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Original Research Article

Association between nocturnal heart rate variability and incident cardiovascular disease events: The HypnoLaus population-based study

Short title: Heart rate variability and cardiovascular disease

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1 **ABSTRACT**

2 **Background:** Although heart rate variability (HRV) is widely used to assess cardiac
3 autonomic function, few studies have specifically investigated nocturnal HRV.

4 **Objective:** We aimed to assess the association between nocturnal HRV and cardiovascular
5 disease (CVD) incidence over 4 years in a population-based sample.

6 **Methods:** 1784 participants (48.2% male, 58±11 years) from the HypnoLaus population-
7 based cohort free of CVD at baseline were included. Polysomnography-based
8 electrocardiograms were exported to analyse time and frequency-domain HRV, Poincaré
9 plots indices, detrended fluctuation analysis, acceleration (AC) and deceleration capacities
10 (DC), entropy, heart rate fragmentation (HRF), and heart rate turbulence. Multivariable-
11 adjusted cox regression analysis was used to assess the association between HRV indices
12 and incident CVD events.

13 **Results:** 67 participants (3.8%) developed a CVD over a mean follow-up time of 4.1±1.1
14 years. In a fully adjusted model, AC (hazard ratio per one SD increase [95% confidence
15 interval]: 1.59 [1.17-2.16]; p=0.004), DC (0.63 [0.47-0.84]; p=0.002) and HRF (1.41 [1.11-
16 1.78]; p=0.005) were the only HRV metrics significantly associated with incident CVD events
17 after controlling for false discovery rate.

18 **Conclusion:** Nocturnal novel HRV parameters such as AC, DC and HRF are better
19 predictors of CVD events than time and frequency traditional HRV parameters. These
20 findings suggest a form of dysautonomia and fragmented rhythms but further experimental
21 studies are needed to delineate the underlying physiological mechanisms of these novel
22 HRV parameters.

23

24 **Key Words:** heart rate variability; heart rate fragmentation; cardiovascular disease;
25 electrocardiogram; sleep; prospective study.

26 INTRODUCTION

27 Sleep is a complex homeostatic and circadian function during which autonomic nervous
28 system (ANS) regulation is largely independent of external stimuli and therefore provides a
29 reasonable representation of basal autonomic regulatory equilibrium.¹ For over three
30 decades, analysis of heart rate variability (HRV) has been proposed as a non-invasive and
31 accessible tool to evaluate cardiac autonomic function (CAF) in health and disease.² In
32 practice, normal beat-to-beat intervals are extracted from the electrocardiogram (ECG) and
33 numerous HRV indices can be calculated using time-domain, frequency-domain and non-
34 linear analysis.³⁻⁵ These indices provide information about sympathetic and parasympathetic
35 modulations as well as randomness of heart rate.^{3, 4} Several epidemiological studies have
36 shown that altered HRV is an independent predictor of cardiovascular outcomes and all-
37 cause mortality.⁶⁻⁸

38 Despite this, HRV is sometimes criticized due to its lack of reproducibility and reliability if
39 standardized procedures are not utilized. To improve reproducibility, HRV indices are usually
40 calculated from 24-h ECG Holter monitoring while participants perform their usual daily
41 activities³ but Holter monitoring is mostly used by cardiologists. Moreover, unrestricted
42 activities for the same participant may vary within and between days, which may have
43 unpredictable effects on 24-h HRV parameters and thus decrease reproducibility.⁹ HRV can
44 also be acquired during daytime using short-term recordings, but methods of measurement
45 need to be highly standardized (position, breathing pace, recording time, etc.) and short-term
46 measurements have lower predictive power than 24-h recordings.^{4, 10, 11}

47 Recently, sleep has been proposed as a highly standardized condition to time-efficiently
48 measure HRV in a setting that is less influenced by environmental factors compared with
49 daytime measures.¹² However, although the association between 24-h HRV and
50 cardiovascular events is well established, it is still largely unknown whether nocturnal HRV
51 metrics can predict the occurrence of cardiovascular diseases (CVD) events.

52 Thus, the primary objective of this study was to determine whether nocturnal HRV indices
53 can predict the incidence of fatal and non-fatal CVD events over a 4-year follow-up period.

54 **METHODS**

55 **Study Population**

56 The HypnoLaus study¹³ is a nested study of CoLaus|PsyCoLaus,^{14, 15} a prospective cohort
57 study including 2,162 participants randomly selected according to the civil register of the city
58 of Lausanne (Switzerland) who completed an ambulatory full polysomnography (PSG) in
59 addition to a full clinical work-up between 2009 and 2013 to assess the prevalence and
60 correlates of sleep characteristics and sleep disorders (more details in Supplemental
61 Appendix).

62

63 **Ethical Statement**

64 Both the CoLaus|PsyCoLaus and HypnoLaus studies were approved by the Ethics
65 Committee of the Vaud Canton (approval numbers 16/03 and 33/09), and written informed
66 consent was obtained from all participants.

67

68 **Heart Rate Variability Measurements**

69 Single-lead ECG data were extracted from the polysomnography recordings in European
70 Data Format (EDF) using Somnologica Studio (version 5.1.1, Embla® Flaga). Each QRS
71 complex was validated and raw RR series were imported to the *HRVanalysis* software
72 version 1.2.¹⁶ An accurate preprocessing was performed as suggested in the HRV Task
73 Force.³ HRV was first analyzed for the whole sleep period using sleep onset and wake-up
74 timestamps from the PSG (including wake after sleep onset periods). In a secondary
75 analysis, HRV was analyzed from the average of 5-minute stable epochs during non-rapid
76 eye movement (NREM, including sleep stages 1, 2 and 3) and rapid eye movement (REM)
77 sleep using the sleep scoring of the PSG. Moreover, HRV was analyzed from the average of
78 5-minute stable epochs without events (arousals, apnea-hypopnea or periodic leg
79 movements) during NREM and REM sleep. HRV analysis included standard time-domain,
80 frequency-domain, non-linear and novel indices according to standard criteria (more details
81 in Supplemental Appendix).^{3, 5}

82

83 **Linear HRV Analysis**

84 In the time-domain, the following indices were reported: mean peak-to-peak R intervals (RR),
85 the standard deviation of normal-to-normal (N-N) intervals (SDNN) and the root mean square
86 of successive N-N differences (rMSSD). In the frequency-domain, a Fast Fourier Transform
87 algorithm was applied based on 5-min epochs and the following spectral power parameters
88 were calculated: very low frequency (VLF), low frequency (LF), high frequency (HF) and the
89 ratio LF/HF.³

90

91 **Nonlinear HRV Analysis**

92 Non-linear measurements provide information on the complexity of autonomic regulations,
93 and included Poincaré plot analysis (short-term [SD1] and long-term variability indices
94 [SD2]),¹⁷ detrended fluctuation analysis (short- and long-term fluctuations indices DFA α_1
95 and α_2)¹⁸ and entropy (approximative entropy [ApEn] and sample entropy [SampEn]).¹⁹

96

97 **Novel HRV analysis**

98 Novel HRV analysis included deceleration capacity (DC) and acceleration capacity (AC),²⁰
99 heart rate fragmentation (HRF; measured as the percentage of inflection point [PIP])²¹ and
100 heart rate turbulence (HRT; quantify by turbulence onset [TO] and turbulence slope [TS]).^{22,}

101 ²³

102

103 **Clinical Assessment**

104 Information on sociodemographic characteristics, medical and treatment history was
105 obtained by trained interviewers using standardized questionnaires during the first follow-up
106 of CoLaus|PsyCoLaus (corresponding to the baseline of HypnoLaus).^{14, 15} Detailed
107 anthropometric measures and definition of comorbidities (diabetes, hypertension,
108 dyslipidemia, depression) are available in Supplementary Appendix.

109

110 **Outcomes**

111 The incidence of a composite cardiovascular disease variable was established based on all
112 the fatal and non-fatal cardiovascular disease events adjudicated by a local expert committee
113 including a panel of cardiologists and neurologists according to international
114 recommendations.²⁴ Fatal events were defined as death from myocardial infarction or stroke.
115 Non-fatal outcomes included the occurrence of non-fatal myocardial infarction, stroke, acute
116 coronary insufficiency (>50% stenosis) needing percutaneous coronary intervention or
117 coronary artery bypass grafting, and peripheral arterial disease defined by angiological
118 examination (US-doppler) and/or revascularization (by peripheral bypass surgery or
119 stenting).

120

121 **Exclusion criteria**

122 Participants with a prior CVD and participants loss to follow-up were excluded (n=198)
123 (Figure 1). Exclusion criteria also included recordings with insufficient ECG quality or
124 technical failure as well as participants with frequent ectopic beats (percentage of
125 supraventricular and ventricular extrasystoles >20% of the recording), atrial fibrillation or
126 flutter, pacemaker, bundle branch block and $\geq 10\%$ RR corrected due to their known influence
127 on HRV (n=149) (Figure 1).

128

129 **Statistical Analysis**

130 All statistical analyses were performed using IBM SPSS Statistics version 26.0 for Macintosh
131 (IMB Corp, Armonk, NY, USA). Baseline characteristics are presented as mean \pm SD,
132 median (interquartile range) or as n (percentage) unless otherwise state. Baseline
133 characteristics and HRV indices between “incident CVD events” and “no incident CVD
134 events” groups were compared using Student’s t-test, Mann Whitney U test or Chi-square
135 test as appropriate. Normality was checked with Q-Q plots. Non-normal HRV parameters
136 were log transformed (log₁₀) prior to analysis. A two-tailed *p*-value <0.05 was considered
137 statistically significant. To determine the associations between HRV indices and CVD events

138 incidence, univariate and multivariable-adjusted COX regressions were used with each HRV
139 parameter tested separately. Model 1 was adjusted for age, sex and body mass index (BMI).
140 Model 2 was additionally adjusted for smoking status, education, depression, hypertension,
141 dyslipidemia, diabetes, sleep drugs, beta-blockers, apnea-hypopnea index (AHI) and periodic
142 leg movement during sleep (PLMSI). Model 3 was additionally adjusted for self-reported
143 treatment for sleep-disordered breathing during the follow-up. Results are expressed as
144 hazard ratio (HR) with 95% CI per one SD increase for each HRV parameter tested. To avoid
145 type 1 error, we corrected for multiple testing using the false discovery rate (FDR)
146 approach.²⁵ To facilitate interpretation, continuous HRV parameters that were statistically
147 significant in the fully adjusted model were also dichotomized according to their median.
148 Potential interaction between the main clinical and demographic parameters (age, sex, BMI,
149 hypertension and diabetes) and incident CVD events were assessed for those significant
150 HRV parameters.

151 Predictive power of these metrics was compared to a base model including age, BMI,
152 diabetes, hypertension, smoking status and dyslipidemia. Discrimination performance of
153 these latter models was assessed with area under the receiver operating characteristic curve
154 (AUROC) while concordance was assessed using Harrell's C index and goodness of fit with
155 Akaike information criterion (AIC) and Bayesian information criterion (BIC).

156 Additional analyses were done to determine: (1) the association of HRV during NREM and
157 REM sleep with incident CVD events; (2) the association of HRV during sleep epochs in
158 NREM and REM without sleep events (arousals, apnea-hypopnea or periodic leg
159 movements) with incident CVD events; (3) the association of HRV with both incidence and
160 recurrence of CVD events; (4) the association between the inability to measure HRV due to
161 frequent ectopic beats, atrial arrhythmia, pacemakers, bundle branch block or lots of
162 corrected RR intervals ($\geq 10\%$) and incident CVD events.

163

164 **RESULTS**

165 **Population Characteristics**

166 Of the 2162 participants from HypnoLaus, 1784 without any CVD at baseline were included
167 in the present study. Detailed exclusion criteria are shown in Figure 1. The mean age was 58
168 years (range 40-84), 46.4% were men and the mean BMI was $26.0 \pm 4.3 \text{ kg.m}^{-2}$.
169 Sixty-seven (3.8%) participants developed a CVD event during follow-up (mean 4.1 ± 1.1
170 years). Of these, 26 (1.5%) had a stroke, 30 (1.7%) developed coronary heart disease
171 (including 1 fatal event), and 11 (0.6%) developed PAD. The mean time to incident CVD
172 event was 2.6 ± 1.4 years. Clinical and sleep characteristics of the participants with and
173 without incident CVD events are shown in Table 1. Participants who developed a CVD event
174 were older, had a higher BMI, were more often smokers, and had a higher prevalence rate of
175 hypertension, dyslipidemia and diabetes. They were also more frequent users of sleep drugs
176 and beta-blockers, and had higher AHI and PLMSI at baseline.

177

178 **Association between HRV and incident CVD**

179 Bivariate analysis for heart rate variability parameters according to cardiovascular status are
180 shown in Table A.1. Numerous HRV indices were significantly associated with incident CVD
181 events in unadjusted analysis and most of them remained significant after adjustment for
182 age, sex and BMI (Model 1) (Table 2). In the Model 2 (additionally adjusted for smoking
183 status, education, depression, hypertension, dyslipidemia, diabetes, sleep drugs, beta-
184 blockers, AHI and PLMSI), AC, DC and PIP were the only significant predictors of incident
185 CVD events after FDR correction. After additional adjustment for self-reported sleep-
186 disordered treatment during follow-up (Model 3), none remains significantly associated with
187 incident CVD events after FDR correction.

188 When AC, DC and PIP were dichotomized according to their median, we found that
189 participants with low AC, low DC and high PIP were at higher risk of incident CVD events
190 compared to those with high AC, high DC and low PIP respectively (Figure 2).

191 No significant interactions were found between the main clinical and demographics
192 parameters (age, sex, BMI, hypertension, diabetes), incident CVD events and AC, DC and
193 PIP (model 3) (Table A.2).

194

195 **Predictive value of HRV metrics**

196 Performance metrics of the risk prediction models are summarized in Table 3. Results
197 showed a minimal improvement in discrimination (AUROC), concordance (Harrell's C), and
198 goodness of fit (AIC and BIC) when AC, DC or PIP were added to the base model.

199

200 **Secondary analysis**

201 When considering HRV during NREM and REM sleep separately, results remained
202 consistent although associations between incident CVD events and AC, DC as well as PIP
203 were only significant in NREM sleep after FDR correction (Table.A.3.). Moreover, DFA α_2
204 was significantly associated with incident CVD events during NREM sleep.

205 When considering only stable epochs without sleep events (arousals, apnea-hypopnea or
206 periodic leg movements), only PIP during NREM remained significantly associated with CVD
207 events after FDR adjustment (Table.A.4.).

208 When considering both incidence and recurrence of CVD (n=86 events), results remained
209 consistent for AC and DC but PIP was no longer associated with the occurrence of CVD
210 events during the follow-up (Table A.5).

211 Lastly, patients excluded from the primary analysis due to the inability to measure HRV
212 because of frequent ectopic beats, atrial arrhythmia, pacemakers, bundle branch block or
213 lots of corrected RR intervals were at higher risk of incident CVD events after adjustment for
214 age, sex and BMI (HR: 1.86 [1.01–3.42]; p=0.046). However, this was no longer significant
215 in the fully adjusted model (1.85 [0.98–3.48]; p=0.059).

216

217

218 **DISCUSSION**

219 To our knowledge, this study is the first to assess the predictive value of a comprehensive
220 panel of linear, non-linear and novel nocturnal HRV indices based on a large population-
221 based sample of middle-to-older age participants. Our findings showed that only novel

222 nocturnal HRV metrics were independently associated with incident fatal and non-fatal CVD
223 events over 4 years of follow-up. By contrast, traditional HRV parameters during sleep were
224 not associated with incident CVD event. Although widely used in both research and clinical
225 practice, nocturnal SDNN, rMSSD, HF and oldest non-linear indices (DFA, entropy) did not
226 provide consistent results in the fully adjusted model after FDR-correction. However, our
227 findings corroborate one recent study performed in the community-based MESA cohort²⁶ and
228 provide further evidence that nocturnal traditional HRV indices are not as accurate in
229 participants without CVD as they can be in the cardiac populations in which they were
230 originally developed.^{3, 6} The discrepancy may be due to the paradoxical increase in short-
231 term variability (rMSSD, HF) for some participants at high risk of CVD, which is actually not
232 attributable to vagal tone modulation but rather to erratic sinus rhythms,^{27, 28} and by the fact
233 that they had been developed on 24-h measurement. Moreover, some nocturnal metrics
234 such as VLF may be influenced by sleep disordered breathing. Even though the analysis was
235 adjusted for the AHI, we cannot exclude that this interference could influence its predictive
236 value.²⁹

237 In contrast, some more novel metrics such as nocturnal PIP, AC and DC appear to be better
238 predictors of incident CVD events. Nocturnal HRF already showed promising results as a
239 predictor of CVD events at 3 years in the MESA cohort²⁶ with an adjusted hazard ratio for
240 PIP in that community-based sample similar to the one obtained in our population-based
241 cohort (HR values of 1.43 and 1.42, respectively), providing additional support for this novel
242 approach. Surprisingly, our results showed that, in addition to DC, AC was strongly
243 associated with incident CVD events whereas previous studies investigating these metrics
244 only found DC to have prognostic value after myocardial infarction.³⁰ These HRV indices
245 likely reflect cardiac dysautonomia not clearly captured by other HRV parameters during
246 sleep but further studies are needed to elucidate the exact underlying physiological
247 mechanisms. Furthermore, unlike previous studies in CVD populations,²² we found no
248 association between HRT indices and the incidence of CVD events in our sample,
249 suggesting that HRT is a better predictor in cardiac populations than in general population.

250 Lastly, secondary analysis showed that HRV during NREM sleep did not improve the
251 prediction of cardiovascular risk compared to HRV during the whole night. This suggests that
252 respiratory polygraphy recordings without sleep stage scoring and with a single-lead ECG
253 may be used in clinical practice to analyze HRV and predict cardiovascular risk.

254

255 **Strengths and Limitations**

256 This study has several strengths including its prospective design in a large population-based
257 cohort with CVD events adjudicated by an expert panel. Moreover, sleep was analyzed using
258 gold-standard in-home PSG and the most commonly used HRV metrics were analyzed and
259 adjusted according to comorbidities, CVD risk factors and treatments.

260 Nonetheless several limitations should be mentioned. First, we used a composite CVD
261 events endpoint to evaluate CVD risk and therefore could not determine whether HRV
262 parameters were better able to predict the incidence of cardiovascular or cerebrovascular
263 diseases due to a relatively low number of events at four years. Further studies with more
264 events are needed to shed light on CVD subgroup analysis. Second, this study might not be
265 applicable to participants with arrhythmia, frequent ectopic beats and/or an implanted cardiac
266 pacemaker because participants with these characteristics were excluded due to the
267 alteration of the cardiac autonomic response. However, these subjects are known to be at
268 risk of CVD and our findings confirmed that the inability to measure HRV may be a predictor
269 of CVD events *per se*. Third, although we found significant prospective associations between
270 some HRV metrics and incident CVD events, the predictive capacity of HRV appears to be
271 relatively low when added to the base risk prediction model, but further studies are needed to
272 confirm these results.

273

274 **Conclusions**

275 Our study showed that “novel” HRV indices are probably better predictors of incident CVD
276 events than traditional HRV indices. In particular, low acceleration and deceleration capacity
277 as well as high heart rate fragmentation showed the strongest associations with incident

278 CVD events in our population-based cohort. Such features can be easily captured by HRV
279 analysis of the PSG ECG channel. Further experimental studies are needed to shed light on
280 the putative underlying physiological mechanisms of these novel HRV parameters.

281

282

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296

297 **Author's contributions**

298 MB, VP, FR and RH designed the study. VP performed HRV analysis. MB and PMV made
299 the statistical analysis. MB wrote the initial draft. All authors interpreted the data and critically
300 reviewed the manuscript.

301

302 **Data access**

303 Due to the sensitivity of the data and the lack of consent for online posting, individual data
304 cannot be made accessible. Only metadata will be made available in digital repositories.
305 Metadata requests can also be made via the study website: www.colaus-psycolaus.ch.

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397

398 **Figure legends**

399

400 **Figure 1.** Study flow chart. Afib indicates atrial fibrillation; CV, cardiovascular; CVD,
401 cardiovascular disease; ECG, electrocardiogram; HRV, heart rate variability; SVES,
402 supraventricular extrasystoles; VES, ventricular extrasystoles. *Frequent VES and SVES
403 were defined by a rate >20% of the recording.

404

405 **Figure 2.** Adjusted risk curves for the incidence of cardiovascular disease (CVD) according
406 to high and low: A) acceleration capacity (AC); B) deceleration capacity (DC); and C)
407 percentage of inflection points (PIP).
408

409 Curves were obtained after adjustment for age, sex, body mass index, education, smoking,
410 depression, hypertension, dyslipidemia, diabetes, sleep drugs, beta-blockers, apnea-
411 hypopnea index and periodic leg movement index during sleep. High and low values for each
412 parameter was determined according to the median in the whole population.

413 HR: hazard ratio; Ref, reference.

414

415 **Table 1.** Population characteristics according to cardiovascular status at 4 years

	No incident CVD events (N=1717)	Incident CVD events (N=67)	p-value
Age (years)	56.1 (40.5-84.1)	67.6 (55.4-72.9)	<0.001
BMI (kg.m ⁻²)	25.5 (23.0-28.3)	26.6 (24.3-29.2)	0.022
Male (%)	790 (46.0%)	38 (56.7%)	0.085
Education (years)	13.8 ± 4.0	12.8 ± 4.0	0.054
Alcohol, units/week	4 (1-9)	4 (0-10)	0.128
Coffee (mL/day)	156 ± 100	148 ± 95	0.502
Smoking status (%)			<0.001
Current smoker	301 (17.7%)	24 (35.8%)	
Former smoker	669 (39.4%)	27 (40.3%)	
Depression (%)	327 (19.0%)	18 (26.9%)	0.112
Hypertension (%)	625 (36.4%)	45 (67.2%)	<0.001
Dyslipidemia (%)	448 (26.1%)	25 (37.3%)	0.042
Diabetes (%)	133 (7.8%)	14 (20.9%)	<0.001
Medication (%)			
Sleep drugs (%)	133 (7.7%)	13 (19.4%)	0.001
Beta-blockers (%)	101 (5.9%)	10 (14.9%)	0.003
ACE inhibitors	83 (4.8%)	11 (16.4%)	<0.001
Angiotensin receptor blockers	190 (11.1%)	12 (17.9%)	0.083
Calcium channel blockers	76 (4.4%)	7 (10.4%)	0.022
Diuretics	96 (5.6%)	13 (19.4%)	<0.001
Antiarrhythmic [†]	4 (0.2%)	0 (0%)	1.000
Sleep characteristics			
Total sleep time (min)	403 ± 71	402 ± 83	0.882
Sleep efficiency (%)	88.5 (81.0-93.0)	83.4 (77.2-89.1)	0.002
N1 (%)	10.1 (7.2-14.4)	12.2 (8.5-16.5)	0.020
N2 (%)	46.2 ± 10.2	50.1 ± 11.1	0.002
N3 (%)	20.1 ± 8.4	16.2 ± 8.5	<0.001
REM (%)	22.1 ± 6.0	20.3 ± 6.9	0.018
AHI (events/h)	9.4 (3.9-19.0)	16.3 (6.5-26.8)	<0.001
ODI (events/h)	9.4 (4.1-18.2)	15.1 (7.1-27.1)	0.001
Mean SpO ₂ (%)	94.2 ± 2.9	93.4 ± 1.6	0.021
PLMSI (events/h)	1.8 (0-17.2)	13.5 (0.9-37.6)	<0.001
OSA treatment (%) ^{††}	116 (7.2%)	15 (27.3%)	<0.001

416

417 Values are presented as mean ± standard deviation or median (interquartile range) for normal and
418 non-normal continuous data, respectively. Categorical data are presented as number of patients (%).
419 P-values <0.05 are in bold.

420 ACE indicates angiotensin-converting enzyme; AHI, apnea-hypopnea index; ODI, oxygen desaturation
421 index; OSA: obstructive sleep apnea; PLMSI, periodic leg movement during sleep; SpO₂, oxygen
422 saturation. Detailed definitions of anthropometrics and comorbidities are presented in the
423 supplementary method.

424 †Antiarrhythmic drugs include amiodarone (C01BD01) and flecainide (C01BC04). ††Self-reported OSA
425 treatment was available in only 1645 participants.

Table 2. Nocturnal heart rate variability predictors of incident fatal and non-fatal adjudicated cardiovascular disease events

	Crude (n=1717/67)		Model 1 (n=1711/64)		Model 2 (n=1686/64)		Model 3 (n=1593/52)	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Mean RR (ms)	0.83 (0.65-1.07)	0.154	0.78 (0.60-1.02)	0.068	0.90 (0.69-1.18)	0.448	0.91 (0.67-1.23)	0.529
SDNN (ms)	0.69 (0.52-0.91)	0.008	0.76 (0.56-1.02)	0.064	0.85 (0.63-1.14)	0.280	0.84 (0.60-1.16)	0.286
RMSSD (ms)	0.46 (0.32-0.67)	<0.001	0.57 (0.39-0.84)	0.005	0.66 (0.45-0.98)	0.037	0.69 (0.45-1.05)	0.082
VLF (ms ² /Hz)	0.64 (0.46-0.91)	0.011	0.69 (0.48-1.01)	0.055	0.77 (0.54-1.10)	0.154	0.77 (0.51-1.15)	0.195
LF (ms ² /Hz)	0.51 (0.34-0.77)	0.001	0.67 (0.44-1.01)	0.056	0.75 (0.50-1.13)	0.169	0.80 (0.52-1.22)	0.296
HF (ms ² /Hz)	0.29 (0.14-0.57)	<0.001	0.43 (0.22-0.84)	0.013	0.53 (0.28-1.00)	0.049	0.61 (0.32-1.16)	0.135
LF/HF ratio	1.17 (0.95-1.44)	0.129	1.11 (0.88-1.39)	0.396	1.09 (0.84-1.40)	0.526	1.09 (0.82-1.45)	0.573
SD1 (ms)	0.47 (0.32-0.68)	<0.001	0.57 (0.39-0.84)	0.005	0.67 (0.46-0.98)	0.039	0.69 (0.46-1.06)	0.087
SD2 (ms)	0.71 (0.54-0.94)	0.015	0.78 (0.58-1.04)	0.041	0.87 (0.65-1.16)	0.337	0.85 (0.61-1.17)	0.312
SD1/SD2 ratio	0.50 (0.35-0.70)	<0.001	0.63 (0.45-0.89)	0.008	0.69 (0.49-0.98)	0.036	0.73 (0.50-1.06)	0.100
DFA α 1	1.37 (1.05-1.79)	0.020	1.30 (0.98-1.71)	0.067	1.25 (0.94-1.67)	0.128	1.20 (0.87-1.66)	0.269
DFA α 2	1.58 (1.23-2.04)	<0.001	1.30 (1.01-1.67)	0.044	1.26 (0.98-1.63)	0.074	1.25 (0.93-1.67)	0.140
AC (ms)	2.31 (1.75-3.04)	<0.001	1.89 (1.39-2.57)	<0.001	1.58 (1.16-2.16)	0.004	1.53 (1.09-2.15)	0.015
DC (ms)	0.44 (0.33-0.57)	<0.001	0.53 (0.39-0.72)	<0.001	0.63 (0.47-0.85)	0.002	0.64 (0.46-0.88)	0.006
ApEn	0.76 (0.60-0.96)	0.022	0.90 (0.70-1.16)	0.396	0.93 (0.71-1.21)	0.589	0.92 (0.68-1.25)	0.610
SampEn	0.81 (0.63-1.04)	0.099	0.95 (0.74-1.22)	0.707	0.99 (0.76-1.29)	0.950	0.97 (0.72-1.31)	0.839

PIP (%)	1.64 (1.31-2.05)	0.001	1.46 (1.15-1.85)	0.002	1.42 (1.11-1.82)	0.005	1.39 (1.06-1.81)	0.016
TO (%)*	0.89 (0.70-1.14)	0.350	0.74 (0.56-0.98)	0.037	0.75 (0.55-1.01)	0.057	0.72 (0.52-0.99)	0.040
TS (ms/RR)*	0.50 (0.33-0.75)	0.001	0.68 (0.45-1.04)	0.076	0.82 (0.54-1.25)	0.353	0.94 (0.61-1.44)	0.785
HRT severity						0.896		0.992
Normal	Ref	-	Ref	-	Ref	-	Ref	-
TO or TS abnormal	1.66 (0.97-2.84)	0.065	1.13 (0.64-2.00)	0.683	1.15 (0.64-2.08)	0.642	0.98 (0.51-1.90)	0.954
TO & TS abnormal	2.69 (1.32-5.46)	0.006	1.25 (0.59-2.65)	0.565	1.09 (0.51-2.32)	0.827	0.94 (0.37-2.38)	0.903

Values presented are standardized hazard ratios (HR) per one-standard deviation (SD) increase in the independent variable, with 95% confidence intervals (CI). n indicates number of participants free of events / number of participants with incident cardiovascular disease in each model.

Model 1 was adjusted for age, sex and body mass index. Model 2: Model 1 + adjustment for educational level, smoking, depression, hypertension, dyslipidemia, diabetes, sleep drugs, beta-blockers, apnea-hypopnea index and periodic leg movement index during sleep. Model 3: Model 1 + adjustment for self-reported sleep-disordered treatment at follow-up. Detailed definitions of confounding factors are presented in the supplementary method.

False discovery rate (FDR) corrected significant results are in bold.

AC indicates acceleration capacity; ApEn, approximative entropy; DC, deceleration capacity; DFA α 1, detrended fluctuation analysis describing short term fluctuations (4 to 11 beats); DFA α 2, detrended fluctuation analysis describing long-term fluctuations (>11 beats); HF, high frequency power; LF, low frequency power; PIP, percentage of inflection points; rMSSD, square root of mean squared differences between successive NN intervals; SampEn, Sample entropy; SD1, Poincaré plot standard deviation perpendicular the line of identity; SD2, Poincaré plot standard deviation along the line of identity; SDNN, standard deviation in normal-to-normal (NN) RR intervals; TO, turbulence onset; TS, turbulence slope; VLF, very-low frequency power.

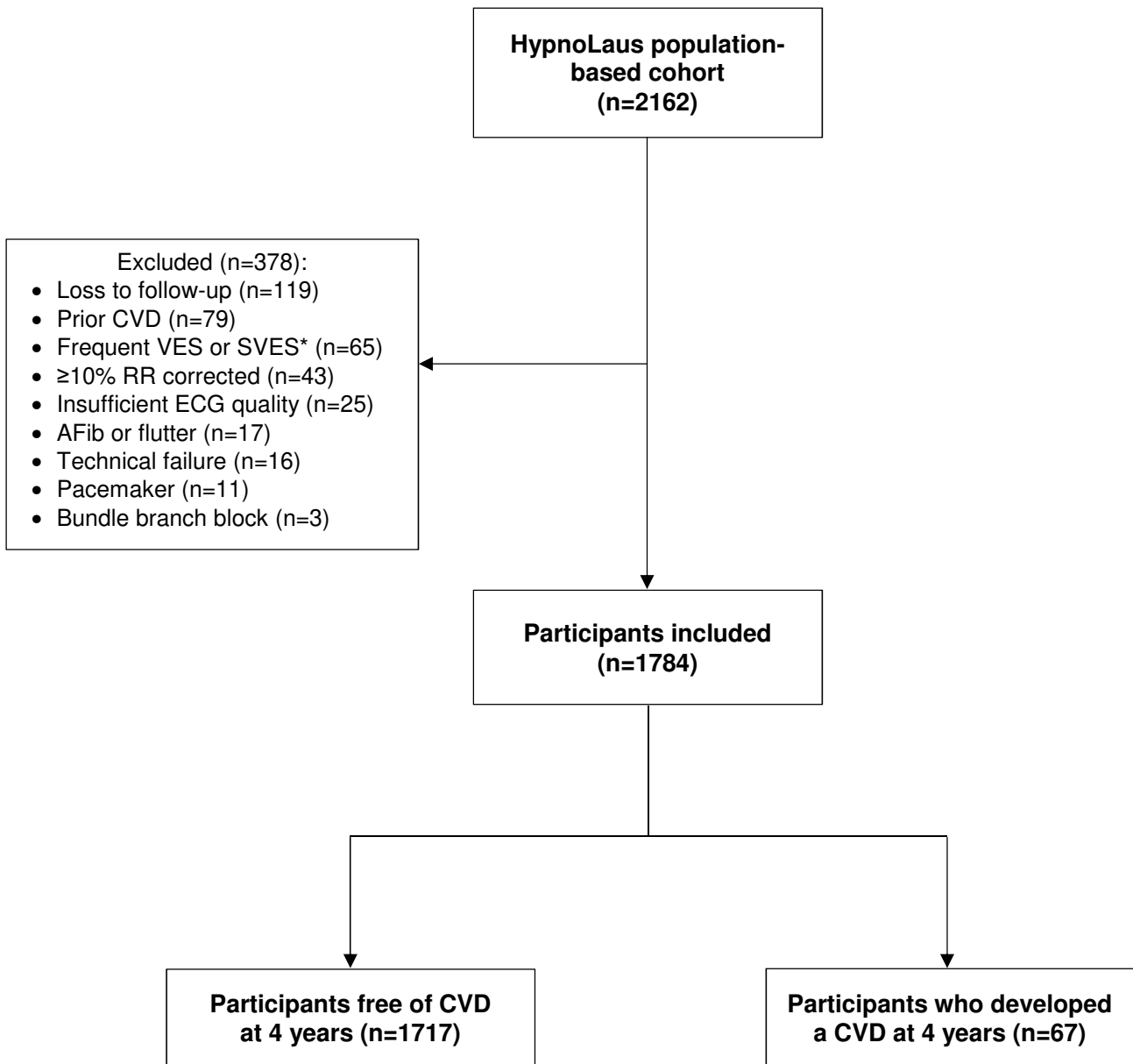
For TO and TS, $n=1069/57$ for crude; $n=1067/55$ for model 1, $n=1056/55$ for model 2 and $n=993/45$ for model 3.

Table 3. Predictive risk metrics for the model with cardiovascular risk factors (base model) and the model with cardiovascular risk factors plus HRV markers.

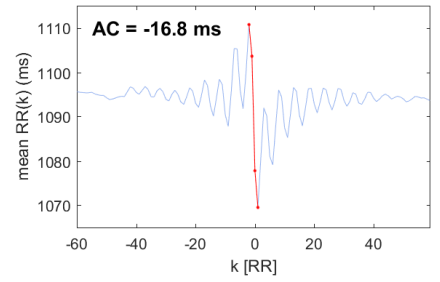
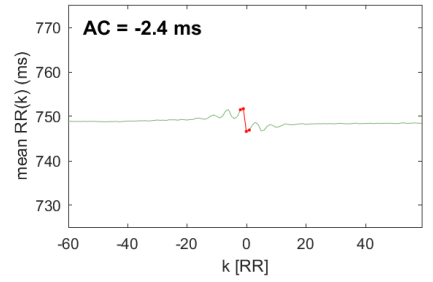
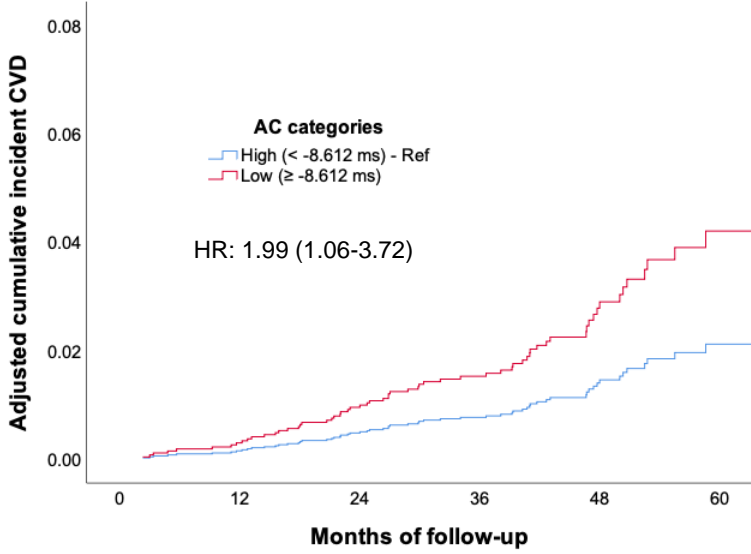
	AUROC	Harrell's C	AIC	BIC
Base model [†]	0.752 (0.690 - 0.814)	0.772 (0.709 - 0.835)	848.0	891.9
+ PIP	0.764 (0.705 - 0.823)	0.779 (0.719 - 0.840)	843.4	892.8
+ AC	0.763 (0.698 - 0.828)	0.780 (0.713 - 0.846)	840.8	890.2
+ DC	0.766 (0.701 - 0.831)	0.783 (0.718 - 0.849)	839.9	889.3

Results are presented as value and (95% confidence interval). AUROC, area under the receiver operating curve; AIC, Akaike's information criterion; BIC, Bayesian information criterion. PIP: percentage of inflection point; AC: acceleration capacity; DC: deceleration capacity.

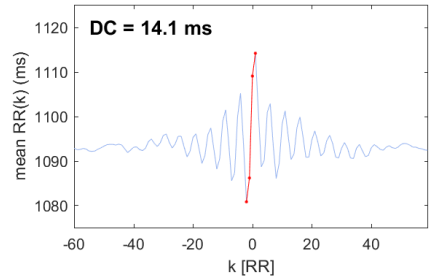
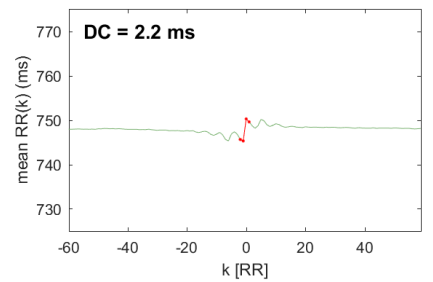
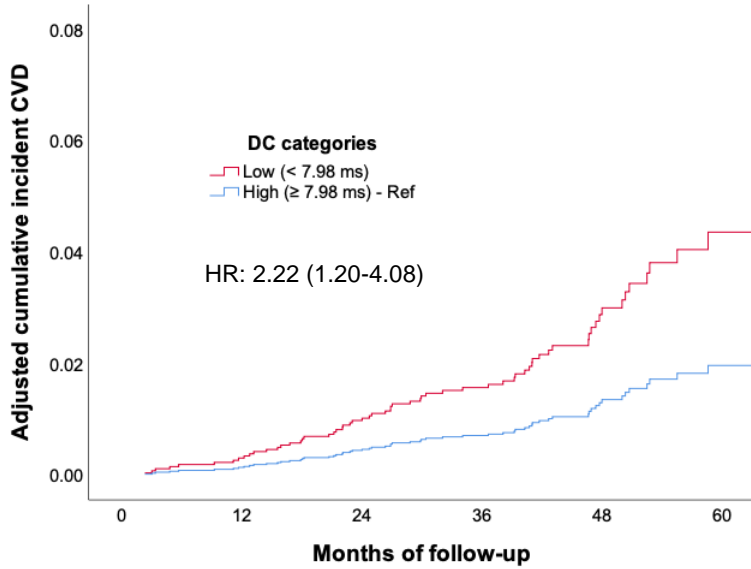
[†]Base model included age, body mass index, diabetes, hypertension, smoking status and dyslipidemia.



A.



B.



C.

