate of TP, supporting the physiological basis of these markers.[Electrophysiology1996b] Acceleration capacity and deceleration capacity were also included where available, based on signal quality and recording length, as they also reflect clinically relevant sympathetic and vagal activity (59).

These metrics are well-known as physiologic markers of acute and chronic stress, and measure slightly different aspects of autonomic nervous system function. HRV was also analyzed hourly through the commercial HeartTrends algorithm (Lev-El Diagnostics Ltd, Israel), which generated the *Dyx* measure. *Dyx* is derived from heart rate time series analysis and measures the variability and randomness of the heart rhythm. *Dyx* is generated through the multipole method analysis of Poincaré plot, in which beat-to-beat (RR) interval lengths are plotted as a function of prior RR intervals to form an ellipse, as seen in Figure A.1.2. *Dyx* is calculated as the ratio of the kurtosis along the y-axis (long-term variability) and the x-axis (beat-to-beat random variation) of the ellipse, and higher values indicate more beat-to-beat randomness and/or decreased variability (60,61).

In addition to summary and hourly assessments of HRV, diurnal rhythms were examined using cosinor metrics within the *Twins* (62). Morphological assessment of ECG changes were also performed in the *MIMS/MIPS* with T-wave area.

6.2.2 Psychological Measures

In all cohorts, chronic psychological variables were measured through patient interviews. In the *Biobank*, depressive symptoms were assessed via the 9-question Primary Care Evaluation of Mental Disorders Brief Patient Health Questionnaire (PHQ-9) (63). Moderate-severe depression is considered when the PHQ-9 score is 10 points or higher (out of 27), with this cutpoint having a sensitivity and specificity of 88% for major depression. Within the *Twins* and *MIMS/MIPS* cohorts, depressive symptomers were assessed with Beck's Depression Inventory, which includes 21 items with 4 statements scored 0-3, with higher scores indicating higher severity of depression (64). A cut-off of $\geq$ 14 points was used to identify patients with moderate-to-severe depressive symptom burden. The diagnosis of post-traumatic stress disorder (PTSD) was defined using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) (65).

In the *MIMS/MIPS* cohort, there was a separate protocol for acute mental stress

challenge. Patients were initially allowed to rest for 30 minutes in a calm, quiet, dimly lit, and temperature-regulated room. After the resting period, mental stress was induced by a standardized public speaking task, as previously described (66). The patients were asked to imagine a real-life stressful situation, such as a close relative having been mistreated in a nursing home, and then asked to make up a realistic story around this scenario. They were given two minutes to prepare the statement, and three minutes to present it in front of a video camera and an audience. The patients were told that the speech would be evaluated for content, quality, and duration.

6.2.3 Cardiac Measures

All cohorts underwent imaging of the heart through different modalities, which all assess complementary aspects of myocardial blood flow.

The *Biobank* used direct coronary angiography through cardiac catheterization. Obstructive CAD was defined as $\geq 70\%$ stenosis or hemodynamic significance by fractional flow reserve. Coronary angiography was used to determine the Gensini score, which is a visual estimation of luminal narrowing in multiple segments based on a modified form of the American Heart Association classification of the coronary tree by trained cardiologists (67). Coronary angiography was also evaluated using the Coronary Artery Surgery Study (CASS), which evaluates the number of major epicardial vessels that have a certain percent stenosis, e.g. the CASS-50 score determines the number of vessels with $\geq 50\%$ stenosis (68). Importantly, direct coronary angiography is limited to visualization of the large, epicardial conduit vessels.

The *Twins* study used MPI using underwent MPI using nitrogen-13-ammonia positron emission tomography (PET) with adenosine as the pharmacologic stressor. Adenosine doses were calculated to induce maximal coronary vasodilation (69). Areas of diminished uptake indicate reduced capacity to maximally vasodilate, thereby causing relative coronary hypoperfusion. Images were visually interpreted by experienced

cardiologists and radiologists with training in nuclear cardiology. Quantitative analysis was performed with the Emory Cardiac Toolbox to generate: a) coronary flow reserve (CFR) for absolute myocardial blood flow during stress and rest, and b) the stress total severity score (STSS), which measures the sum of the number of standard deviations below the expected value for each pixel compared to a database of normal controls (70). CFR was defined as the ratio of mean stress to rest myocardial blood flow (mL/min/g), and low CFR was defined as a ratio < 1.5 (71). Abnormal MPI findings were defined as $> 5\%$ MPI deficit. For generalizability, semi-quantitative assessments were used.

In the *MIMS/MIPS*, MPI was also used but with single-photon emission computered tomography (SPECT), using sestamibi radio-labelled with Technetium-99. SPECT was performed at rest, after mental stress, and after physical stress (either exercise or pharmacological). Myocardial perfusion abnormalities were quantified using the Emory Cardiac Toolbox software, similar to the *Twins*, and semi-quantitative assessments were determined.

6.3 Sample Size and Power Considerations

The subjects for the *Biobank* were enrolled from September 2019 to February 2020, however further enrollment was limited due to the COVID-19 pandemic. It was expected that approximately 200 patients would be needed for adequate power, but due to limitations, the analyses were conducted on available data. The enrollment for *MIMS/MIPS* and *Twins* was completed prior to the initiation of this analysis, and provided a robust population to expand upon and answer the specific aims.

7 Analysis

Statistical analysis was performed using R 4.0.0 (R Core Team 2020, Vienna, Austria). The analytical approach was guided by the specific aims (Section 5).

7.1 Clinical Characteristics

In the *Biobank*, HRV was summarized over the recording length and at 15-30 minute intervals at the time of coronary angiography. In the *Twins*, HRV was computed hourly, including *Dyx*. The 24-hour data was used to generate cosinor statistics as well using published software (72). In the *MIMS/MIPS*, HRV was computed for each phase of the mental stress challenge. Power spectral density measures of HF, LF, and VLF HRV were log-transformed for normal distribution. Each of the clinical cohorts was described by clinical covariates, including frequency of comorbid diseases and summary statistics of continuous measures.

7.2 Myocardial Ischemia

The purpose of **Aim I** was to identify the relationship of autonomic dysfunction with CAD, as measured by both coronary angiography and MPI. Within the *Biobank*, summary HRV metrics were compared between those with obstructive CAD versus nonobstructive CAD using the Wilcoxon rank sum test. HRV was then assess in a similar manner between those receiving revascularization and those that did not. HRV was segmented by timing of coronary angiography. HRV was compared at the start of catheterization (or initial intervention in those that were revascularized) to the 30 minutes before the procedure, by intervention status.

To evaluate the relationship with myocardial perfusion, HRV was also assessed within the *MIMS/MIPS* cohort, which had both mental and physical stress MPI as

outcomes. Logistic regressions were used with HRV during stress and rest as the exposures, not adjusted for clinical covariates. The area under the receiver-operator characteristic curves were generated.

$$
\begin{aligned}
\text{global\_cfr}_i &\sim N\left(\mu, \sigma^2\right) \\
\mu &= \alpha_{j[i],k[i]} + \beta_1(\text{lf}) + \beta_2(\text{age}) + \beta_3(\text{bmi}) + \\
&\quad \beta_4(\text{race}_{\text{African American}}) + \beta_5(\text{race}_{\text{Asian}}) + \beta_6(\text{smoking}_1) + \beta_7(\text{prevchd}_1) + \\
&\quad \beta_9(\text{hptn}_1) + \beta_0(\text{dm}_1) \\
\alpha_j &\sim N\left(\mu_{\alpha_j}, \sigma^2_{\alpha_j}\right), \text{ for vetrid j} = 1, \ldots, \text{J} \\
\alpha_k &\sim N\left(\gamma^\alpha_0 + \gamma^\alpha_1(\text{chf}_1), \sigma^2_{\alpha_k}\right), \text{ for pair k} = 1, \ldots, \text{K}
\end{aligned}
$$

Within the *Twins*, similar analyses were performed with MPI and hourly HRV. Early morning, approximately 7 AM, HRV was used based on previous work suggesting the strong temporal relationship of HRV with clinical outcomes (26). Generalized linear mixed-effects models with Laplace approximations were used to account for clustering within twin pairs and within repeat study visits (73). Coronary flow reserve was treated as the outcome variable in linear models, and abnormal MPI was treated as the outcome variable in logistic models. With the mixed-effects model, with random effects or conditioning for both twin pair status and repeat study visits. The models were sequentially adjusted for demographic variables (age, BMI, and race), and then for cardiovascular risk factors (smoking, hypertension, cardiovascular disease). HRV was summarized as cosinor metrics of MESOR (midline estimating statistic of rhythm), amplitude, and acrophase. These metrics were treated as exposures, with CFR and MPI as outcomes, in mixed effects models as above.

7.3 Psychological Stress

The purpose of **Aim II** was to establish the relationship between psychological stress and autonomic dysfunction. Acute psychological stress was defined as the

mental stress challenge in the *MIMS/MIPS* cohorts. Chronic psychological stress was defined as diagnoses of depression and PTSD, and/or symptom burden.

In the *Biobank*, the distribution of HRV was compared between those with depression, as measured by PHQ-9, using Wilcoxon rank sum test. In the *Twins*, using the above described generalized linear mixed-effects models, regression analysis was performed with early morning HRV as the exposure and either PTSD or depression as the outcome. Sequential adjustment was performed for demographic factors and cardiovascular risk factors. Cosinor metrics were also used as additional exposure variables in additional mixed-models.

The *MIMS/MIPS* cohort offered the opportunity to assess both acute and chronic psychological stress. The distribution of HRV was visually compared between phases of the mental stress challenge for all subjects. HRV was then analyzed within subjects by comparing the rest phase to the both the stress and recovery phase using paired T-test. The distribution of HRV was then compared by phase in those with MSIMI and those without MSIMI using the Wilcoxon rank sum test. Logistic regression models were used with PTSD and depression as outcomes and HRV by stress phase as exposures, without adjustment, and concordance statistics were generated. Logistic regression models were then built with MSIMI as the outcome and stress HRV as the exposure, along with concordance statistics. These models were sequentially adjusted for demographic factors (age, BMI, sex, race), cardiovascular risk factors (smoking, hypertension, diabetes, hyperlipidemia), known cardiovascular disease (coronary/peripheral artery disease), and then with chronic psychological stress (depression, PTSD).

7.4 Clinical Outcomes

The purpose of **Aim III** was to evaluate the role of autonomic dysfunction in clinical outcomes, and understand the effect of autonomic dysfunction on the relationship between outcomes and both psychological stress and myocardial ischemia. Clinical

outcomes were available in the *Twins* and *MIMS/MIPS* cohorts. Proportional hazard assumptions were assessed visually in both cohorts.

The clinical outcomes of interest were overall mortality and death by cardiovascular disease in the *Twins*. Early morning HRV was treated as the exposure, with the survival time before censoring event as the outcome in Cox proportional hazard models. The models were sequentially adjusted for myocardial ischemia, demographic factors (age, BMI, race), cardiovascular factors (known cardiovascular disease, hypertension, diabetes, smoking), and psychological factors (depression, PTSD). In a similar fashion, cosinor metrics for HRV were used as exposure variables in models that were sequentially adjusted as above.

In the *MIMS/MIPS*, the clinical outcomes of interest were overall mortality, cardiovascular mortality, and recurrent cardiovascular events including myocardial infarction or revascularization. Cox proportional hazard models were used for both overall and cardiovascular mortality. Recurrent event analysis used proportional hazard models with strata for events and individuals using marginal models, Prentice-Williams-Peterson models (both total and gap time), and Anderson-Gill models (74). These models were sequentially adjusted for demographic factors (age, BMI, sex, race), cardiovascular risk factors (smoking, hypertension, diabetes, hyperlipidemia), known cardiovascular disease (coronary/peripheral artery disease), and then with chronic psychological stress (depression, PTSD).

RESULTS

8 Study Overview

The study populations in the three cohorts are uniquely suited for these analyses. They are complementary in their description of cardiovascular disease, autonomic function, and psychological factors, and are described here.

The *Biobank* cohort, as described in Table A.1.3, had 56 participants, with a mean (95% CI) age in years of 62 (52, 70). 9 (17%) were female, and 14 (26%) were Black. There were 34 (71%) that had obstructive CAD on coronary angiography, and 10 (21%) had depression.

The *MIMS/MIPS* cohort had 958 participants. The mean age was 59 (52, 68), 323 (34%) were female, and 385 (41%) were Black. 700 (84%) had obstructive CAD. 273 (30%) had a diagnosis of depression, and 87 (9.5%) had a diagnosis of PTSD. In this population, 238 (25%) had MSIMI. Additional breakdown by study group is described in **??**.

The *Twins* cohort, as described in both Table A.1.4 and A.1.6, had 1012 participants over 4 follow-up visits, with 610 unique participants. The mean age was 55.0 (52.0, 57.0) during the initial enrollment period, and was 68.4 (66.8, 69.5) during the final enrollment period. All participants were male, and 95.75% were White. The average rate over the enrollment periods of abnormal MPI was 12.13%. The average rate of PTSD was 16.52% and the average rate of depression was 13.38%.

9 Psychological Stress

Acute mental stress was assessed primarily using the *MIMS/MIPS* cohorts. The distribution of HRV metrics based on the phase of acute mental stress challenge was evaluated, as seen in Figure A.2.1. There were small differences between stress and rest HRV, as seen in Table A.2.2. The difference in distribution of HRV was compared between those that had MSIMI and those that did not, as described in A.3.7. There was a decrease in HRV in those with MSIMI compared to those without, except with heart rate.

The association between HRV during acute mental stress and chronic mental stress was also assessed (Table A.2.3). Every 10 beat/minute increase in resting heart rate had an OR = 1.33 (95% CI 1.11, 1.58) for PTSD and an OR = 1.15 (95% CI 1.01, 1.3) for depression. Every 1 unit increase in LF HRV during recovery had an OR = 0.51 (95% CI 0.26, 1.07) for depression. No other HRV metrics were strongly associated.

Chronic psychological stressors were analyzed using all three cohorts. In the *Biobank* cohort, there were no significant differences seen in HRV by depressive symptoms as measured on the PHQ-9.

In the *Twins*, early morning HRV was measured against both PTSD and depression. There was a significant relationship between HRV and both depression and PTSD as seen in Table A.2.5. In adjusted logistic models for PTSD, every 1 unit increase in HF HRV had an OR = 0.57 (95% CI 0.4, 0.82), and LF HRV had an OR = 0.65 (95% CI 0.45, 0.94). In adjusted models logistic models for depression, every 1 unit of increase in VLF HRV had an OR = 0.04 (95% CI 0, 0.92). *Dyx* and VLF HRV were not strongly associated with PTSD.

When assessing circadian autonomic variability, measured by cosinor analysis,

significant relationships were seen with both depression and PTSD in the MESOR and amplitude (Table A.2.6). For example, every 1 unit increase in the MESOR of LF HRV had an OR = 0.46 (95% 0.31, 0.69) and every 1 unit increase in the amplitude had an OR = 0.31 (95% 0.13, 0.72) for PTSD. Every 1 unit increase in the MESOR of LF HRV had an OR = 0.26 (95% 0.15, 0.45) and every 1 unit increase in the amplitude had an OR = 0.31 (95% 0.14, 0.68) for depression.

10 Myocardial Ischemia

The relationship of autonomic dysfunction to CAD as measured by coronary angiography was assessed in the *Biobank* cohort. When comparing summary HRV metrics between those with obstructive CAD versus nonobstructive CAD, there were no significant differences between HRV distributions (A.3.1). When comparing those that had revascularization of the CAD and those that did not (A.3.2), there was a difference seen in RR interval. Those that underwent revascularization had a mean (95% CI) RR interval of 868 (775, 932), while those that did not had a mean RR interval of 648 (608, 872). There was a trend towards an increased *Dyx* in those that underwent revascularization (2.03 (1.52, 2.71)) than those that did not (1.36 (1.17, 1.78)). No other HRV metrics were associated with revascularization. To effect of the timing of revascularization on the subsequent changes in HRV acutely were assessed, as described in Table A.3.3. No differences were seen between HRV before or after cardiac catheterization.

The relationship of autonomic dysfunction to myocardial perfusion was assessed using both mental stress and physical stress in the *MIMS/MIPS* cohorts. ECG and HRV metrics did not have an association with abnormal MPI with combined mental and physical stress nor with physical stress. Both HF HRV and LF HRV most prominently had an association with MSIMI, with stress HRV HRV having an odds ratio (OR) = 0.48 (95% CI 0.31, 0.76) and LF HRV having an OR = 0.45 (95% CI 0.27, 0.74). The other associations are described in Table A.3.4.

This relationship between myocardial perfusion and autonomic dysfunction was further explored using quantitative MPI in the *Twins* cohort. Morning HRV at approximately 7 AM was predominately associated with coronary flow reserve, as described in Table A.3.5. A change in 1 unit of LF HRV was associated with an 1.16 (95% CI 1.04, 1.28) in adjusted models. *Dyx* had an OR = 0.7 (95% CI 0.51, 0.98)

for abnormal MPI.

Within the *Twins*, circadian autonomic variability was measured using cosinor analysis. The relationship of the MESOR, amplitude, and acrophase with abnormal MPI and coronary flow reserve were evaluated (Table A.3.6). The MESOR in particular showed a consistent relationship with coronary flow reserve, with a 0.88 (0.58, 1.32) increase in every 1 unit increase in LF HRV, and a 0.89 (0.61, 1.31) increase for every 1 unit increase in *Dyx*.

To assess the relationship of acute mental stress with myocardial perfusion abnormalities, the relationship between HRV and MSIMI was assessed. As seen in Table A.3.8, there was a robust association between LF and HF HRV during rest and stress with MSIMI. In fully adjusted models, including adjustment for both cardiovascular and psychological risk factors, every 1 unit increase in stress HF HRV had an OR = 0.47 (95% CI 0.29, 0.77) and stress LF HRV had an OR = 0.47 (95% CI 0.3, 0.91) for MSIMI.

11 Clinical Outcomes

Clinical outcome data was available in both the *Twins* and the *MIMS/MIPS* cohorts. With the *Twins*, early morning HRV showed a robust association with overall mortality and with cardiovascular disease, as seen in Table A.4.1. In fully adjusted models for overall mortality, *Dyx* and VLF HRV had the strongest association. With every 1 unit of increase in *Dyx*, there was a hazard ratio (HR) = 0.41 (95% CI 0.27, 0.64), and with every 1 unit increase in VLF HRV, there was a HR = 0.49 (95% CI 0.27, 0.88). When evaluating the relationship of circadian autonomic variability and clinical outcomes, *Dyx* was a significant predictor of both overall and cardiovascular mortality. The MESOR of *Dyx* had a HR = 0.34 (95% CI 0.21, 0.56) and the amplitude of *Dyx* had a HR = 0.42 (95% CI 0.22, 0.79). Further relationships are outlined in A.4.2.

Using the *MIMS/MIPS* cohorts, stress HRV was compared with clinical outcomes. There was a robust relationship between stress HRV and overall mortality, cardiovascular mortality, and recurrent cardiovascular events as described in Table A.4.3. In fully adjusted models for cardiovascular mortality, including primary adjustment for MSIMI, 1 unit increase in stress LF HRV had a HR = 0.25 (95% CI 0.11, 0.56) and HF HRV had a HR = 0.25 (95% CI 0.11, 0.56).

DISCUSSION

12 Principal Findings

This was a multi-cohort study evaluating the relationship between physiological and psychological stress with autonomic dysfunction, enriched for subjects both with cardiovascular and psychiatric illness. The use of three varied cohorts allowed for specific testing of the hypothetical pathways between stress and cardiovascular disease, and leveraged the importance of stress reactivity with clinical outcomes, as described in Section 5. We found a strong relationship between autonomic dysfunction and both acute and chronic mental stress and with myocardial ischemia. We found that autonomic dysfunction not only predicted clinical outcomes, but also mediated the increased mortality in those with mental stress. We summarize the major findings below.

12.1 Psychological Stress

In **Aim I**, we studied the potential autonomic mechanisms that may underlie both acute and chronic mental stress. Psychological stress has an increased risk of cardiovascular mortality beyond that of maladaptive health behaviors (14,15). Both depression and PTSD show an excess risk of cardiovascular disease that cannot be explained by symptom burden (23,75). Stress and its effect on autonomic function may play a role in this excess risk (76), thus our aim to study underlying autonomic mechanisms may help to explain this disparity.

We found that within subjects, there was a significant difference in autonomic metrics during stress, with return towards baseline during recovery. Heart rate, as expected, increased during acute stress, however both HF and LF HRV decreased in power. On the other hand, T wave area remained persistently decreased during recovery as compared to rest. This suggests that the autonomic mechanisms at hand have a

range of effects of differing duration, with electrical and morphological changes being the most persistent, and frequency-domain metrics, such as HF HRV, being the most rapid and prominent. Even shorter-term HRV metrics have been used, recorded under < 1 minute, and have shown differences for mental stress activities during daily living (76), however the clinical importance of this is unknown. Our approach and findings are more robust due to the controlled experimental environment, measurement during recovery, and ability to adjust for relevant clinical covariates. The likely mechanism that occurs during acute mental stress is sympathetic activation and parasympathetic withdrawal. These findings allow us to utilize the ECG-based response to stress as an additional exposure for neurocardiac disease and clinical outcomes.

We sought to identify if the change in autonomic function in response to acute stress would associate with the chronic mental stress of depression or PTSD. We found that elevated resting heart rate was associated with both psychiatric diseases, and decreases in LF HRV during recovery were associated with only depression. This was contrary to what we expected, as there is a known relationship of HRV with both depression and PTSD (51,77). Prior studies have shown the relationship of HRV and depression with longer-duration ECG recordings, such as 24 hour Holter monitor (78). Our findings are most likely limited by the short duration of recordings. Autonomic modulation is a dynamic phenomenon, and by measuring during stress and recovery, the underlying abnormalities may be suppressed.

Due to the multiple available data sets, we were able to ask this question about the relationship of autonomic dysfunction and chronic mental stress using longer term recordings. We found that HRV, particularly during morning hours, was more robustly associated with HRV. The strongest association for depression was LF and VLF HRV, and for PTSD was acceleration capacity and HF and LF HRV. Although these associations are supported in the literature (51,78), our findings are unique in that they were selected at purely early morning hours. Due to the auto-regressive nature of ECG signal, 24 hour data could not easily be assessed in a similar manner, and thus cosinor analysis was performed (72). We evaluated circadian autonomic

variability and found a much more robust relationship between autonomic function and both depression and PTSD. The global or equatorial measure of autonomic tone, described by the MESOR, and the overall amplitude of autonomic tone, were decreased in patients with both depression and PTSD with frequency-domain HRV and acceleration capacity. *Dyx* variability had less changes in amplitude than other variables. There were no changes in the acrophase, best described as the time to peak amplitude of the signal, in any of these measures. This suggest that the relationship between autonomic function and psychiatric disease may more strongly have circadian characteristics than previously thought (79,80). Depressive symptom burden, for example, has a level of diurnal variation (81), which may be quantified by autonomic metrics.

12.2 Myocardial Ischemia

In **Aim II**, we studied the autonomic mechanisms underlying ischemic heart disease. IHD is not fully understood, as half of sudden cardiac deaths occur in men and women without known cardiovascular disease (82–84). There are important levels to the coronary artery system, including epicardial conduit vessels and the myocaridal resistance vessels. The difference between the two has started to become more clinically relevant as we begin to understand that myocardial ischemia is not only a problem of epicardial atherosclerotic disease (71). The ANS however has been implicated for its prominent role in regulation of cardiac electrophysiology, contractility, coronary vasomotor tone, amongst other effects (29). Both macrovascular and microvascular systems are heavily innervated and respond to different autonomic inputs, and also have afferent systems, allowing for bidirectional communication (27). Thus, understanding the differences and quantifying the effect of autonomic dysfunction as it relates to myocardial ischemia will help us to better understand and differentiate between these diseases burdens and their corresponding increased risks.

We first sought to evaluate the role of obstructive CAD on autonomic function. By

assessing obstructive CAD through direct angiography, we were limited to assessing purely epicardial disease. We found no strong relationship between autonomic metrics and obstructive CAD, however there was a trend towards higher *Dyx* in subjects with obstructive CAD. We found that those who underwent revascularization had a lower heart rate than those who did not, and similarly, *Dyx* trended higher in those patients than those without revascularization. This is a counterintuitve finding, in that literature there has been increasing support that HRV metrics such as *Dyx* may correlate with abnormal stress tests (26,42,85). However, in these studies, subjects had no known CAD, and were of low to intermediate pretest probability. The subjects we found with obstructive CAD would have been excluded, which may explain the findings or that obstructive CAD may modify autonomic tone in a non-linear manner.

We extended the prior analysis to review HRV during the process of revascularization. During revascularization, we compared the time after initial angioplasty to the 30 minutes preceding the case. We found no differences between HRV before or after cardiac catheterization was initiated, regardless of revascularization was performed. This finding is even more surprising in that revascularization is known to lead to autonomic changes immediately, such as an increased transient risk for ventricular arrhythmias (86) This may be based on the chronicity of the epicardial disease, as a majority of these subjects were outpatient at the time of catheterization.

We evaluated the epicardial vessels through myocardial perfusion, using either exercise or pharmacological stress, and assessed the relationship with autonomic function. We used acute stress changes, early morning changes, and circadian autonomic variability as metrics of autonomic tone. We found that short-term recordings of autonomic metrics had no association with physical stress-induced myocardial ischemia (PSIMI), either at rest, stress, or during recovery. When we extended the recording duration to 1 hour, selected at 7 AM based on prior findings (26), we found associations primarily with *Dyx*, even after adjustment for cardiovascular risk factors and known cardiovascular disease. When using the full 24 hour recording,

there were no prominent relationships with abnormal MPI. These findings do show a pattern that increased *Dyx* decreases the risk for having an abnormal MPI, which is similar in that of the ECG metrics available, it is the only one to show a consistent relationship with suspected epicardial disease. However, the relationship is inverse than that of the findings of obstructive CAD on angiography, which begs the question of the mechanism by which *Dyx* is associated with epicardial disease.

To fully delineate the pathways involved in IHD, we evaluated the myocardial blood flow through coronary flow reserve, and assessed the relationship with autonomic dysfunction as previously. Using quantitative PET, we were able to measure coronary flow reserve, which may be related by the autonomic activity (87). We found that early morning HRV was reliably related to CFR, even after adjustment for traditional risk factors. The most prominent association was with LF HRV and the other frequency-domain HRV metrics. When assessed using circadian autonomic variability, the relationship was consistent, and showed not only a relationship with the MESOR, but also that of the amplitude. These findings suggest that CFR, as compared to MPI, may be more regulated by autonomic tone, which supports the circadian distribution of major adverse cardiovascular events (47).

The relationship of stress reactivity and myocardial perfusion was systematically assessed to understand if there were common, autonomic mechanisms underlying both. By measuring HRV during acute mental stress challenge, we were able to create a surrogate for stress reactivity. This marker was used as a predictor for the development of mental stress-induced myocardial ischemia (MSIMI). We found that HRV, but not heart rate or T wave area, was significantly lower in those with MSIMI, particularly during the stress phase. We found a robust association in logistic models, particularly with HF and LF HRV during stress, for the risk of MSIMI. Every 1 unit increase in HRV lead to approximately half the risk of developing MSIMI. Even when adjusted for cardiovascular risk factors, the relationship remained robust, and was unchanged by the inclusion of chronic mental stress variables of PTSD and depression. This finding is the first of its kind, as it shows a direct relationship

between MSIMI and autonomic dysfunction in a non-invasive, quantifiable manner, and highlights the important autonomic mechanisms underlying MSIMI.

12.3 Clinical Outcomes

In **Aim III**, we sought to understand the autonomic mechanisms underlying the risk of major adverse cardiovascular events (MACE). In large, population studies, LF HRV has been seen to associate with an increased risk for overall mortality (88,89). Autonomic dysfunction has become an increasingly recognized additional risk factor for overall cardiovascular mortality (90). Certain measures, such as *Dyx* have even shown increased mortality after myocardial infarction similar in effect to that of decreased ejection fraction (41). This suggests that autonomic testing is just at its nacency at helping to predict cardiovascular events (91).

We examined the utility of a morning autonomic changes with both overall and cardiovascular mortality. We found that both LF and VLF HRV were consistently associated with mortality, however *Dyx* was the most prominent marker of outcomes. When measuring circadian autonomic variability, the MESOR of both LF and VLF HRV were both strongly associated with mortality. *Dyx* once again was most strongly associated, with a robust relationship of both MESOR and amplitude. Heart rate was also seen to be related, both through MESOR and amplitude, with increased heart rate leading to increased mortality, with decreased amplitude associated with decreased mortality. These findings were robust through adjustment of abnormal MPI, cardiovascular risk factors, known IHD, as well as chronic mental stress. This supports the idea that autonomic dysfunction is a unique, independent risk factor for mortality. The relationship of autonomic function with mortality reflects that changes in diurnal rhythm is a clinical important entity, and either may predict or share a common pathology that leads to increased cardiovascular risk.

Similar to circadian changes, acute mental stress may serve as a method to elicit autonomic dysfunction. This type of autonomic dysfunction may also increase risk for

MACE. We found that stress HRV was a robust predictor of not only cardiovascular death, but also recurrent cardiovascular events, with a hazard ratio ranging from 0.3 – 0.5. When adjusted for MSIMI, there was no change in the effect size of stress HRV on mortality or recurrent MACE, and MSIMI was not found to be a significant covariate. These findings were similar after adjustment for cardiovascular disease and risk factors, as well as chronic mental stress. This suggests that not only does HRV strongly associate with MSIMI, but explains the known increased cardiovascular risk that is associated with MSIMI. This supports the concept that not only does autonomic dysfunction lead to MSIMI and explain its increased risk of mortality, but is likely the mechanism by which MSIMI occurs (92).

13 Limitations and Future Direction

Each of the cohorts had unique strengths and limitations. First, the generalizability of these findings is someone limited, in that the populations had different baseline characteristics, such as frequency of CAD, sex, and age. ECG metrics are likely not able to be compared between groups due to these class differences. However, there is some evidence that HRV is not readily made to be standardized across populations (93), and there may be reason to compare findings within an individual instead. Globally, the method of sampling of ECG signal and the generation of HRV is fraught with potential errors, including interpolation methods, signal duration, noise. ECG signal was also recording using different technologies, from the VivaLNK ECG patch to single and multi lead Holter monitors. Our signal recordings between cohorts is not of a similar length, thus the exact metrics, such as cosinor analysis, could not be used equivalently between groups. The amount of noise, differences in sampling frequencies, and overall quality led to exclusion of signal that was considered unusuable. These are common errors faced by researchers within the field, which we minimized using standardized, open-source techniques (56). However, as we have worked through these errors in prior studies, there is confidence that there are similar patterns between groups even with the cohort variability.

Chronic psychological stress was measured in the binary form of a single diagnosis. Treating depression and PTSD as continuous variables may have provided a more useful outcome in assessing the effect of autonomic dysfunction but was limited by the frequency of symptom burden. In addition, symptom burden was assessed differently between cohorts, such as using the PHQ-9 in the *Biobank* and Beck's Depression Inventory in the *Twins*. However, the correlation between the two forms are high, and allow for some level of generalizability and extrapolation of autonomic function to its relationship with depressive symptom burden.

This is similar to limitations in assessing CAD, in that each cohort used a separate method for assessing myocardial perfusion, from direct coronary angiography to PET and SPECT. Coronary artery calcium was not available in nuclear imaging, thus confirming the presence of epicardial coronary disease was not possible. Additional factors, such as prior coronary revascularization, reason for angiography, and clinical context, were not able to be accounted for as easily due to the limited number of events. At the same time, the differences in imaging modalities assesses different components of neurocardiac axis, from epicardial disease to microvascular disease, giving a more nuanced understanding of cardiovascular physiology.

Clinically, although the follow-up events were adjudicated, primary data on the cause of death was not always able to be obtained. Between cohorts, cardiovascular events were not necessarily measured in the same way, such as recurrent events recorded in the *MIMS/MIPS* cohort, but only initial events in the *Twins*. In addition, follow-up data may have led to conflicting or overlapping events, such as onset of atrial fibrillation during hospitalization for decompensated heart failure, and based on the modeling approaches used, we would not be able to control for same-day events. However, the use of recurrent events was strengthened particularly by using a series of recurrent event modeling approaches, all with similar findings suggesting a robust and persistent relationship with autonomic dysfunction and clinical outcomes.

The largest strengths of this study are the use of multiple cohorts, the rigor applied to each hypothesis, and advanced statistical techniques to control for confounding, such as mixed effects models to control for twins. The ability to share similar ECG metrics between cohorts also allowed for some translation of study findings between groups.

This study reveals a number of important clinical questions. Autonomic changes may not only associate with overall depression or PTSD, but may also precede or correlate with changes in symptom burden, or may an intervenable metric to help control symptoms, such as with biofeedback (94). Autonomic dysfunction appears to have a strong associationi with coronary microvascular dysfunction. Assessing microvascular

dysfunction during coronary angiography with intracoronary acetylcholine, or through cardiac magnetic resonance imaging, may allow for more precise measurements of coronary flow reserve, and provide a higher fidelity metric for comparison. In addition to baseline HRV predicting future adverse events, repeat HRV metrics would be helpful to assess autonomic function as a dynamic variable that may change over time. This could be expanded to include interventions aimed at improving autonomic function, from cognitive behavioral therapy to vagal nerve stimulation, which would help to assess the role of autonomic dysfunction as a modifiable risk factor. The study of the neurocardiac axis is likely in its infancy, with significant room for expansive questions to help tackle this challenging field.

CONCLUSIONS

The objective of this study was to evaluate how stress reactivity effects cardiovascular disease and clinical outcomes. By leveraging several methods of assessing stress, as described in the specific aims, we were able to assess the relationship of stress with autonomic dysfunction along the neurocardiac axis. We were able to demonstrate the association of autonomic metrics with both psychological stress and myocardial ischemia, and using nuanced differences between our datasets, evaluate specific components of psychological stress and coronary artery disease.

The most prominent finding was the relationship of autonomic metrics with MSIMI. This ties together the effect of acute mental stress on myocardial ischemia through autonomic mechanisms that are readily quantifiable. Not only is there a relationship with MSIMI, but autonomic dysfunction in response to stress may in fact mediate the increased mortality seen with MSIMI. An additional important discovery was the relationship of circadian autonomic variability to abnormal myocardial blood flow, psychological stress, and overall clinical outcomes. This diurnal pattern supports the known circadian pattern of MACE, most commonly seen between 6 AM and 10 AM, and subsequently between 6 PM and 8 PM (47).

These findings are part and parcel of a quantifiable approach to measuring stress reactivity, supporting the clinical significance on multiple levels of the neurocardiac axis, including mortality. The next steps will be to identify if the autonomic response to stress can be modulated, and whether this will lead to decreases in cardiovascular disease, psychological distress, and overall mortality.

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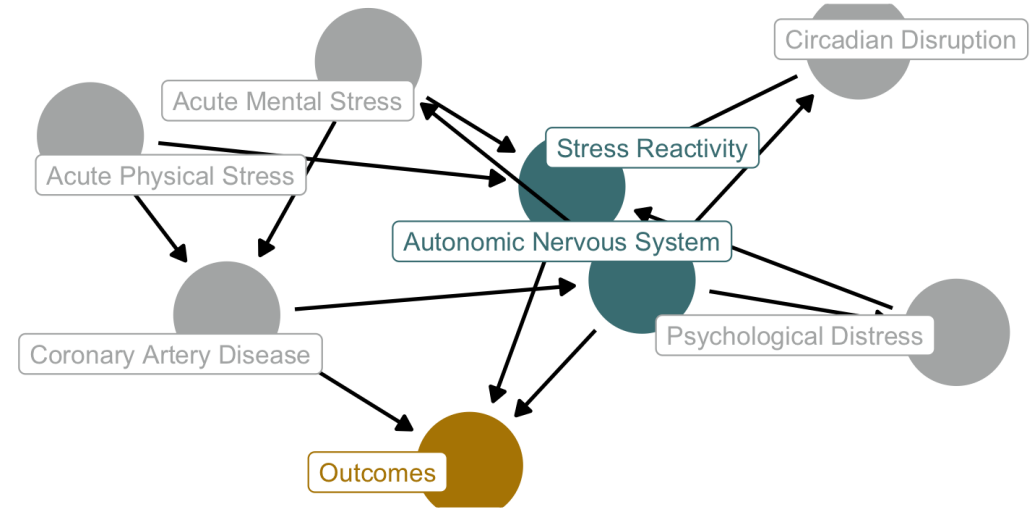
APPENDIX

A TABLES AND FIGURES

A.1 Clinical Overview

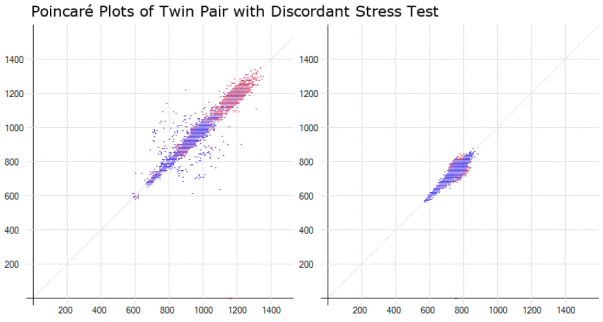
The follow section divides the relevant figures and tables into those describing the study, aims, and clinical cohorts.

A.1.1 Overview of Stress Reactivity



Directed acyclic graph of the relationship between neurocardiac stressors and pote

A.1.2 Poincaré Plot of HRV



A.1.3 Biobank Cohort Description

Emory Cardiovascular Biobank
Cohort Description

Characteristic	**N = 56**[1]
Age (years)	62 (52, 70)
Race	
African American Black	14 (26%)
Asian	2 (3.8%)
Caucasian White	37 (70%)
BMI (kg/m^2)	29.3 (26.2, 34.0)
Sex	
Female	9 (17%)
Male	44 (83%)
PHQ-9 Score	4.5 (1.0, 9.0)
Depression	10 (21%)
Gensini Score	26 (20, 51)
Stenosis	34 (71%)
CASS-70 Score	
0	21 (44%)
1	13 (27%)
2	9 (19%)
3	5 (10%)

[1]Median (IQR); n (%)

A description of subjects undergoing left heart catheterization with coronary angiography, including burden of coronary artery disease. CASS = Coronary Artery Surgery Score, PHQ = Patient Health Questionnaire, BMI = Body Mass Index.

A.1.4 Twin Cohorts Description

Emory Twins Study
Cohort Discription

Characteristic	**THS1**, N = 361[1]	**SAVEIT**, N = 206[1]	**THS2**, N = 165[1]	**ETSF**, N = 280[1]
Age (years)	55.0 (52.0, 57.0)	57.0 (56.0, 59.0)	61.0 (59.0, 62.0)	68.4 (66.8, 69.5)
BMI (kg/m^2)	28.0 (26.0, 32.0)	30.0 (27.0, 33.0)	30.0 (27.0, 33.0)	29.0 (27.0, 32.0)
Race				
White	345 (96%)	198 (96%)	157 (95%)	269 (96%)
African American	12 (3.3%)	8 (3.9%)	6 (3.6%)	7 (2.5%)
Asian	4 (1.1%)	0 (0%)	2 (1.2%)	4 (1.4%)
Current Smoker	230 (64%)	155 (76%)	120 (74%)	178 (64%)
Known IHD	34 (9.4%)	30 (15%)	29 (18%)	11 (3.9%)
Congestive Heart Failure	2 (0.6%)	0 (0%)	3 (1.8%)	4 (1.4%)
Hypertension	106 (29%)	69 (33%)	91 (55%)	165 (59%)
Diabetes Mellitus	33 (9.1%)	34 (17%)	27 (16%)	64 (23%)
Post-Traumatic Stress Disorder	22 (6.1%)	59 (29%)	45 (27%)	41 (15%)
Depression	40 (11%)	42 (20%)	26 (16%)	27 (9.7%)
Abnormal Myocardial Perfusion	40 (13%)	10 (5.9%)	29 (18%)	32 (12%)

[1]Median (IQR); n (%)

Description of the veteran twin subjects within each follow-up period. They were evaluated for clinical characteristics, including quantitative myocardial perfusion imaging. THS = Twins Heart Study, SAVEIT = Stress and Vascular Evaluation in Twins, ETSF = Emory Twins Study Follow-Up.

A.1.5 Mental Stress Cohorts Description

MIMS and MIPS
Cohort Discription

Characteristic	MIMS		MIPS	
	MSIMI = 0, N = 256[1]	**MSIMI = 1**, N = 50[1]	**MSIMI = 0**, N = 440[1]	**MSIMI = 1**, N = 188[1]
Age (years)	52.0 (47.0, 56.2)	51.5 (46.6, 54.7)	66 (58, 71)	64 (57, 71)
Sex (Female)	117 (46%)	33 (66%)	92 (21%)	76 (40%)
Race				
White	79 (31%)	9 (18%)	308 (70%)	115 (61%)
Black	165 (64%)	36 (72%)	110 (25%)	67 (36%)
Other	12 (4.7%)	5 (10%)	22 (5.0%)	6 (3.2%)
BMI (kg/m^2)	30 (26, 35)	30 (26, 38)	29.1 (25.6, 32.1)	29.5 (26.2, 32.8)
Current Smoker	62 (25%)	11 (22%)	215 (49%)	84 (45%)
Obstructive Coronary Artery Disease	201 (84%)	41 (89%)	316 (83%)	132 (85%)
Diabetes Mellitus	79 (31%)	18 (36%)	137 (31%)	69 (37%)
Coronary Artery Bypass Graft	51 (20%)	12 (24%)	139 (32%)	75 (40%)
Percutaneous Coronary Intervention	177 (69%)	35 (70%)	226 (51%)	100 (53%)
Hyperlipidemia	206 (80%)	40 (80%)	369 (84%)	151 (80%)
Hypertension	205 (80%)	42 (84%)	325 (74%)	147 (78%)
PSIMI	49 (20%)	20 (40%)	121 (28%)	96 (53%)
Depression	92 (37%)	16 (32%)	111 (26%)	51 (28%)
Post-Traumatic Stress Disorder	32 (13%)	12 (24%)	35 (8.2%)	8 (4.4%)

[1]Median (IQR); n (%)

MSIMI = Mental Stress Induced Myocardial Ischemia; PSIMI = Physical Stress Induced Myocardial Ischemia, MIMS = Myocardial Infarction and Mental Stress, MIPS = Mental Stress Ischemia Mechanisms and Prognosis Study

A.1.6 HRV in Twins Cohorts

Description of HRV
Emory Twins Study

ECG/HRV Metric	THS1[1]	SAVEIT[1]	THS2[1]	ETSF[1]
RR Interval	918 (816, 1,018)	870 (774, 973)	923 (828, 1,025)	915 (806, 1,020)
SDNN	60 (46, 74)	52 (40, 68)	53 (40, 68)	48 (36, 62)
RMSSD	27 (20, 35)	24 (17, 33)	25 (18, 35)	25 (18, 37)
PNN50	0.05 (0.02, 0.11)	0.03 (0.01, 0.09)	0.04 (0.01, 0.10)	0.03 (0.01, 0.09)
Ultra Low Frequency	6.60 (5.87, 7.21)	6.39 (5.68, 7.08)	6.42 (5.73, 7.11)	6.00 (5.30, 6.67)
Very Low Frequency	7.81 (7.27, 8.25)	7.55 (7.03, 8.08)	7.54 (7.02, 8.05)	7.29 (6.76, 7.79)
Low Frequency	6.79 (6.28, 7.23)	6.57 (6.00, 7.08)	6.45 (5.85, 6.95)	6.28 (5.70, 6.86)
High Frequency	5.48 (4.94, 6.00)	5.30 (4.64, 5.92)	5.31 (4.66, 6.03)	5.32 (4.64, 6.11)
Low/High Frequency Ratio	4.13 (2.63, 6.05)	4.02 (2.50, 5.92)	3.24 (2.02, 5.16)	3.01 (1.71, 4.83)
Total Power	8.45 (7.94, 8.86)	8.20 (7.70, 8.70)	8.18 (7.69, 8.67)	7.97 (7.47, 8.46)
Acceleration Capacity	-11.0 (-14.1, -7.9)	-9.5 (-12.5, -6.9)	-9.4 (-12.2, -6.7)	-8.1 (-11.6, -6.1)
Deceleration Capacity	10.3 (7.0, 13.5)	8.8 (6.1, 11.8)	8.5 (5.9, 11.4)	7.3 (5.2, 10.8)
Sample Entropy	1.52 (1.33, 1.69)	1.50 (1.32, 1.70)	1.53 (1.32, 1.72)	1.55 (1.35, 1.77)
Approximate Entropy	0.93 (0.87, 1.00)	0.95 (0.89, 1.03)	0.94 (0.87, 1.01)	0.96 (0.89, 1.04)
DYX	2.91 (2.37, 3.47)	2.80 (2.31, 3.33)	2.81 (2.30, 3.34)	2.58 (2.03, 3.13)

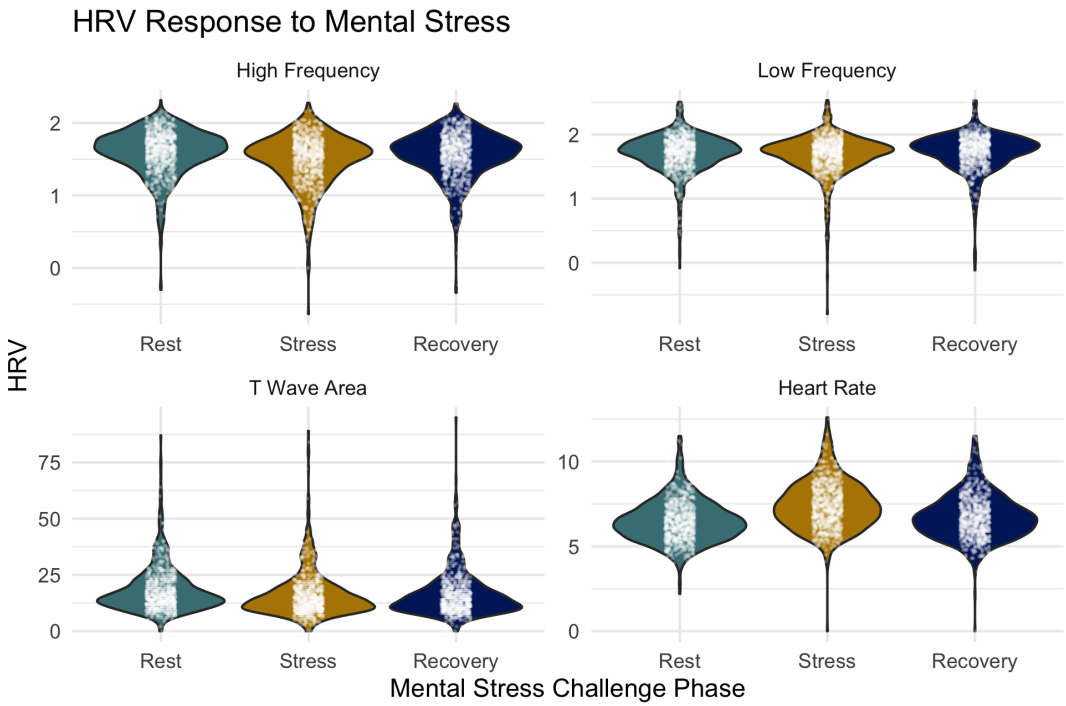
[1]Median (IQR)

Heart rate variability is described in each of the follow-up periods. HRV = heart rate variability, Dyx = kurtosis of Poincare plot, SDNN = the standard deviation of normally conducted RR intervals, RMSSD = the root mean square of successive differences in normally conducted RR intervals, PNN50 = proportion of normally conducted RR intervals that differ by more than 50 ms divided by the total number of normally conducted RR intervals

A.2 Psychological Stress

The follow section divides the relevant figures and tables into those that pertain to the relationship of autonomic function and psychological stress, including both acute mental stress and chronic psychological stress.

A.2.1 HRV and Mental Stress Challenge



The distribution of HRV and ECG findings in different phases of mental stress challenges.

A.2.2 Distribution of HRV and Mental Stress Challenge

Difference between Mental Stress Challenge Phases and ECG Metrics
MIMS/MIPS Cohorts

	Mean (95% CI)	T-statistic
Heart Rate		
Stress	1.0 (0.9, 1.0)	22.1
Recovery	0.3 (0.2, 0.3)	8.2
High Frequency HRV		
Stress	−0.1 (−0.1, −0.1)	−11.5
Recovery	−0.0 (−0.1, −0.0)	−5.7
Low Frequency HRV		
Stress	−0.0 (−0.0, −0.0)	−3.0
Recovery	0.0 (−0.0, 0.0)	1.9
T Wave Area		
Stress	−3.7 (−5.9, −1.5)	−3.4
Recovery	−3.2 (−5.2, −1.3)	−3.3

HRV summarised during stress and recovery phase of the mental stress challenge were compared to rest HRV. HRV = heart rate variability.

A.2.3 Depression and PTSD with Mental Stress Challenge

Mental Stress Challenge HRV and Chronic Psychological Stress
MIMS/MIPS Cohorts

ECG/HRV Metric	Depression[1]	PTSD[1]
Heart Rate		
Rest	1.15 (1.01, 1.3), AUC 0.55	1.33 (1.11, 1.58), AUC 0.6
Stress	1.01 (0.9, 1.13), AUC 0.51	1.04 (0.88, 1.22), AUC 0.53
Recovery	1.09 (0.97, 1.22), AUC 0.54	1.14 (0.96, 1.35), AUC 0.56
T Wave Area		
Rest	1 (0.99, 1.01), AUC 0.54	1 (0.99, 1.01), AUC 0.56
Stress	1.01 (1, 1.02), AUC 0.5	1.01 (1, 1.03), AUC 0.5
Recovery	1.01 (1, 1.02), AUC 0.54	1.02 (1, 1.03), AUC 0.58
High Frequency HRV		
Rest	0.98 (0.6, 1.64), AUC 0.48	0.6 (0.31, 1.23), AUC 0.54
Stress	0.79 (0.51, 1.21), AUC 0.51	0.63 (0.35, 1.17), AUC 0.54
Recovery	0.71 (0.44, 1.15), AUC 0.52	0.53 (0.28, 1.04), AUC 0.55
Low Frequency HRV		
Rest	0.71 (0.42, 1.19), AUC 0.52	0.58 (0.29, 1.21), AUC 0.57
Stress	0.74 (0.46, 1.21), AUC 0.53	0.63 (0.34, 1.26), AUC 0.55
Recovery	0.5 (0.29, 0.83), AUC 0.54	0.51 (0.26, 1.07), AUC 0.56

[1]Logistic regression model, OR with 95% CI and concordance statistic.

The association between HRV during mental stress challenge and the chronic psychological stressors of depression and PTSD are described. HRV = heart rate variability.

A.2.4 Depression by PHQ-9 and HRV

HRV and Depression by PHQ-9
Emory Cardiovascular Biobank

HRV Metric	**No Depression**, N = 38[1]	**Depression**, N = 10[1]	**p-value**[2]
RR Interval	872 (738, 929)	727 (689, 920)	0.6
SDNN	37 (21, 54)	26 (16, 44)	0.6
RMSSD	25 (19, 36)	16 (13, 26)	0.13
PNN50	0.03 (0.01, 0.09)	0.01 (0.01, 0.04)	0.089
Ultra Low Frequency	233 (108, 405)	173 (81, 358)	>0.9
Very Low Frequency	887 (310, 1,613)	444 (227, 1,561)	0.7
Low Frequency	486 (109, 725)	138 (67, 617)	0.6
High Frequency	306 (117, 824)	99 (51, 316)	0.14
Low/High Frequency Ratio	1.35 (0.64, 1.90)	1.28 (1.01, 1.98)	0.9
Total Power	1,865 (660, 3,914)	929 (438, 2,786)	0.5
Acceleration Capacity	-6.52 (-10.22, -4.21)	-3.88 (-7.42, -2.64)	0.3
Deceleration Capacity	5.2 (4.0, 9.0)	4.0 (3.1, 7.5)	0.4
Sample Entropy	1.41 (1.14, 1.62)	1.34 (1.07, 1.50)	0.4
Approximate Entropy	0.92 (0.85, 1.04)	0.99 (0.93, 1.04)	0.4
Dyx	1.75 (1.29, 2.57)	2.07 (1.76, 2.69)	0.4

[1]Median (IQR)
[2]Wilcoxon rank sum exact test

In patients undergoing angiography, HRV metrics were described in those with moderate to severe depressive symptoms to those with mild to minimal symptoms by PHQ-9. HRV = Heart Rate Variability, PHQ-9 = Patient Health Questionnaire.

A.2.5 HRV and Chronic Mental Stress in Twins

Morning HRV and Chronic Psychological Stress
Emory Twins Study

	AC	*Dyx*	HF	LF	VLF
PTSD					
Model 1	1.11 (1.03, 1.21)	0.90 (0.67, 1.20)	0.69 (0.50, 0.94)	0.60 (0.42, 0.86)	0.70 (0.48, 1.03)
Model 2	1.11 (1.02, 1.20)	2.12 (2.12, 2.13)	0.70 (0.51, 0.97)	0.63 (0.44, 0.92)	0.75 (0.75, 0.75)
Model 3	1.15 (1.06, 1.26)	1.98 (0.44, 8.89)	0.57 (0.40, 0.82)	0.65 (0.45, 0.94)	0.61 (0.40, 0.94)
Depression					
Model 1	1.25 (1.12, 1.39)	0.60 (0.25, 1.47)	0.53 (0.16, 1.78)	0.46 (0.46, 0.46)	0.22 (0.12, 0.42)
Model 2	1.25 (1.11, 1.39)	0.54 (0.16, 1.86)	0.52 (0.35, 0.78)	0.25 (0.15, 0.40)	0.20 (0.11, 0.36)
Model 3	1.22 (0.71, 2.08)	0.32 (0.10, 0.98)	0.41 (0.04, 4.61)	0.05 (0.00, 1.21)	0.04 (0.00, 0.92)

[1]Model 1 = HRV
[2]Model 2 = Model 1 + Age + BMI + Race
[3]Model 3 = Model 2 + Smoking + HTN + Cardiovascular Disease

Depression is measured as a binary outcome with Beck Depression Inventory score > 14. PTSD = Post-Traumatic Stress Disorder, HRV = heart rate variability, LF = low frequency HRV, HF = high frequency HRV, VLF = very low frequency HRV, AC = acceleration capacity

A.2.6 Circadian HRV and Chronic Mental Stress

Circadian HRV and Chronic Psychological Stress
Emory Twins Study

	MESOR	Amplitude	Phi
PTSD			
High Frequency HRV	0.61 (0.42, 0.88)	0.28 (0.09, 0.84)	1.16 (0.96, 1.4)
Low Frequency HRV	0.46 (0.31, 0.69)	0.31 (0.13, 0.72)	1.02 (0.86, 1.21)
Very Low Frequency HRV	0.56 (0.36, 0.87)	0.49 (0.28, 0.86)	1.1 (0.87, 1.39)
Acceleration Capacity	1.13 (1.03, 1.25)	0.75 (0.6, 0.94)	0.87 (0.71, 1.08)
Heart Rate	1.27 (0.98, 1.63)	0.92 (0.54, 1.58)	1.06 (0.79, 1.42)
Dyx	0.67 (0.47, 0.95)	0.93 (0.57, 1.51)	1.16 (0.94, 1.44)
Depression			
High Frequency HRV	0.43 (0.27, 0.69)	0.42 (0.22, 0.82)	1.09 (0.89, 1.35)
Low Frequency HRV	0.26 (0.15, 0.45)	0.31 (0.14, 0.68)	1.06 (0.86, 1.31)
Very Low Frequency HRV	0.24 (0.13, 0.45)	0.26 (0.13, 0.5)	1.12 (0.83, 1.53)
Acceleration Capacity	1.25 (1.11, 1.41)	0.79 (0.67, 0.94)	0.94 (0.73, 1.21)
Heart Rate	1.36 (1.03, 1.78)	1.25 (0.63, 2.47)	1.32 (0.96, 1.8)
Dyx	0.35 (0.23, 0.55)	0.61 (0.33, 1.12)	0.95 (0.75, 1.21)

Depression is measured as a binary outcome with Beck Depression Inventory score > 14. The HRV metrics are measured over 24 hours using cosinor statistics. PTSD = Post-Traumatic Stress Disorder, HRV = heart rate variability, LF = low frequency HRV, HF = high frequency HRV, VLF = very low frequency HRV, AC = acceleration capacity, MESOR = midline estimating statistic of rhythm, Amplitude = maximum distance from MESOR, Phi = shift of acrophase

A.3 Myocardial Ischemia

The follow section divides the relevant figures and tables into those that pertain to the relationship of autonomic function and myocardial ischemia, including both obstructive coronary artery disease and myocardial perfusion.

A.3.1 Relationship Between Obstructive and Non-Obstructive Coronary Artery Disease

HRV and Obstructive CAD
Emory Cardiovascular Biobank

Characteristic	**Nonobstructive CAD**, N = 29[1]	**Obstructive CAD**, N = 27[1]	**p-value**[2]
RR Interval	733 (655, 932)	868 (786, 922)	0.12
SDNN	27 (17, 54)	43 (26, 52)	0.3
RMSSD	21 (15, 33)	29 (19, 43)	0.3
PNN50	0.02 (0.01, 0.07)	0.06 (0.02, 0.10)	0.2
Ultra Low Frequency	141 (86, 294)	202 (133, 497)	0.2
Very Low Frequency	474 (158, 1,687)	887 (444, 1,347)	0.3
Low Frequency	184 (56, 923)	486 (138, 704)	0.4
High Frequency	193 (94, 865)	327 (148, 687)	0.4
Low/High Frequency Ratio	1.08 (0.49, 1.76)	1.42 (0.73, 1.92)	0.3
Total Power	1,005 (378, 4,144)	1,915 (929, 3,914)	0.4
Acceleration Capacity	-4.71 (-9.54, -3.85)	-7.04 (-10.08, -4.25)	0.4
Deceleration Capacity	4.84 (3.85, 9.36)	6.20 (3.98, 8.96)	0.6
Sample Entropy	1.36 (1.07, 1.47)	1.37 (1.18, 1.61)	0.3
Approximate Entropy	0.94 (0.87, 1.04)	0.88 (0.85, 1.03)	0.3
Dyx	1.72 (1.19, 2.11)	2.07 (1.60, 2.66)	0.093

[1]Median (IQR)
[2]Wilcoxon rank sum exact test

In patients undergoing angiography, HRV metrics were described in those with both obstructive (>70%) and nonobstructive CAD, and evaluated for differences in distribution. HRV = Heart Rate Variability, CAD = Coronary Artery Disease.

A.3.2 Effective of Revascularization on Autonomic Function

HRV and Revascularization
Emory Cardiovascular Biobank

HRV Metric	**No Revascularization** N = 14[1]	**Revascularization** N = 34[1]	**p-value**[2]
RR Interval	648 (608, 872)	868 (775, 932)	0.019
SDNN	18 (15, 49)	37 (26, 51)	0.11
RMSSD	16 (13, 32)	28 (20, 40)	0.11
PNN50	0.01 (0.01, 0.02)	0.05 (0.01, 0.10)	0.086
Ultra Low Frequency	99 (56, 269)	200 (130, 477)	0.11
Very Low Frequency	205 (94, 1,465)	826 (414, 1,336)	0.2
Low Frequency	70 (42, 833)	383 (145, 689)	0.2
High Frequency	96 (89, 480)	306 (140, 620)	0.2
Low/High Frequency Ratio	0.99 (0.41, 1.28)	1.45 (0.65, 2.00)	0.2
Total Power	431 (216, 3,156)	1,865 (881, 3,562)	0.2
Acceleration Capacity	-4.12 (-7.06, -2.08)	-6.52 (-9.39, -4.06)	0.3
Deceleration Capacity	4.83 (2.05, 6.49)	5.07 (4.00, 8.58)	0.4
Sample Entropy	1.14 (1.06, 1.39)	1.37 (1.16, 1.56)	0.15
Approximate Entropy	0.95 (0.91, 1.11)	0.92 (0.85, 1.00)	0.2
Dyx	1.36 (1.17, 1.78)	2.03 (1.52, 2.71)	0.063

[1]Median (IQR)
[2]Wilcoxon rank sum exact test

In patients undergoing angiography, HRV metrics were described in those that received revascularization, and evaluated for differences in distribution. HRV = Heart Rate Variability, CAD = Coronary Artery Disease.

A.3.3 HRV by Timing of Revascularization

HRV and Timing of Myocardial Reperfusion
Emory Cardiovascular Biobank

	No Revascularization			Revascularization		
ECG Metrics	**Angiography** N = 6[1]	**Before** N = 5[1]	**p-value**[2]	**Balloon** N = 15[1]	**Before** N = 20[1]	**p-value**[2]
RR Interval	711.7 (688.2, 855.9)	749.3 (723.6, 869.5)	0.8	849.5 (746.4, 949.6)	865.8 (801.1, 925.2)	0.6
SDNN	38.2 (16.8, 60.9)	47.4 (19.0, 49.0)	>0.9	30.7 (22.4, 62.4)	32.9 (25.5, 51.3)	0.8
RMSSD	28.8 (14.4, 48.6)	30.2 (20.6, 38.7)	>0.9	21.1 (16.3, 35.2)	20.7 (15.7, 27.8)	0.9
PNN50	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	>0.9	0.0 (0.0, 0.1)	0.0 (0.0, 0.0)	0.6
Ultra Low Frequency	110.3 (36.3, 177.9)	96.2 (92.7, 185.2)	0.8	151.6 (78.6, 623.7)	99.3 (52.1, 368.8)	0.5
Very Low Frequency	684.7 (115.1, 2,018.6)	1,000.1 (118.5, 1,340.7)	>0.9	507.3 (313.6, 1,643.5)	490.8 (230.2, 1,425.3)	0.7
Low Frequency	608.7 (74.9, 1,139.7)	867.6 (48.6, 875.5)	0.8	241.8 (83.9, 530.6)	276.2 (77.5, 551.9)	>0.9
High Frequency	539.6 (132.5, 967.8)	387.0 (127.5, 591.6)	0.8	107.7 (68.8, 579.8)	150.6 (92.7, 322.5)	>0.9
Low/High Frequency Ratio	0.6 (0.4, 1.1)	1.8 (0.4, 2.2)	0.4	1.2 (0.4, 1.8)	1.1 (0.5, 2.9)	0.6
Total Power	1,941.4 (360.8, 4,653.2)	2,559.9 (363.5, 3,097.1)	>0.9	1,208.3 (600.6, 4,185.2)	1,109.0 (672.4, 2,980.9)	0.7
Acceleration Capacity	-7.3 (-9.5, -4.6)	-4.8 (-11.5, -4.3)	>0.9	-5.0 (-7.1, -3.8)	-6.4 (-8.8, -3.7)	0.6
Deceleration Capacity	7.1 (4.7, 9.5)	6.4 (4.4, 12.1)	>0.9	4.4 (3.6, 6.8)	5.9 (3.8, 7.5)	0.7
Sample Entropy	1.0 (0.7, 1.4)	1.4 (0.8, 1.5)	0.7	1.2 (1.0, 1.4)	1.3 (1.2, 1.5)	0.2
Approximate Entropy	0.8 (0.7, 1.1)	0.8 (0.8, 0.9)	>0.9	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)	0.9

[1]Median (IQR)
[2]Wilcoxon rank sum exact test

HRV was measured before the procedure started and during the time of coronary angiography (versus intervention). Coronary arteries with obstructive disease are reperfused using balloon angioplasty and potential stenting. HRV = Heart Rate Variability, CAD = Coronary Artery Disease.

A.3.4 Relationship of HRV with both Mental and Physical Stress

Myocardial Perfusion Imaging with Physical and Mental Stress
MIMS/MIPS Cohorts

ECG/HRV Metric	Combined MSIMI/PSIMI[1]	MSIMI[1]	PSIMI[1]
Heart Rate			
Rest	1.03 (0.92, 1.16), AUC 0.51	1.15 (1.01, 1.32), AUC 0.54	0.97 (0.85, 1.1), AUC 0.51
Stress	1 (0.9, 1.1), AUC 0.49	1.08 (0.96, 1.21), AUC 0.54	1.02 (0.91, 1.14), AUC 0.5
Recovery	0.98 (0.88, 1.1), AUC 0.52	1.08 (0.96, 1.23), AUC 0.52	0.95 (0.84, 1.06), AUC 0.53
T Wave Area			
Rest	1 (0.99, 1), AUC 0.49	1 (0.98, 1), AUC 0.51	1 (0.99, 1), AUC 0.5
Stress	1 (0.99, 1.01), AUC 0.51	1 (0.98, 1.01), AUC 0.5	1.01 (0.99, 1.02), AUC 0.52
Recovery	1 (0.99, 1.01), AUC 0.51	0.98 (0.97, 1), AUC 0.56	1 (0.99, 1.01), AUC 0.5
High Frequency HRV			
Rest	0.71 (0.45, 1.13), AUC 0.55	0.57 (0.34, 0.95), AUC 0.56	0.71 (0.43, 1.17), AUC 0.54
Stress	0.7 (0.47, 1.05), AUC 0.54	0.48 (0.31, 0.76), AUC 0.58	0.85 (0.55, 1.31), AUC 0.52
Recovery	0.82 (0.52, 1.27), AUC 0.53	0.62 (0.38, 1.02), AUC 0.55	0.85 (0.53, 1.39), AUC 0.52
Low Frequency HRV			
Rest	0.67 (0.41, 1.1), AUC 0.55	0.53 (0.31, 0.92), AUC 0.56	0.64 (0.37, 1.08), AUC 0.54
Stress	0.64 (0.4, 1.01), AUC 0.56	0.45 (0.27, 0.74), AUC 0.59	0.63 (0.38, 1.03), AUC 0.54
Recovery	0.64 (0.39, 1.04), AUC 0.56	0.43 (0.25, 0.74), AUC 0.59	0.64 (0.38, 1.08), AUC 0.55

[1]Logistic regression model, OR with 95% CI and concordance statistic.

HRV was measured during the three stages of mental stress challenge and compared in logistic regression models with the results of myocardial perfusion imaging. HRV = heart rate variability, MSIMI = mental stress-induced myocardial ischemia, PSIMI = physical stress-induced myocardial ischemia, AUC = area under receiver-operator curve. Bolded text signifies a p-value < 0.05.

A.3.5 Quantitative Myocardial Perfusion and HRV

Myocardial Perfusion Imaging and Morning HRV
Emory Twins Study

	AC	*Dyx*	HF	LF	VLF
Coronary Flow Reserve					
Model 1	0.96 (0.95, 0.98)	1.13 (1.05, 1.22)	1.10 (1.02, 1.20)	1.23 (1.11, 1.35)	1.18 (1.06, 1.31)
Model 2	0.97 (0.95, 0.99)	1.09 (1.01, 1.17)	1.10 (1.02, 1.20)	1.21 (1.10, 1.34)	1.17 (1.05, 1.30)
Model 3	0.97 (0.95, 0.99)	1.04 (0.96, 1.12)	1.09 (1.01, 1.18)	1.16 (1.04, 1.28)	1.11 (0.99, 1.24)
Abnormal MPI					
Model 1	0.96 (0.89, 1.03)	0.72 (0.53, 0.99)	1.20 (0.87, 1.64)	0.93 (0.63, 1.37)	0.78 (0.51, 1.20)
Model 2	0.96 (0.90, 1.04)	0.71 (0.51, 0.97)	1.19 (0.87, 1.65)	0.90 (0.61, 1.34)	0.76 (0.49, 1.19)
Model 3	0.95 (0.88, 1.03)	0.70 (0.51, 0.98)	1.21 (0.87, 1.66)	0.94 (0.61, 1.43)	0.79 (0.50, 1.26)

[1]Model 1 = HRV
[2]Model 2 = Model 1 + Age + BMI + Race
[3]Model 3 = Model 2 + Smoking + HTN + Cardiovascular Disease

Relationship between abnormal MPI and CFR with HRV. HRV = heart rate variability, MPI = myocardial perfusion imaging, CFR = coronary flow reserve, LF = low frequency HRV, HF = high frequency HRV, VLF = very low frequency HRV, AC = acceleration capacity

A.3.6 Circadian HRV and Myocardial Perfusion

Circadian HRV and Myocardial Perfusion Abnormalities
Emory Twins Study

	MESOR	Amplitude	Phi
Coronary Flow Reserve			
High Frequency HRV	1.1 (1.01, 1.2)	1.1 (0.98, 1.23)	0.98 (0.94, 1.03)
Low Frequency HRV	1.21 (1.09, 1.34)	1.13 (0.96, 1.34)	1.02 (0.97, 1.06)
Very Low Frequency HRV	1.12 (1.02, 1.24)	1.13 (1.01, 1.26)	1.02 (0.96, 1.09)
Acceleration Capacity	0.97 (0.95, 0.99)	1.01 (0.98, 1.04)	1.03 (0.97, 1.08)
Heart Rate	0.91 (0.85, 0.97)	1.05 (0.92, 1.2)	1.07 (0.99, 1.15)
Dyx	1.19 (1.08, 1.31)	1.14 (0.99, 1.3)	0.98 (0.92, 1.04)
Abnormal MPI			
High Frequency HRV	1.32 (0.9, 1.92)	1.88 (0.93, 3.8)	0.99 (0.81, 1.2)
Low Frequency HRV	0.88 (0.58, 1.32)	1.14 (0.61, 2.15)	0.92 (0.76, 1.1)
Very Low Frequency HRV	0.88 (0.57, 1.36)	1 (0.63, 1.6)	0.96 (0.75, 1.24)
Acceleration Capacity	0.98 (0.91, 1.05)	1.14 (1.01, 1.28)	0.89 (0.7, 1.15)
Heart Rate	0.95 (0.74, 1.21)	1.16 (0.72, 1.86)	1.15 (0.86, 1.53)
Dyx	0.89 (0.61, 1.31)	0.78 (0.4, 1.5)	0.88 (0.7, 1.09)

Myocardial perfusion was quantified as a ccontinuous variable and as a binary of abnormal or normal. The HRV metrics are measured over 24 hours using cosinor statistics. MPI = myocardial perfusion imaging, CFR = coronary flow reserve, HRV = heart rate variability, LF = low frequency HRV, HF = high frequency HRV, VLF = very low frequency HRV, AC = acceleration capacity, MESOR = midline estimating statistic of rhythm, Amplitude = maximum distance from MESOR, Phi = shift of acrophase

A.3.7 Distribution of HRV and MSIMI

HRV distribution by MSIMI
MIMS/MIPS cohorts

Characteristic	**MSIMI = 0**, N = 710[1]	**MSIMI = 1**, N = 243[1]	**p-value**[2]
Heart Rate			
Rest	6.40 (5.60, 7.20)	6.40 (5.88, 7.50)	0.090
Stress	7.30 (6.40, 8.30)	7.50 (6.60, 8.50)	0.092
Recovery	6.65 (5.90, 7.40)	6.60 (5.90, 7.80)	0.5
T Wave Area			
Rest	16 (12, 23)	16 (12, 23)	0.8
Stress	14 (10, 19)	14 (10, 20)	0.9
Recovery	15 (10, 20)	13 (9, 19)	0.024
High Frequency HRV			
Rest	1.65 (1.48, 1.81)	1.61 (1.39, 1.76)	0.017
Stress	1.57 (1.34, 1.74)	1.48 (1.22, 1.65)	<0.001
Recovery	1.62 (1.43, 1.78)	1.55 (1.35, 1.74)	0.034
Low Frequency HRV			
Rest	1.76 (1.60, 1.89)	1.70 (1.49, 1.86)	0.010
Stress	1.74 (1.59, 1.87)	1.66 (1.48, 1.81)	<0.001
Recovery	1.79 (1.61, 1.91)	1.71 (1.52, 1.85)	<0.001

[1]Median (IQR)
[2]Wilcoxon rank sum test

The distribution of HRV between those with MSIMI and those without. The HRV metric are stratified by phase of mental stress challenge. MSIMI = mental stress-induced myocardial ischemia, HRV = heart rate variability.

A.3.8 Modeling Mental Stress-Induced Myocardial Ischemia and HRV

Mental Stress-Induced Myocardial Ischemia and HRV
MIMS/MIPS Cohorts

Sequential Models	Stress LF	Rest LF	Stress HF	Rest HF
Model 1	0.45 (0.27, 0.74), AUC 0.59	0.53 (0.31, 0.92), AUC 0.56	0.48 (0.31, 0.76), AUC 0.58	0.57 (0.34, 0.95), AUC 0.56
Model 2	0.49 (0.29, 0.81), AUC 0.64	0.59 (0.34, 1.04), AUC 0.62	0.45 (0.28, 0.72), AUC 0.64	0.49 (0.29, 0.85), AUC 0.62
Model 3	0.51 (0.3, 0.87), AUC 0.63	0.64 (0.36, 1.13), AUC 0.62	0.48 (0.29, 0.77), AUC 0.64	0.53 (0.3, 0.93), AUC 0.62
Model 4	0.53 (0.31, 0.91), AUC 0.65	0.65 (0.36, 1.15), AUC 0.63	0.49 (0.3, 0.79), AUC 0.65	0.54 (0.31, 0.96), AUC 0.64
Model 5	0.52 (0.3, 0.91), AUC 0.65	0.66 (0.36, 1.18), AUC 0.63	0.47 (0.29, 0.77), AUC 0.66	0.54 (0.3, 0.95), AUC 0.63

[1]Model 1 = MSIMI ~ HRV
[2]Model 2 = Model 1 + Age + BMI + Sex + Race
[3]Model 3 = Model 2 + Smoking + Diabetes + Hypertension + Hyperlipidemia
[4]Model 4 = Model 3 + Known Coronary/Peripheral Artery Disease
[5]Model 5 = Model 4 + Depression + Post-Traumatic Stress Disorder

The association between the exposure of HRV with the finding of MSIMI is described. The HRV metric are stratified by phase of mental stress challenge. MSIMI = mental stress-induced myocardial ischemia, HRV = heart rate variability.

A.4 Clinical Outcomes

The follow section divides the relevant figures and tables into those describing the relationship between autonomic dysfunction and clinical outcomes.

A.4.1 Outcomes in Twins

Clinical Outcomes by HRV
Emory Twins Study

	Acceleration Capacity	Dyx	High Frequency HRV	Low Frequency HRV	Very Low Frequency HRV
Cardiovascular Death					
Model 1	1.04 (0.97, 1.11)	0.64 (0.51, 0.81)	0.84 (0.62, 1.13)	0.75 (0.54, 1.03)	0.64 (0.44, 0.92)
Model 2	1.03 (0.96, 1.1)	0.65 (0.5, 0.84)	0.81 (0.59, 1.12)	0.83 (0.58, 1.18)	0.67 (0.45, 1.01)
Model 3	1.03 (0.96, 1.1)	0.66 (0.51, 0.85)	0.82 (0.6, 1.13)	0.86 (0.59, 1.25)	0.68 (0.45, 1.04)
Model 4	1.03 (0.96, 1.12)	0.69 (0.53, 0.9)	0.79 (0.58, 1.09)	0.86 (0.57, 1.29)	0.76 (0.5, 1.18)
Model 5	1.03 (0.95, 1.12)	0.68 (0.52, 0.89)	0.8 (0.58, 1.11)	0.86 (0.57, 1.29)	0.77 (0.49, 1.21)
All Cause Mortality					
Model 1	1.12 (1.01, 1.23)	0.49 (0.35, 0.68)	0.72 (0.48, 1.09)	0.5 (0.33, 0.75)	0.43 (0.27, 0.68)
Model 2	1.12 (1, 1.26)	0.44 (0.3, 0.65)	0.64 (0.4, 1.01)	0.49 (0.31, 0.79)	0.4 (0.23, 0.69)
Model 3	1.13 (1.01, 1.27)	0.39 (0.26, 0.6)	0.64 (0.41, 1)	0.51 (0.32, 0.83)	0.41 (0.23, 0.73)
Model 4	1.12 (1, 1.26)	0.41 (0.27, 0.64)	0.66 (0.43, 1.03)	0.54 (0.32, 0.9)	0.44 (0.25, 0.8)
Model 5	1.11 (0.98, 1.24)	0.41 (0.27, 0.64)	0.71 (0.45, 1.12)	0.55 (0.32, 0.95)	0.49 (0.27, 0.88)

[1]Model 1 = HRV
[2]Model 2 = Model 1 + Myocardial Perfusion Imaging
[3]Model 3 = Model 2 + Age + BMI + Race
[4]Model 4 = Model 3 + Cardiovascular Disease + Hypertension + Diabetes + Smoking
[5]Model 5 = Model 4 + Depression + PTSD

Every unit increased in HRV had the associated hazard ratio (95% CI) for both overall and cardiovascular mortality. HRV = heart rate variability.

A.4.2 Circadian Outcomes in Twins

Clinical Outcomes by Circadian HRV
Emory Twins Study

	MESOR	Amplitude	Phi
All Cause Mortality			
High Frequency HRV	0.64 (0.32, 1.26)	0.73 (0.29, 1.82)	1.38 (0.95, 1.99)
Low Frequency HRV	0.32 (0.16, 0.67)	0.37 (0.12, 1.15)	1.08 (0.79, 1.47)
Very Low Frequency HRV	0.36 (0.15, 0.89)	0.31 (0.05, 1.91)	1.32 (0.81, 2.16)
Acceleration Capacity	1.15 (0.98, 1.36)	0.83 (0.61, 1.13)	1.05 (0.74, 1.5)
Heart Rate	1.64 (1.19, 2.26)	0.5 (0.17, 1.43)	0.85 (0.58, 1.24)
Dyx	0.34 (0.21, 0.56)	0.42 (0.22, 0.79)	0.93 (0.67, 1.28)
Cardiovascular Death			
High Frequency HRV	0.83 (0.53, 1.32)	0.7 (0.17, 2.85)	1.13 (0.91, 1.42)
Low Frequency HRV	0.6 (0.33, 1.07)	0.66 (0.26, 1.65)	0.96 (0.76, 1.21)
Very Low Frequency HRV	0.64 (0.32, 1.3)	0.31 (0.04, 2.27)	1.05 (0.77, 1.42)
Acceleration Capacity	1.1 (0.97, 1.25)	0.8 (0.59, 1.1)	1.11 (0.88, 1.39)
Heart Rate	1.57 (1.22, 2.03)	0.34 (0.14, 0.83)	1.02 (0.76, 1.35)
Dyx	0.42 (0.28, 0.62)	0.54 (0.34, 0.85)	0.88 (0.68, 1.13)

The HRV metrics are measured over 24 hours using cosinor statistics. Every unit increase in HRV had an associated hazard ratio (95% CI) for both overall and cardiovascular mortality. HRV = heart rate variability, LF = low frequency HRV, HF = high frequency HRV, VLF = very low frequency HRV, AC = acceleration capacity, MESOR = midline estimating statistic of rhythm, Amplitude = maximum distance from MESOR, Phi = shift of acrophase

A.4.3 Outcomes in MIMS/MIPS

Outcomes Analysis for Mental Stress and HRV
Traditional and Recurrent Event Models in MIMS/MIPS

	Death	Cardiovascular Death	Marginal	PWP Total Time	PWP Gap Time	Anderson Gill
Stress Low Frequency HRV						
Model 1	0.39 (0.24, 0.64)	0.32 (0.18, 0.57)	0.52 (0.34, 0.8)	0.49 (0.29, 0.8)	0.51 (0.31, 0.81)	0.52 (0.34, 0.8)
Model 2	0.42 (0.25, 0.68)	0.32 (0.18, 0.58)	0.54 (0.35, 0.84)	0.5 (0.3, 0.85)	0.53 (0.32, 0.85)	0.54 (0.35, 0.84)
Model 3	0.37 (0.22, 0.63)	0.24 (0.12, 0.46)	0.52 (0.33, 0.82)	0.47 (0.27, 0.84)	0.5 (0.3, 0.84)	0.52 (0.33, 0.82)
Model 4	0.38 (0.21, 0.7)	0.25 (0.12, 0.52)	0.58 (0.36, 0.95)	0.48 (0.27, 0.86)	0.51 (0.3, 0.85)	0.58 (0.36, 0.95)
Model 5	0.37 (0.2, 0.7)	0.24 (0.11, 0.51)	0.51 (0.31, 0.84)	0.52 (0.28, 0.94)	0.53 (0.31, 0.92)	0.51 (0.31, 0.84)
Model 6	0.4 (0.2, 0.77)	0.25 (0.11, 0.56)	0.5 (0.3, 0.85)	0.54 (0.29, 1)	0.55 (0.31, 0.97)	0.5 (0.3, 0.85)
Stress High Frequency HRV						
Model 1	0.45 (0.26, 0.77)	0.32 (0.17, 0.61)	0.65 (0.43, 0.98)	0.51 (0.32, 0.8)	0.57 (0.38, 0.85)	0.65 (0.43, 0.98)
Model 2	0.48 (0.27, 0.83)	0.32 (0.16, 0.62)	0.68 (0.44, 1.04)	0.53 (0.33, 0.85)	0.6 (0.4, 0.9)	0.68 (0.44, 1.04)
Model 3	0.44 (0.25, 0.78)	0.28 (0.14, 0.56)	0.64 (0.42, 0.99)	0.54 (0.33, 0.87)	0.59 (0.39, 0.91)	0.64 (0.42, 0.99)
Model 4	0.5 (0.27, 0.92)	0.3 (0.14, 0.65)	0.71 (0.45, 1.14)	0.55 (0.34, 0.89)	0.61 (0.4, 0.94)	0.71 (0.45, 1.14)
Model 5	0.5 (0.26, 0.93)	0.3 (0.13, 0.66)	0.65 (0.4, 1.07)	0.57 (0.35, 0.95)	0.63 (0.41, 0.98)	0.65 (0.4, 1.07)
Model 6	0.57 (0.3, 1.12)	0.31 (0.14, 0.72)	0.66 (0.39, 1.1)	0.61 (0.37, 1.03)	0.67 (0.43, 1.05)	0.66 (0.39, 1.1)

[1]Model 1 = MSIMI ~ HRV
[2]Model 2 = Model 1 + MSIMI
[3]Model 3 = Model 2 + Age + BMI + Sex + Race
[4]Model 4 = Model 3 + Smoking + Diabetes + Hypertension + Hyperlipidemia
[5]Model 5 = Model 4 + Known Coronary/Peripheral Artery Disease
[6]Model 6 = Model 5 + Depression + Post-Traumatic Stress Disorder

This summarises the Cox proportional hazard models for both censoring events and for recurrent event analyses. Estimates = HR (95% CI). Bolded terms signify p-value < 0.05. PWP = Prentice, Williams, and Peterson models, MSIMI = Mental Stress-Induced Myocardial Ischemia, LF = Low Frequency, HF = High Frequency, HRV = Heart Rate Variability