Autonomic reactivity to mental stress is associated with cardiovascular mortality

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Aims	The mechanisms linking acute psychological stress to cardiovascular disease (CVD) mortality are incompletely understood. We studied the relationship of electrocardiographic measures of autonomic dysfunction during acute mental stress provocation and CVD death.
Methods and results	In a pooled cohort of 765 participants with stable CVD from two related studies, we collected Holter data during standardized laboratory-based mental stress testing with a speech task and followed them for events. We assessed autonomic function using low-frequency (LF) heart rate variability (HRV) in 5-min intervals before, during, and after stress induction, and specifically examined changes from rest to stress. We employed cause-specific survival models to examine its association with CVD and all-cause mortality, controlling for demographic and CVD risk factors. The mean (SD) age was 58 (10) years, 35% were women, and 44% self-identified as Black. After a median follow-up of 5.6 years, 37 (5%) died from CVD causes. A stress-induced LF HRV decrease (67% of sample), vs. increase, was associated with a hazard ratio (HR) of 3.48 (95% confidence interval—3.25, 3.73) for CVD mortality. Low rest LF HRV (bottom quartile) was also independently associated with CVD mortality, HR = 1.75 (1.58, 1.94), vs. normal rest LF HRV (upper three quartiles). The combination of stress-induced LF HRV decrease and low rest LF HRV was associated with HR = 5.73 (5.33, 6.15) vs. the normal stress/rest LF HRV reference. We found similar results with HF HRV.
Conclusion	Stress-induced LF HRV decrease and low rest LF HRV are both independently and additively associated with a higher CVD mortality risk. Additional research is needed to assess whether targeting autonomic dysfunction may improve CVD outcomes.

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Structured graphical abstract
**Key question**

In individuals with stable cardiovascular disease, is stress-induced autonomic dysfunction, as measured by a decrease in low-frequency heart rate variability (LF HRV) during stress, associated with increased cardiovascular mortality risk?

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Key finding

A decrease in LF HRV induced by mental stress was associated with a three-fold increased hazard of cardiovascular mortality after multivariable adjustment. The combination of abnormal rest (bottom quartile) and mental stress–induced decrease in LF HRV was additive, resulting in a five-fold increased hazard compared to normal rest and mental stress–induced increase in LF HRV (reference).

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Take-home message

Stress-related autonomic dysfunction, measured by reductions in LF HRV during stress, is an important contributing factor in cardiovascular disease mortality. The effects of mental stress–induced HRV change are also independent of baseline autonomic dysfunction, emphasizing the importance of stress pathways in risk stratification.
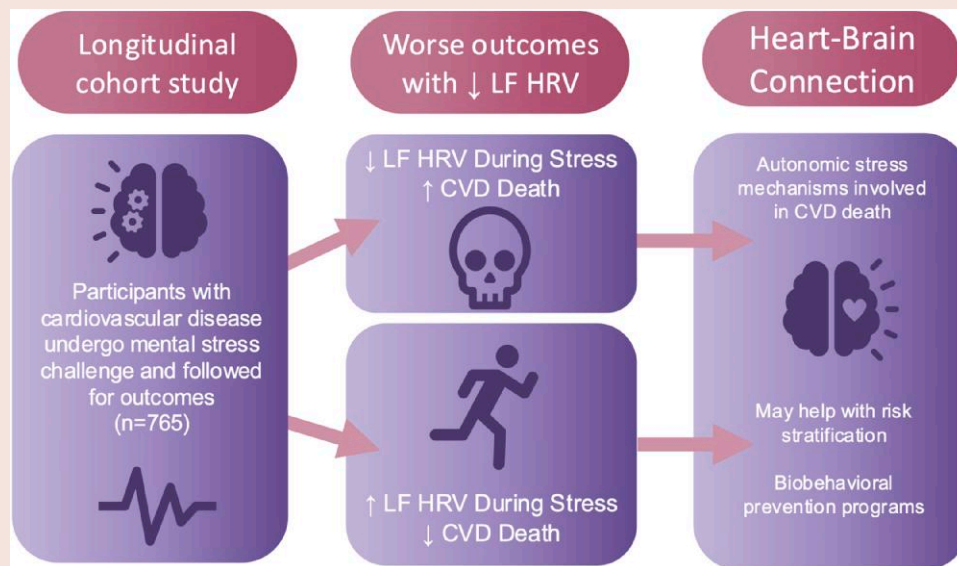
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Introduction

Cardiovascular disease (CVD) remains prevalent and risk factors for its progression to adverse outcomes are incompletely understood.[1–3] Traditional risk factors do not fully explain the risk of mortality,[1–4] and high-risk individuals, including those with coronary artery disease (CAD), are especially vulnerable to the deleterious effects of stress.[5] Neuropsychological mechanisms may play a critical role,[6] as witnessed by the several-fold increase in sudden cardiac death observed immediately *after* the 1994 Northridge earthquake.[7] The combination of a stressful trigger and pathological cardiac substrate underlies such mortality events, and evaluating the components of this paradigm may help explain this excess risk.[8] The autonomic nervous system (ANS) serves as a key mediator, with possible downstream consequences including mental stress–induced myocardial ischaemia and electrical instability that may precede CVD mortality events.[4,8]

We can study ANS changes in response to stress with heart rate variability (HRV), an ambulatory electrocardiographic (ECG) digital biomarker that measures beat-to-beat changes in heart rate over time.[9–12] Frequency-based HRV measures reflect the sympathetic and parasympathetic response to respiration, baroreflex activity, and hormonal activity,[9,13] allowing these measures to serve as surrogate markers of ANS function.

High-frequency (HF) HRV is a known measure of parasympathetic function and low-frequency (LF) HRV has been associated with baroreflex sensitivity.[9] The LF band in particular may reflect maladaptive stress responses underlying increased CVD risk,[14] with lower levels of HRV associating with ventricular tachyarrhythmias,[15] abnormal myocardial perfusion,[16] and sudden cardiac death.[17] While decreased long-term HRV is known to predict all-cause mortality,[18–20] the role of acute psychological stress in leading to cardiovascular events, as demonstrated by the earthquake study and others,[7] suggests the acute autonomic response to stress may also play a role. We know little of how stress, and the neurobiological flexibility to respond to psychological stress, contributes additive risk for CVD mortality.[21] Understanding autonomic reactivity may inform CVD risk reduction interventions aimed at mechanisms involving cardiovascular adaptation to acute psychological stress.[22]

In this study, we examined whether stress-induced changes in two ANS markers, LF HRV (primary) and HF HRV (secondary), were associated with CVD mortality in individuals with stable CVD.[23,24] We hypothesized that a stress-induced HRV decrease is associated with CVD and all-cause mortality after adjustment for baseline autonomic function, as measured by resting HRV. We also explored potential confounding and mediating effects of adjustment for sociodemographic and cardiovascular characteristics.

Methods

Enrolment

The research protocol for both cohorts was approved by the Institutional Review Board of Emory University, and all participants provided written informed consent. Individuals were enrolled between June 2011 and March 2016 as part of two similar study protocols, the Mental Stress Ischemia Prognosis Study (MIPS)[23] and the Myocardial Infarction and Mental Stress Study 2 (MIMS2).[24] Both studies recruited individuals with stable CVD from hospitals and clinics affiliated with Emory University. Ambulatory electrocardiographic (ECG) recordings were added in an ancillary study of both parent studies 2 years after the initial enrolment began. For MIPS, individuals were enrolled if they were between the ages of 30 and 79 years with a documented history of CAD.[23] For MIMS2, individuals were enrolled if they were between the ages of 18 and 60 years and had been hospitalized with a myocardial infarction (MI) within the prior 8 months. Individuals were excluded if they were pregnant or had a poor prognosis due to medical co-morbidities. Additionally, individuals were excluded if they were enrolled prior to the ancillary study with ambulatory ECG recordings, if they had inadequate ambulatory ECG recordings, or were not able to complete myocardial perfusion imaging.

Mental stress provocation

Participants underwent a mental stress provocation test after a 12-h fast. They were also asked to withhold beta-blocker and nitrate medications the morning of their research visit. Mental stress was evoked using a public speaking task,[23] with continuous ECG recorded throughout the test. Although mental stress responses to speech stressors may vary, we did not evaluate differences in perceived stress responses to the mental stress challenge, as previous analyses found no difference between high- and low-risk individuals. A speech-based mental stressor was chosen as it is often superior to other forms of mental stress provocation, as it includes both cognitive and social stress components, and is readily reproducible.[25]

Three technetium Tc 99 m sestamibi single-photon emission computed tomography (SPECT) scans (Philips Cardio MD) were performed, at rest, during mental stress, and during conventional stress. Conventional stress testing included either a standard Bruce protocol or pharmacological stress test with regadenoson. Testing was performed on two separate days 1 week apart. Single-photon emission computed tomography scans were interpreted by two experienced readers blinded to the stress type, with ischaemia defined as a summed difference score of four or more for conventional stress.[26]

Heart rate variability

Each participant wore an ambulatory electrocardiographic (Holter) monitor (GE Marquette SEER digital system; GE Medical Systems, Waukesha, Wisconsin) during 15-min baseline rest, 5-min mental stress, and 15-min recovery periods while in a seated position. Recordings were stored digitally at 128 Hz and edited for noise and arrhythmia detection prior to automated detection for normal and aberrant QRS complexes in GE MARS 8.0.2 software. Variations in heart rate can be assessed by a number of mathematical measures, usually divided into time and frequency domains,[11] of which we chose the frequency domain for analysis due to its physiological relevance.[9] Heart rate variability was measured using the MARS commercial algorithm by extracting the intervals between sinus beats. The interval data were segmented into 5-min windows using power spectral density through fast Fourier transform and divided into two discrete frequency bands: LF 0.05 to <0.15 Hz and HF 0.15 to <0.40 Hz. Heart rate variability in the frequency domain is intrinsically dependent on the recording duration, and thus, only similar recording windows can be compared. These frequency bands integrate heart rate fluctuations in response to physiological stimuli, including influences of baroreceptor activity (LF) and respiration (HF).[9] We chose LF HRV as the independent variable of primary interest as it reflects increased CVD death risk in other studies,[27] is influenced by both cardiac sympathetic and parasympathetic activities, and associates with baroreflex sensitivity,[9] which in turn also predicts increased adverse cardiovascular events.[28–30] We chose HF HRV as a secondary independent variable due to the body of evidence supporting it as a more pure cardiac parasympathetic measure.[11,31–34]

We chose to examine both rest and stress HRVs because the context of HRV measurement is critical in its relationship with pathology, such as differences in circadian rhythm and time-of-day.[16,35] Resting and post-stress HRVs are rarely accounted for together in traditional evaluations of HRV with CVD mortality.[18] Rest HRV was defined as an average value of the 5-min window during the baseline rest period that ended 5 min before speech, when pre-stress anxiety may occur. Peak stress HRV was defined as the average value during the 5-min mental stress challenge, during which time the participant prepared for the speech (2 min) and delivered it over the next 3 min. At lower respiratory rates, respiratory-related efferent vagally mediated influences are prominent in the LF band and can be affected in speech-based stress tasks;[31,36] however, this effect would be expected uniformly among participants.

Other measures

Sociodemographic and past medical history were obtained through standardized questionnaires, clinical interview, and chart review. Depression and post-traumatic stress disorder (PTSD) were assessed via structured clinical interviews from the Fourth Diagnostic and Statistical Manual of Mental Health Disorders.[37] Race and ethnicity were self-reported by participants. Because only few participants were of race other than non-Hispanic Black or non-Hispanic White, only two categories were used in the models (non-Hispanic Black vs. all others). Height and weight were used to calculate body mass index (BMI). Angiographic data were obtained from the most recent coronary angiogram in the participants's chart. The left ventricular ejection fraction was obtained at the time of SPECT. Heart failure (with reduced ejection fraction) was defined as left ventricular ejection fraction < 40% and a previous diagnosis of clinical heart failure. Follow-up data were collected through participant contacts, medical records, and the social security death index up until the last follow-up in February 2020. Mortality events and incident MI events were adjudicated by a committee of study cardiologists who were blinded to HRV values. Our definition of cardiovascular mortality was adapted from the Multi-Ethnic Study of Atherosclerosis study and defined as a mortality event secondary to either MI, arrhythmia, heart failure, or major stroke.[38] Cardiovascular mortality was considered the primary outcome because of previous studies showing that HRV is a more important prognostic marker for fatal arrhythmias, rather than non-fatal atherosclerotic events.[39] As HRV has been shown to predict total deaths, all-cause mortality was a secondary endpoint.[27]

Statistical analysis

We explored both the potential compatibility between cohorts by comparing the key characteristics and their distributions, as well as subgroup differences by mental stress-induced LF HRV change status. Our comparison included demographic, clinical, and psychosocial factors as well as haemodynamic responses to mental stress. For skewed continuous variables, we presented the median values and inter-quartile range. Otherwise, we examined mean values and standard deviations. For categorical variables, we presented the number and proportion. Statistical testing was not performed for group differences as not an *a priori* component of our hypothesis,[40] in line with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[41] Because of the low incidence of CVD deaths, we focused our analysis on the combined cohort after ensuring that they were compatible in our baseline evaluation. We also examined for statistical interaction and clustering by cohort using mixed effect models.

We focused on LF HRV as our primary measure of interest, and HF HRV as our secondary measure of interest. Both measures are commonly evaluated on Holter ECG data, and their physiological significance is generally better understood than time domain and non-linear HRV measures. Heart rate variability measures were natural log-transformed to achieve a Gaussian distribution prior to analysis to facilitate interpretation of the estimates. We defined stress-induced HRV change as the difference in HRV from rest to peak stress. We dichotomized the change as either an increase or decrease of HRV with stress, similar to previous studies.[42] We defined abnormal (low) rest HRV as the bottom quartile and normal (high) rest HRV as the upper three quartiles.[27] We also examined the additive effects of rest and stress HRV by creating four categories: (i) normal rest HRV and mental stress–induced HRV increase, (ii) low rest HRV and mental stress–induced HRV increase, (iii) normal rest HRV and mental stress–induced

Table 1 Cohort characteristics

	Patients with mental stress–induced LF HRV decrease					
	MIPS		MIMS2		Pooled	
	Yes (*n* = 286)[a]	No (*n* = 165)[a]	Yes (*n* = 217)	No (*n* = 85)	Yes (*n* = 503)[a]	No (*n* = 250)[a]
Demographic factors						
Age (years)	65 (57, 71)	64 (55, 69)	52 (47, 56)	52 (47, 56)	57 (52, 66)	57 (51, 67)
Sex						
Men	208 (73%)	127 (77%)	116 (53%)	38 (45%)	324 (64%)	165 (66%)
Women	78 (27%)	38 (23%)	101 (47%)	47 (55%)	179 (36%)	85 (34%)
Race[b]						
Asian or Pacific Islander	13 (4.5%)	4 (2.4%)	12 (5.5%)	5 (5.9%)	25 (5.0%)	9 (3.6%)
Black or African American	85 (30%)	49 (30%)	139 (64%)	57 (67%)	224 (45%)	106 (42%)
White	188 (66%)	112 (68%)	66 (30%)	23 (27%)	254 (50%)	135 (54%)
Cardiovascular risk factors						
Hypertension	236 (83%)	119 (72%)	181 (83%)	65 (76%)	417 (83%)	184 (74%)
Hyperlipidaemia	252 (88%)	131 (79%)	178 (82%)	65 (76%)	430 (85%)	196 (78%)
Diabetes	108 (38%)	39 (24%)	78 (36%)	16 (19%)	186 (37%)	55 (22%)
Smoking status						
Current	38 (13%)	17 (10%)	52 (24%)	19 (22%)	90 (18%)	36 (14%)
Former	126 (44%)	90 (55%)	75 (35%)	21 (25%)	201 (40%)	111 (45%)
Never	121 (42%)	57 (35%)	90 (41%)	45 (53%)	211 (42%)	102 (41%)
BMI (kg/m^2)	29.4 (25.8, 32.0)	29.3 (25.8, 32.9)	31 (27, 36)	29 (26, 34)	30 (26, 34)	29 (26, 33)
Clinical characteristics						
≥1 Coronary vessel with 70% stenosis[c]	210 (86%)	115 (82%)	165 (83%)	72 (87%)	375 (85%)	187 (84%)
Gensini score[c]	27 (10, 65)	21 (8, 53)	36 (12, 64)	20 (8, 42)	32 (11, 64)	20 (8, 48)
Myocardial infarction	82 (29%)	59 (36%)	217 (100%)	85 (100%)	299 (59%)	144 (58%)
LVEF (%)[d]	69 (61, 77)	72 (64, 77)	53 (43, 58)	55 (48, 60)	60 (50, 71)	65 (55, 74)
Heart failure[e]	28 (9.8%)	15 (9.3%)	62 (29%)	18 (21%)	90 (18%)	33 (13%)
Conventional stress–induced myocardial ischaemia[f]	93 (34%)	46 (29%)	56 (27%)	20 (24%)	149 (31%)	66 (27%)
Selected medications						
Aspirin	238 (84%)	143 (87%)	172 (80%)	72 (86%)	410 (82%)	215 (86%)
Statins	249 (87%)	141 (85%)	174 (81%)	80 (95%)	423 (84%)	221 (89%)
Beta-blockers	215 (75%)	120 (73%)	180 (83%)	75 (89%)	395 (79%)	195 (78%)
Antidepressants	73 (26%)	39 (24%)	38 (18%)	13 (15%)	111 (22%)	52 (21%)
Mental stress testing[g]						
Heart rate, change (beats/minute)[h]	15 (10, 22)	15 (9, 20)	21 (13, 29)	18 (13, 25)	17 (11, 26)	16 (10, 22)
Systolic blood pressure, change (mmHg)[h]	40 (29, 55)	38 (29, 49)	42 (30, 50)	40 (28, 51)	40 (29, 52)	39 (29, 49)
Diastolic blood pressure, change (mmHg)[h]	22 (16, 31)	23 (18, 29)	27 (20, 36)	27 (21, 35)	24 (18, 33)	25 (19, 32)
RPP, change (per 1000 units)[h]	5.09 (3.60, 6.85)	4.73 (3.30, 5.92)	5.67 (4.25, 8.01)	5.29 (4.02, 6.89)	5.32 (3.92, 7.40)	4.82 (3.57, 6.22)
Rest LF HRV (ln ms^2)[i]	5.63 (4.59, 6.63)	5.32 (4.59, 5.96)	6.29 (5.27, 7.05)	5.66 (5.23, 6.27)	5.87 (4.88, 6.78)	5.53 (4.81, 6.08)
Stress LF HRV (ln ms^2)[i]	5.04 (4.32, 5.82)	6.10 (5.49, 6.94)	5.45 (4.44, 6.18)	6.49 (6.02, 7.20)	5.22 (4.36, 6.02)	6.27 (5.70, 7.01)
Stress-induced LF HRV changes (ln ms^2)[j]	−0.43 (−0.95, −0.04)	0.75 (0.41, 1.34)	−0.55 (−1.06, −0.19)	0.67 (0.32, 1.21)	−0.50 (−1.01, −0.09)	0.74 (0.39, 1.27)
Psychological factors						
Beck Depression Inventory II[k]	6 (2, 11)	6 (2, 10)	9 (4, 19)	8 (5, 17)	7 (3, 14)	7 (3, 13)
Post-Traumatic Stress Disorder Score[l]	22 (18, 31)	22 (19, 29)	27 (22, 39)	25 (21, 40)	24 (19, 34)	23 (20, 32)

Continued

Table 1 *Continued*

	Patients with mental stress–induced LF HRV decrease					
	MIPS		MIMS2		Pooled	
	Yes (n = 286)[a]	No (n = 165)[a]	Yes (n = 217)	No (n = 85)	Yes (n = 503)[a]	No (n = 250)[a]
Clinical outcomes						
Follow-up time (years)	6.30 (5.50, 6.60)	6.20 (5.60, 6.60)	4.50 (3.40, 5.30)	5.00 (4.30, 5.60)	5.50 (4.10, 6.40)	5.80 (5.00, 6.40)
Cardiovascular mortality	18 (6.3%)	3 (1.8%)	14 (6.5%)	2 (2.4%)	32 (6.4%)	5 (2.0%)
All-cause mortality	35 (12%)	9 (5.5%)	19 (8.8%)	2 (2.4%)	54 (11%)	11 (4.4%)
	18 (6.3%)	14 (8.5%)	22 (10%)	13 (15%)	40 (8.0%)	27 (11%)

HRV, heart rate variability; HF, high frequency; LF, low frequency; LVEF, left ventricular ejection fraction; MIMS2, Myocardial Infarction and Mental Stress 2; MIPS, Mental Stress Ischemia Prognosis Study; PTSD, post-traumatic stress disorder; RPP, rate pressure product.

[a]Median (IQR); *n* (%).

[b]Race was self-reported using predetermined categories.

[c]Coronary angiography, if performed, occurred during initial MI and was missing for ~10% of participants. The Gensini score measures the burden of disease by including the number, location, and degree of stenosis of each lesion.

[d]In MIPS, LVEF was derived from myocardial perfusion imaging, while in MIMS2 it was derived from echocardiography, ventricular angiogram, or myocardial perfusion imaging (based on availability) at time of enrolment of initial MI.

[e]Defined as previous diagnosis of heart failure with LVEF ≤ 40%.

[f]Participants underwent standard Bruce protocol if able to tolerate, otherwise switched to pharmacological stress according to standard, pre-specified protocol.

[g]Mental stress testing was performed after a period of rest using a public speaking task. Measurements were made continuously during the rest and stress periods.

[h]Difference between the maximum value during mental stress (the 3 min of the speaking task) and minimum values during rest.

[i]HRV was generated through power spectral analysis of RR intervals obtained through cardiac telemetry. Values represent the average during the rest and stress period, respectively.

[j]HRV values at rest were subtracted from HRV at stress to calculate the amount of stress-induced HRV change.

[k]Measures depressive symptoms on a continuous scale using 21 questions, each scored from 0 to 3 (maximum score of 63), with higher scores representing more depressive symptoms. A score ≥ 14 suggests at least mild depression.

[l]Measures the burden of PTSD symptoms and serves as a screening tool for the diagnosis, composed of 20 questions scored from 0 to 4 (maximum score of 80). A score ≥ 31 suggests probably PTSD.

HRV decrease, and (iv) low rest HRV and mental stress–induced HRV decrease. We also examined LF and HF HRV as continuous variables, which generally provide more statistical power but also assume a linear dose–response relationship.

Our primary outcome variable was CVD mortality. We used Cox proportional hazard models to examine the relationship between HRV and CVD mortality and include frailty terms to account for the clustering by original cohort. We focused on cause-specific,[43] rather than competing risk analysis,[44] since we sought to examine an aetiologic question of whether mental stress–induced HRV change influences the risk of CVD mortality. Although high-risk individuals may be at risk of non-CVD mortality as well, our goal was to obtain estimates of the risk of stress autonomic dysfunction as a predictor of CVD mortality occurring as a first event. We examined the relationships with all-cause mortality as a secondary outcome. We did not perform a competing risk analysis, as previous studies have suggested that cause-specific models are more appropriate for mechanistic research.[43] We did not examine non-fatal atherosclerotic outcomes, such as incident MI,[39] given the strength of research supporting autonomic dysfunction as a risk factor for ventricular tachyarrhythmias is significantly stronger than for atherosclerotic progression.[45–48]

We evaluated serial models starting with the unadjusted relationship of HRV with CVD mortality (model 1). We then adjusted for age, sex, and race (model 2). In model 3, we adjusted for cardiovascular clinical factors including hypertension, hyperlipidaemia, smoking, diabetes, and heart failure, as well as psychosocial variables and medications. Medications included a history of anti-hypertensive and beta-blocker usage. We then adjusted for myocardial ischaemia based on SPECT with exercise or vasodilator stress (model 4). We then restricted the terms to only significant variables or terms that influenced the primary independent variable by >10% to reduce the ratio of outcomes to predictors to less than five for each CVD mortality event. Models with too many predictors may bias the estimates upwards,[49] and in our analysis, most predictors were balanced equally between high- and low-risk groups. The disclosure of the full and parsimonious models is reported in the supplement. Similar analyses were conducted for the secondary independent variable of HF HRV and secondary outcome of

all-cause mortality. In all analyses, cohort status was adjusted for using mixed effect models.

We explored multiple imputation (iterations = 20) to assess the impact of missingness in a sensitivity analysis. We performed model diagnostics by inspecting Schoenfeld residuals and by testing time interaction terms to examine the proportional hazard assumption, which were met. Martingale residuals were used to assess non-linearity of continuous terms, which were found to be acceptable. Goodness-of-fit testing was performed on the adjusted models using their Martingale residuals. Internal model validation and calibration were assessed using bootstrapping methods. To assess strength of association, net reclassification and concordance testing was performed. Net reclassification improvement was assessed using additional comparative models to assess impact of HRV.[50,51] Model discrimination was calculated with C-statistics, with confidence intervals built using the infinitesimal jackknife variance. We performed all analyses in R (version 4.2.1).[52]

Results

Cohort description

In the combined cohort pool of 765 participants with available ECG data, 452 were from the MIPS cohort, and 313 were from the MIMS2 cohort. Among these, 12 participants were excluded as HRV data could not be generated due to poor signal quality or >20% non-sinus beats. Therefore, the analytical sample included 753 participants. The mean (standard deviation, SD) age was 58.2 (10.2) years, 35% were women, and 44% were Black. Participants in MIMS2 were younger and more often women than in MIPS. Other demographic and clinical data were similar (*Table 1*). The overall mean (SD) of ln(LF) HRV decreased from rest, 5.7 (2), to stress, 5.6 (1.9); however, this was not significantly different (*P* = 0.28). In addition, 503 (67%) participants had a mental stress–induced LF HRV decrease. The prevalence of CVD risk factors
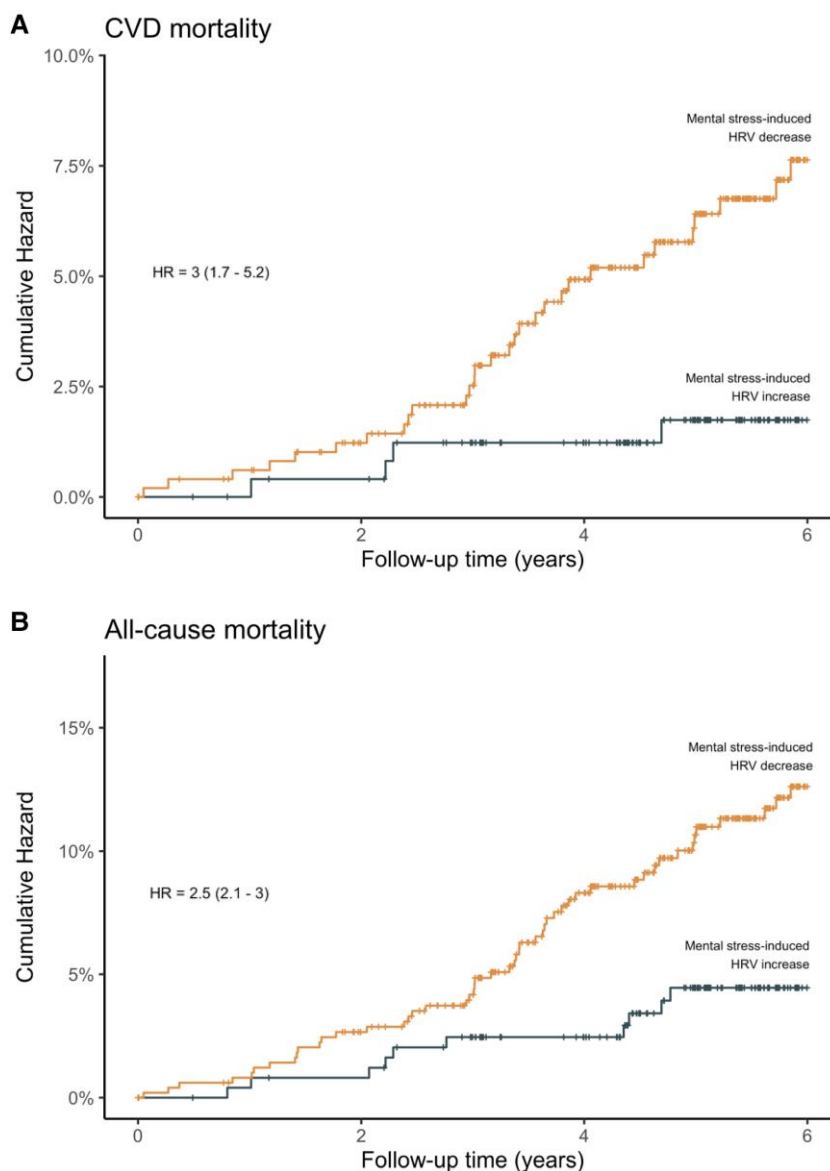
Figure 1 Cumulative incidence of the study endpoints in the pooled cohort by mental stress–induced low-frequency heart rate variability change. The cumulative incidence per outcome type is shown, with hazard ratio (95% robust confidence intervals) for fully adjusted models. The median observation period was 5.6 (IQR, 3.6-7.6). (*A*) There were 37 cardiovascular disease events for participants with mental stress-induced heart rate variability decrease. (*B*) There were 65 all-cause mortality events for participants with mental stress-induced heart rate variability increase. CVD, cardiovascular disease; HR, hazard ratio; HRV, heart rate variability.

was slightly higher in the group with mental stress–induced LF HRV decrease compared with than LF HRV increase, by 10–20% for most comorbidities. The group-wise differences were similar when substituting HF HRV for LF HRV (see Supplementary material online, *Table S1*). Other stress-related metrics, such as changes in heart rate and blood pressure, were similar between those with mental stress-induced HRV decrease compared with HRV increase.

Association of mental stress-induced HRV change with adverse cardiovascular events

During a median (IQR) follow-up time of 5.6 (4.4, 6.4) years, we recorded 37 (4.8%) CVD mortality events and 65 (8.5%) all-cause

mortality events. Cumulative incidence curves that divided the cohort based on mental stress-induced HRV change were drawn for both endpoints. See *Figure 1* for LF HRV and Supplementary material online, *Figure S1* for HF HRV.

A mental stress-induced LF HRV decrease was associated with an increased risk of CVD and all-cause mortality; HR of 3.44 (95% CI, 3.21, 3.68) and 2.64 (1.93, 3.60), respectively (*Table 2*). Estimates did not change substantially in models adjusting for sociodemographic factors (model 2), cardiovascular risk factors (model 3), or conventional stress-induced myocardial ischaemia (model 4). The effect of cohort status was not significant. We compared the original, full model with the parsimonious, restricted-term model (disclosure of all parameter estimates shown in Supplementary material online, *Table S2*). The

Table 2 Association between mental stress–induced LF HRV change and rest LF HRV and the study endpoints in the pooled cohort

	Mental stress–induced LF HRV decrease				Low rest HRV			
	Yes (n = 498)	No (n = 250)	Rate difference per 100 person-years (95% CI)	HR (95% CI)[a]	Yes (n = 189)	No (n = 559)	Rate difference per 100 person-years (95% CI)	HR (95% CI)[b]
Cardiovascular mortality[c]								
Total no. of events	32	5	–	–	14	23	–	–
Rate per 100 person-years	1.25	0.36	0.88 (0.36, 1.41)	–	1.37	0.79	0.58 (−0.19, 1.35)	–
Model 1	–	–	–	3.48 (3.25, 3.73)	–	–	–	1.75 (1.58, 1.94)
Model 2 = Model 1 + demographic factors[d]	–	–	–	3.54 (3.04, 4.12)	–	–	–	2.14 (1.69, 2.72)
Model 3 = Model 2 + cardiovascular risk factors[e]	–	–	–	2.92 (2.03, 4.21)	–	–	–	1.64 (1.25, 2.17)
Model 4 = Model 3 + stress testing[f]	–	–	–	3.02 (1.73, 5.25)	–	–	–	1.8 (1.46, 2.22)
All-cause mortality[c]								
Total no. of events	54	11	–	–	22	43	–	–
Rate per 100 person-years	2.1	0.8	1.3 (0.58, 2.02)	–	2.15	1.47	0.68 (−0.3, 1.66)	–
Model 1	–	–	–	2.66 (1.93, 3.67)	–	–	–	1.47 (0.96, 2.25)
Model 2 = Model 1 + demographic factors[d]	–	–	–	2.68 (1.92, 3.73)	–	–	–	1.43 (0.85, 2.41)
Model 3 = Model 2 + cardiovascular risk factors[e]	–	–	–	2.67 (2.17, 3.27)	–	–	–	1.34 (0.81, 2.2)
Model 4 = Model 3 + stress testing[f]	–	–	–	2.51 (2.08, 3.02)	–	–	–	1.38 (0.8, 2.4)

LF, low frequency; HRV, heart rate variability; HR, hazard ratio.

[a]The HR compares the incidence of outcome events between participants based on those who had mental stress–induced HRV decrease vs. those with mental stress–induced HRV increase (reference). These models adjusted for low rest HRV. Robust confidence intervals are reported.
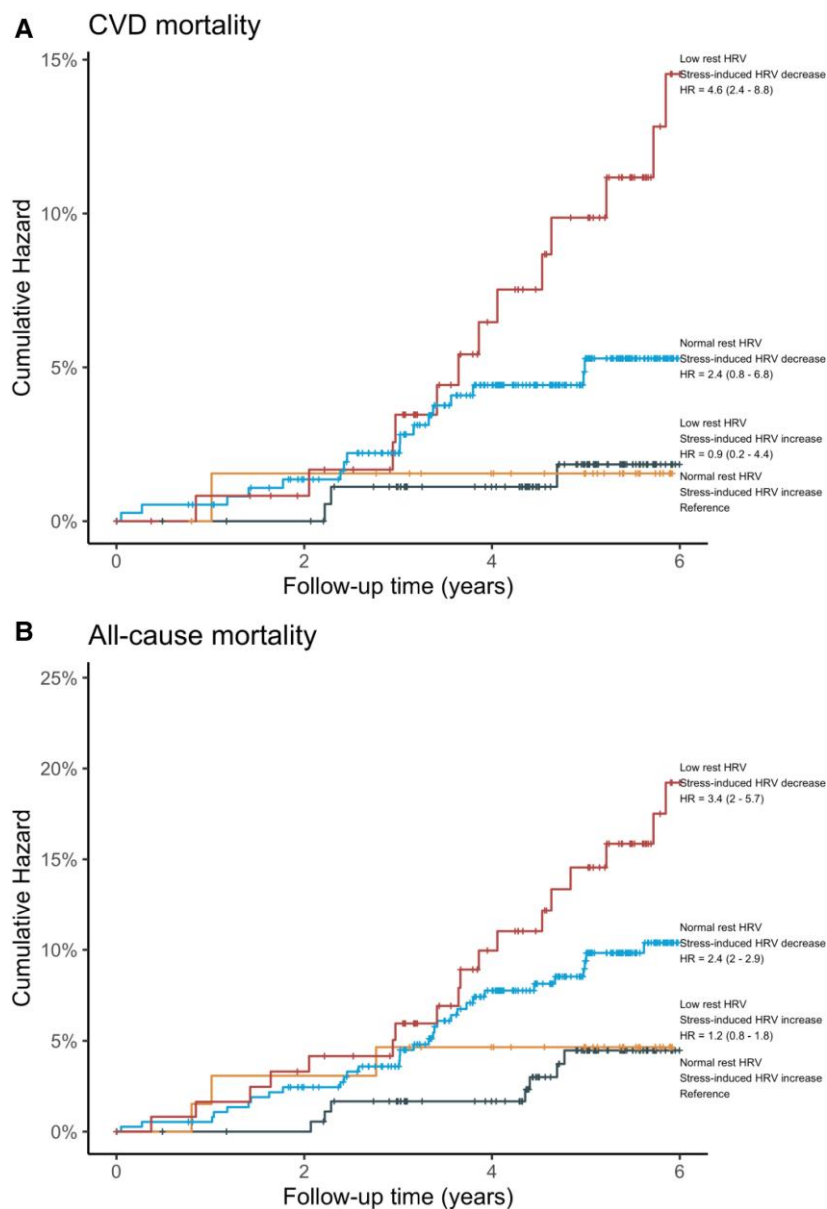
[b]The HR compares the incidence of outcome events between participants who had low rest HRV, measured as the bottom quartile, and those with normal rest HRV, defined as those in the upper three quartiles. These models adjusted for mental stress–induced HRV decrease. Robust confidence intervals are reported.

[c]The median observation period was 5.6 (IQR, 3.6–7.6).

[d]Age, sex, and race (Black v. non-Black participants).

[e]Body mass index, smoking status, history of hypertension, history of diabetes, history of dyslipidaemia, previous myocardial infarction (MIPS) or ischaemia (MIMS2) with ST changes, and heart failure were originally included. Variables that did not significantly change the effect size were removed, and only hyperlipidaemia and heart failure were retained.

[f]Abnormal myocardial perfusion by conventional stress testing was defined as > 4 difference between the summed rest and stress scores.

Figure 2 Cumulative incidence of the study endpoints in the pooled cohort by mental stress–induced low-frequency heart rate variability change and rest low-frequency heart rate variability. The cumulative incidence per outcome type is shown, with hazard ratio (95% robust confidence intervals) for fully adjusted models. The median observation period was 5.6 (IQR, 3.6–7.6). The reference group was defined by those with normal heart rate variability and mental stress-induced HRV increase. (*A*) There were 37 cardiovascular disease events for participants with mental stress-induced heart rate variability decrease. (*B*) There were 65 all-cause mortality events for participants with mental stress-induced heart rate variability increase. CVD, cardiovascular disease; HR, hazard ratio; HRV, heart rate variability.

estimates were similar and did not attenuate with adjustment, and the association with CVD mortality for mental stress–induced LF HRV decrease compared to increase remained significant. Low rest LF HRV (bottom quartile of resting distribution) was associated with a HR of 1.75 (1.58, 1.94) for CVD mortality and 1.47 (0.96, 2.25) for all-cause mortality. Fully adjusted and parsimonious models were similar (see Supplementary material online, *Table S2*). Imputed models showed similar results (not shown).

We then evaluated the additive risk of mental stress–induced LF HRV change and rest LF HRV by combining them into a four-level

variable as described in the Methods. We present cumulative hazard curves describing the relationship for CVD and all-cause mortality by HRV response types in *Figure 2*. In the highest risk group (low rest LF HRV and mental stress–induced LF HRV decrease), as compared to the lowest risk group (normal rest LF HRV and mental stress–induced LF HRV increase), the risk of CVD mortality was 4.63 (2.44, 8.76) in adjusted models (*Table 3*). We show similar results for HF HRV in Supplementary material online, *Figure S2*.

When analysing stress and rest LF HRV as continuous variables together in fully adjusted models, each unit decrease in mental stress–

Table 3 Association of combined rest and stress categories of low-frequency heart rate variability with study endpoints in the pooled cohort

| | Normal rest & stress-induced increase[a] | LF HRV response category v. reference | | | | | |
| | | Low rest & stress-induced increase | | Normal rest & stress-induced decrease | | Low rest & stress-induced decrease | |
	Reference, n = 184	n = 66	HR (95% CI)[b]	n = 375	HR (95% CI)[b]	n = 123	HR (95% CI)[b]
Cardiovascular mortality[c]							
Total no. of events	4	1	–	19	–	13	–
Rate per 100 person-years	0.4	0.27	–	0.99	–	2.01	–
Model 1	–	–	0.79 (0.35, 1.81)	–	2.57 (1.92, 3.45)	–	5.73 (5.33, 6.15)
Model 2 = Model 1 + demographic factors[d]	–	–	0.78 (0.28, 2.16)	–	2.49 (1.65, 3.77)	–	6.1 (5.79, 6.42)
Model 3 = Model 2 + cardiovascular risk factors[e]	–	–	0.7 (0.24, 2.08)	–	2.21 (1.12, 4.34)	–	4.06 (3.12, 5.29)
Model 4 = Model 3 + stress testing[f]	–	–	0.93 (0.2, 4.38)	–	2.36 (0.82, 6.79)	–	4.63 (2.44, 8.76)
All-cause mortality[c]							
Total no. of events	8	3	–	35	–	19	–
Rate per 100 person-years	0.8	0.8	–	1.82	–	2.93	–
Model 1	–	–	0.95 (0.83, 1.09)	–	2.27 (1.97, 2.62)	–	3.52 (1.71, 7.23)
Model 2 = Model 1 + demographic factors[d]	–	–	0.92 (0.79, 1.07)	–	2.25 (1.99, 2.55)	–	3.52 (1.71, 7.26)
Model 3 = Model 2 + cardiovascular risk factors[e]	–	–	1.03 (0.77, 1.39)	–	2.41 (2.24, 2.59)	–	3.38 (1.97, 5.8)
Model 4 = Model 3 + stress testing[f]	–	–	1.22 (0.82, 1.82)	–	2.38 (1.97, 2.88)	–	3.37 (1.98, 5.74)

HRV, heart rate variability; LF, low frequency; HR, hazard ratio.

[a]Reference category for comparison, defined as normal rest HRV (upper three quartiles) and mental stress–induced HRV increase (increase in HRV from rest to stress).

[b]The HR compares the incidence of outcome events between participants with the labelled category and those with normal rest HRV and mental stress-induced HRV increase (reference). Robust confidence intervals are reported.

[c]The median observation period was 5.6 (IQR, 3.6–7.6).

[d]Age, sex, and race (Black v. non-Black participants).

[e]Body mass index, smoking status, history of hypertension, history of diabetes, history of dyslipidaemia, previous myocardial infarction (MIPS) or ischemia (MIMS2) with ST changes, and heart failure were originally included. Variables that did not significantly change the effect size were removed, and only hyperlipidaemia and heart failure were retained.

[f]Abnormal myocardial perfusion by conventional stress testing was defined as greater than or equal to four difference between the summed rest and stress scores.

induced LF HRV change was associated with a HR of 1.10 (95% CI 1.00, 1.20) for CVD mortality and 1.07 (1.00, 1.14) for all-cause mortality. The rest HRV measures were not significantly associated with either mortality metric.

For HF HRV, the results were similar to those of LF HRV for both dichotomous (*Table 4*) and combined categorical analyses (*Table 5*), although the estimates for CVD mortality and all-cause mortality were of smaller magnitude as compared to LF HRV.

Discrimination and reclassification analyses

The expected and observed event rates were similar in goodness-of-fit and model calibration testing. We also examined the additive effects of both rest HRV and mental stress–induced LF HRV change on Harrell's C-index for predicting mortality over traditional risk factors and found that they improved the C-statistic from 0.77 (0.67, 0.87) to 0.79 (0.69, 0.90) for CVD mortality (see Supplementary material online, *Table S3*). For HF HRV, improvements in C-statistic were similar. Compared to baseline models with traditional risk factors, adding low rest HRV and mental stress–induced HRV decrease showed a 30.5% (95% CI 4.7–44.5%, *P* = 0.013) improvement in continuous net reclassification for prediction of CVD mortality. In stratified analyses by age, sex, race, hypertension, diabetes, obstructive CAD, LV function, myocardial ischaemia, and study cohort, we found no significant interactions and similar effect sizes across subgroups.

Discussion

In this prospective cohort of adult individuals with stable CVD, we found robust associations between mental stress–induced HRV decrease and increased CVD mortality risk with both LF and HF HRV, suggesting that stress-related autonomic mechanisms play an important prognostic role in this large and growing high-risk population. The effect size was large (HR ∼ 3.5) despite multivariable adjustment for confounders, rest HRV, and cohort status, which is both novel and meaningful. The highest risk group with low rest LF HRV and mental stress–induced LF HRV decrease combined had the highest hazard (∼ 5 vs. controls), which was similar in magnitude to heart failure observed in our fully adjusted multivariable model (see Supplementary material online, *Table S2*). In contrast, the event rates were extremely low in the group with normal rest HRV and mental stress–induced HRV increase (0.27 events per 100 person-years, *Table 4*). This low event rate in individuals with preserved autonomic stress reactivity is especially impressive considering that in most clinical contexts, a history of CVD will, by itself, rank most individuals as high risk regardless of their other risk factors. As such, our findings suggest stress-related autonomic physiology is important when evaluating both fatal CVD risk and resilience.

The strengths of these findings are supported by previous research involving autonomic dysfunction as upstream to the development of cardiac electrophysiological instability and fatal ventricular arrhythmias.[53] Previous studies have suggested stress may acutely increase repolarization heterogeneity as measured by microvolt T-wave alternans,

Table 4 Association between mental stress–induced HF HRV change and rest HF HRV and the study endpoints in the pooled cohort

	Mental stress–induced HF HRV decrease				Low rest HRV			
	Yes (n = 644)	No (n = 104)	Rate difference per 100 person-years (95% CI)	HR (95% CI)	Yes (n = 188)	No (n = 560)	Rate difference per 100 person-years (95% CI)	HR (95% CI)
Cardiovascular mortality[a]								
Total no. of events	35	2	–	–	17	20	–	–
Rate per 100 person-years	1.04	0.35	0.69 (0.1, 1.27)	–	1.7	0.68	1.01 (0.17, 1.86)	–
Model 1	–	–	–	2.79 (0.77, 10.19)	–	–	–	2.41 (2.35, 2.48)
Model 2 = Model 1 + demographic factors[b]	–	–	–	3.09 (0.81, 11.86)	–	–	–	2.62 (2.03, 3.39)
Model 3 = Model 2 + cardiovascular risk factors[c]	–	–	–	2.94 (1.12, 7.66)	–	–	–	1.98 (1.75, 2.24)
Model 4 = Model 3 + stress testing[d]	–	–	–	2.63 (1.13, 6.12)	–	–	–	1.87 (1.71, 2.04)
All-cause mortality[a]								
Total no. of events	60	5	–	–	27	38	–	–
Rate per 100 person-years	1.78	0.88	0.9 (0.03, 1.77)	–	2.69	1.29	1.4 (0.33, 2.47)	–
Model 1	–	–	–	1.95 (1.38, 2.77)	–	–	–	2.03 (1.25, 3.31)
Model 2 = Model 1 + demographic factors[b]	–	–	–	2.1 (1.47, 3.01)	–	–	–	2.06 (1.15, 3.69)
Model 3 = Model 2 + cardiovascular risk factors[c]	–	–	–	2 (1.5, 2.67)	–	–	–	1.85 (1.14, 2.99)
Model 4 = Model 3 + stress testing[d]	–	–	–	2.23 (2.18, 2.28)	–	–	–	1.77 (1.02, 3.07)

HF, high frequency; HRV, heart rate variability; HR, hazard ratio.

[a]The median observation period was 5.6 (IQR, 3.6–7.6).

[b]Age, sex, and race (Black v. non-Black participants).

[c]Body mass index, smoking status, history of hypertension, history of diabetes, history of dyslipidaemia, previous myocardial infarction (MIPS) or ischaemia (MIMS2) with ST changes, and heart failure were originally included. Variables that did not significantly change the effect size were removed, and only hyperlipidaemia and heart failure were retained.

[d]Abnormal myocardial perfusion by conventional stress testing was defined as > 4 difference between the summed rest and stress scores.

Table 5 Association of combined rest and stress categories of high-frequency heartrate variability with study endpoints in the pooled cohort

	Normal rest & stress-induced increase	Low rest & stress-induced increase		Normal rest & stress-induced decrease		Low rest & stress-induced decrease	
			HF HRV response category v. reference				
	Reference, n = 83	n = 21	HR (95% CI)[a]	n = 477	HR (95% CI)[a]	n = 167	HR (95% CI)[a]
Cardiovascular mortality[b]							
Total no. of events	1	1	–	19	–	16	–
Rate per 100 person-years	0.22	0.84	–	0.76	–	1.81	–
Model 1	–	–	4.13 (3.12, 5.46)	–	3.42 (0.89, 13.18)	–	8.85 (1.88, 41.6)
Model 2 = Model 1 + demographic factors[c]	–	–	4.13 (3.97, 4.29)	–	3.83 (1.12, 13.15)	–	9.78 (2.1, 45.46)
Model 3 = Model 2 + cardiovascular risk factors[d]	–	–	2.19 (1.5, 3.19)	–	3.09 (1.53, 6.24)	–	6.08 (2.57, 14.41)
Model 4 = Model 3 + stress testing[e]	–	–	2.11 (1.94, 2.29)	–	2.8 (1.32, 5.95)	–	5.19 (2.19, 12.3)
All-cause mortality[b]							
Total no. of events	3	2	–	35	–	25	–
Rate per 100 person-years	0.67	1.67	–	1.41	–	2.83	–
Model 1	–	–	2.46 (1.11, 5.46)	–	2.13 (1.69, 2.68)	–	4.19 (2.68, 6.54)
Model 2 = Model 1 + demographic factors[c]	–	–	2.55 (1.11, 5.88)	–	2.3 (1.81, 2.92)	–	4.66 (2.92, 7.43)
Model 3 = Model 2 + cardiovascular risk factors[d]	–	–	2.03 (0.55, 7.44)	–	2.08 (1.3, 3.34)	–	3.82 (3.25, 4.49)
Model 4 = Model 3 + stress testing[e]	–	–	3.22 (0.74, 14)	–	2.93 (1.11, 7.75)	–	4.96 (3.88, 6.33)

HRV, heart rate variability; HF, high frequency; HR, hazard ratio.

[a]The HR compares the incidence of outcome events between participants with the labelled category and those with normal rest HRV and mental stress-induced HRV increase (reference). Robust confidence intervals are reported.

[b]The median observation period was 5.6 (IQR, 3.6–7.6).

[c]Age, sex, and race (Black v. non-Black participants).

[d]Body mass index, smoking status, history of hypertension, history of diabetes, history of dyslipidaemia, previous myocardial infarction (MIPS) or ischemia (MIMS2) with ST changes, and heart failure were originally included. Variables that did not significantly change the effect size were removed, and only hyperlipidaemia and heart failure were retained.

[e]Abnormal myocardial perfusion by conventional stress testing was defined as greater than or equal to 4 difference between the summed rest and stress scores.

a marker of sudden cardiac death risk.[54,55] Autonomic changes with acute mental stress may precipitate fatal arrhythmias,[56] and both HRV metrics used in this study may help provide new insights. Low-frequency HRV describes the amplitude of HR changes that occur in the range of two to nine times per minute and may help quantify the amplitude of baroreflex-induced sympathetic and parasympathetic pulsatile activities that occur at those frequencies.[34,57] Individuals with reduced baroreflex sensitivity during mental stress challenge, especially if it was low during the pre-stress rest period, may have been at increased risk of ventricular fibrillation due to downstream electrophysiological and ischaemic effects.[58,59] Parasympathetic withdrawal with stress, which is measured by HF HRV, may also play an important role in arrhythmia risk.[60,61] The similarities between our results with LF and HF HRV suggest multiple interrelated autonomic pathways may be involved in the risk of CVD death.

Although many previous studies found that lower HRV (especially LF) predicts mortality, they did not examine HRV during provocative manoeuvers that offer more insight on the possible underlying pathways.[19,20,27,45,46,62–66] This limits the potential to investigate targeted interventions to increase HRV, which can be affected by many other factors, including genetic, psychological, behavioural, or situational.[67] Our evaluation of HRV during mental stress challenge suggests that stress-related pathways are particularly important when examining short-term HRV as a prognostic risk factor.[42] These pathways may involve brain regions including the amygdala, anterior cingulate cortex, and insula, which are activated during the stress response and regulate ANS activity.[68] Decreased stress HF HRV is associated with increased rostromedial prefrontal cortex activation,[69] which regulates activity of the amygdala and vagal nuclei; as such, this region may also be involved in the mechanistic pathway leading to increased CVD death risk.[70] Overall, the literature supports the potential conclusions based on our data that autonomic testing during mental stress provocation may be critical for identifying those with pathophysiological neurocardiac mechanisms which increase their risk of CVD death.

The clinical and public health impacts of our findings are promising as HRV becomes increasingly accessible through portable ECG and wristband wearables with pulse sensors. Although testing with an acute mental stress challenge is not practical in widespread clinical settings, HRV and behavioural stress responses are modifiable and evidence-based interventions that improve autonomic function deserve greater support towards implementation in individuals who at least have reduced HRV at rest.[60] Such data are increasingly available with both newer ECG monitors and consumer wearables. Examples of HRV promoting interventions include biofeedback, yoga, cardiac rehabilitation, neuromodulation, and cognitive behavioural therapy.[71] More research is needed to identify high-risk groups based on their autonomic responses to stress in a way that is more feasible in clinical practices settings. One example may be to use Holter monitors and stress diaries.

Integrative interventions such as biofeedback and yoga have been found to increase LF HRV in previous studies[72] and suggest that more research to specifically examine their potential benefits on CVD death risk is needed.

Limitations

This study is subject to several limitations. Mental stress was assessed in the laboratory, which may not reflect everyday life, although moderate associations have been previously found.[73] Additionally, the mental stress challenge included only a single stressor and thus does not reflect the potential cumulative effects of multiple, sequential stressors.[74] Nonetheless, it does avoid the confounding by stress habituation.[25] Also, our study population included individuals with established CVD. Our findings may not be generalizable to those outside of this population; however, the high prevalence of this condition, and the high morbidity and mortality rates in this group, underscores the public health significance of this work. The definition of CVD was varied between inclusion cohorts, whereas MIPS enrolled those with established CAD while MIMS2 enrolled those with recent MI, which may subtend cohort-specific differences. The use of mixed effect models helped to control for this, and our findings were robust to cohort status (mainly history of MI and age). Third, there are inherent limitations in the assessment of HRV. We used standarized HRV measurements, as limited data on clinical cut points are available. We were not able to adjust for differences in respiration between speech stress and rest, which may have impacted HF HRV and impaired our ability to accurately measure isolated autonomic changes with stress; nonetheless, this limitation was likely uniform across the cohort and likely would have biased the results to the null. Heart rate variability may also have been influenced by medications, such as AV nodal blocking agents, antiarrhythmic drugs, and anti-hypertensive agents. Although beta-blockers were held the day of the study, residual effects may still have been present; however, the medications were balanced between stress reactivity groups, and models adjusting for beta-blockers did not show an association in the analysis. Lastly, we observed a limited number of CVD events, which limited our statistical power. However, we were able to show with sequentially adjusted models that our results were robust, and the estimates in the fully adjusted model and reduced model were similar. Our robust findings with all-cause mortality also decrease the likelihood of bias due to the small number of outcomes.

Conclusion

The results of this longitudinal cohort study suggest a robust relationship between stress-induced autonomic dysfunction and both CVD and all-cause mortality. We also found smaller associations with baseline resting autonomic dysfunction. Our findings were not explained by potential confounding from traditional risk factors. These findings underscore the mechanistic and prognostic importance of autonomic stress pathways in CVD.

Lead author biography

Dr Anish S. Shah, MD, MS, is a cardiac electrophysiology fellow at the University of Utah with a research interest in the interplay between stress and arrhythmias. He received his medical degree at Texas A&M University and completed his medicine training at the J. Willis Hurst Internal Medicine Residency Program at Emory University and a research-track cardiology fellowship at the University of Illinois Chicago. He received an NIH-TL1 award and a subsequent NIH-F32 award in parallel to his clinical training. He has a background and interest in epidemiology, signal processing, and computational genetics.

Data Availability

The data underlying this article cannot be shared publicly for the privacy of the individuals participating in the study. The data will be shared on reasonable request to the corresponding author.

Supplementary material

Supplementary material is available at *European Heart Journal Open* online.

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