

BMJ Open Longitudinal effect of nocturnal R-R intervals changes on cardiovascular outcome in a community-based cohort

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ABSTRACT

Rationale Sleep-disordered breathing (SDB) is strongly linked to adverse cardiovascular outcomes (cardiovascular diseases (CVD)). Whether heart rate changes measured by nocturnal R-R interval (RRI) dips (RRI dip index (RRDI)) adversely affect the CVD outcomes is unknown.

Objectives To test whether nocturnal RRDI predicts CVD incidence and mortality in the Wisconsin Sleep Cohort study (WSCS), independent of the known effects of SDB on beat-to-beat variability.

Methods The study analysed electrocardiograph obtained from polysomnography study to assess the nocturnal total RRDI (the number of RRI dips divided by the total recording time) and sleep RRDI (the number of RRI dips divided by total sleep time). A composite CVD risk as a function of total and sleep RRDI was estimated by Cox proportional hazards in the WSCS.

Results The study sample consisted of 569 participants from the WSCS with no prior CVD at baseline were followed up for up to 15 years. Nocturnal total RRDI (10-unit change) was associated with composite CVD event(s) (HR, 1.24 per 10-unit increment in RRDI (95% CI 1.10 to 1.39), $p < 0.001$). After adjusting for demographic factors (age 58 ± 8 years old; 53% male; and body mass index 31 ± 7 kg/m²), and apnoea–hypopnoea index (AHI 4%), individuals with highest total nocturnal RRDI category (≥ 28 vs < 15 dips/hour) had a significant HR for increased incidence of CVD and mortality of 7.4 (95% CI 1.97 to 27.7), $p = 0.003$. Sleep RRDI was significantly associated with new-onset CVD event(s) (HR, 1.21 per 10-unit increment in RRDI (95% CI 1.09 to 1.35), $p < 0.001$) which remained significant after adjusting for demographic factors, AHI 4%, hypoxemia and other comorbidities.

Conclusion Increased nocturnal RRDI predicts cardiovascular mortality and morbidity, independent of the known effects of SDB on beat-to-beat variability. The frequency of RRDI is higher in men than in women, and is significantly associated with new-onset CVD event(s) in men but not in women.

INTRODUCTION

Sleep-disordered breathing (SDB) is a disorder characterised by the occurrence of recurrent episodes of apnoea and hypopnoea, resulting in a cascade of physiological responses including hypoxemia,

Strengths and limitations of this study

- It used a new method of detecting heart rate accelerations to assess its effect on the incidence of cardiovascular diseases (such as heart attack, heart failure, or need for cardiac procedures) and cardiovascular-related mortality.
- Using secondary analysis of a database of a prospective community cohort from the Wisconsin Sleep Cohort, we found that heart rate accelerations predict cardiovascular mortality and incidence of cardiovascular diseases.
- These results suggest that assessing the nocturnal ECG for heart accelerations may assist in predicting cardiovascular disease early on.
- The study was focused on individuals who had no prior pre-existing cardiac disease and were not on medications that affect heart rate such as Beta blocker; therefore, it may not be applicable to patients with current heart disease.
- This study lacks racial diversity as 95% was reported as a white race. Therefore, the results may not be generalisable to other races.

hypercapnia, intrathoracic pressure swings due to the inspiratory effort, activation of the sympathetic nervous system and arousal from sleep.¹ In clinical practice, SDB is defined by the measurement of apnoea–hypopnoea index (AHI), as the average number of respiratory events divided by the total sleep time. Although AHI is easy to use, this measure discounts other physiological consequences of the respiratory events that may be important, including associated hypoxemia and arousals from sleep, as well as the cardiac autonomic disturbances throughout the night.² Indeed, recent evidence showed that sub-type of excessively sleepy patients with moderate to severe SDB have significantly increased the risk for prevalent and incident cardiovascular events indicating the central role of sleep disruption in increased CVD risk.³ In addition, a recent study found that

varying hypopnoea definitions in the general population can affect the risk stratification of cardiovascular disease in patients with SDB.⁴⁵

While recent reports included measurements of sleep fragmentation and respiratory event duration as a surrogate of arousal threshold⁶ it did not include direct measurements of sympathetic activity and heart rate changes related to these events and its physiological stressors. Nocturnal heart rate variability, not day-time, is a heritable phenotype⁷ independent of covariates, suggesting that genetic factors play an important role in controlling these cardiovascular risk factors.⁸ Therefore, R-R interval (RRI), a time domain measure of heart rate variability, may reflect a physiological trait that predicts the risk of adverse cardiovascular outcomes, otherwise missed by SDB severity classification using traditional AHI and desaturation criteria.⁹ However, the long-term effect of heart rate changes during sleep on the cardiovascular outcome and mortality is unknown.

The objectives of this study were to examine whether RRI or heart rate accelerations can serve as predictors of cardiovascular disease in the Wisconsin Sleep Cohort study (WSCS), a prospective community cohort. We hypothesised that increased nocturnal RRI dip index (RRDI) would be associated with increased cardiovascular disease (CVD) or mortality independent of the known effects of SDB on beat-to-beat variability. Results of this study have been previously reported in the form of an abstract.^{10 11}

METHODS

Participants

We studied individuals from the WSCS. The protocols of the WSCS were approved by the Health Sciences Institutional Review Board of the University of Wisconsin-Madison. All participants provided written informed consent. Sampling and data collection protocols of the WSCS have been described previously.¹²

Cohort description

The WSCS comprises 1546 adult employees of state agencies, ages 30–60 years old at the Cohort's inception, which underwent attended in-laboratory overnight polysomnography (PSG) and provided health-related questionnaires approximately every 4 years. Data presented here were collected from August of 2000 through August 2016 (the period when digital PSG recording systems were in use by the WSCS). The most recent available PSG study was used for analysis. WSCS participants were eligible to be included in the study if they had full PSG with adequate ECG recording, not treated for SDB, had no prior CVD event and did not use beta blockers or chronotropic drugs (online supplementary table 1S) on the night of the sleep study or at any other point during follow-up.

Patient and public involvement

This study was a secondary analysis for pre-existing data from an established cohort of the Wisconsin study. Therefore, participants were not involved in the design, recruitment, or conduct of this study.

Predictor

The main predictor variable is the hourly rate of RRI changes assessed over an entire night's sleep period. The recorded ECG signals were retrieved from PSG to measure the RRI, which are time intervals between successive pairs of QRS complexes, by using software for the detection of R waves in LabChart 7 with heart rate variability Module (AD Instruments, Colorado Springs, Colorado, USA) (figure 1). In this program, ECG signal was examined and retrieved to a MatLab R2017a program (MathWorks, Natick, Massachusetts, USA) developed and validated by our group to obtain RRI signal for the entire night (figure 2).^{13 14} The RRI dips, defined by a decreased RRI compared with the average RRI for the corresponding 1 min segment as a baseline, were collected. Given that 90% dips threshold correlated previously with most respiratory events (apneic and non-apneic respiratory events, defined below),¹⁴ total RRI dips index (RRDI) was defined by the number of RRI dips below the 90% baseline divided by the total PSG recording time in hours (from light on to light out), regardless of wake or sleep stages. Sleep RRDI for non-rapid eye movement (REM) and REM stages combined were defined by the number of RRI dips below the 90% baseline divided by the total sleep time in hours (for both REM and non-REM sleep stages). Subsequently, sleep RRDI was calculated for specific sleep stages for REM and non-REM, respectively. In subgroup analysis, the gender differences in total and sleep RRDI were compared between men and women.

The person performing the analysis was blinded to the participant's demographic information. The RRDI values were examined as a continuous variable and as a categorical variable divided into tertiles (lower 25% (low), middle 50% (medium) and upper 25% (high)).

Outcome

Incident CVD events were death related to CVD, self-reported physician-diagnosed heart attack, heart failure, or a CVD procedure (angioplasty, stent, pacemaker, bypass, or defibrillation). Information on CVD events, including the date of the event, was obtained on the overnight health questionnaire. Death certificates and cause of mortality in the cohort were obtained by matching social security numbers with two data sources: The National Death Index and the Wisconsin State Bureau of Health Information and Policy, Phial Records Section. All included participants in this study were able to complete the follow-up information. The censored analysis was used, and the data is censored at the last visit. If multiple events were reported over the

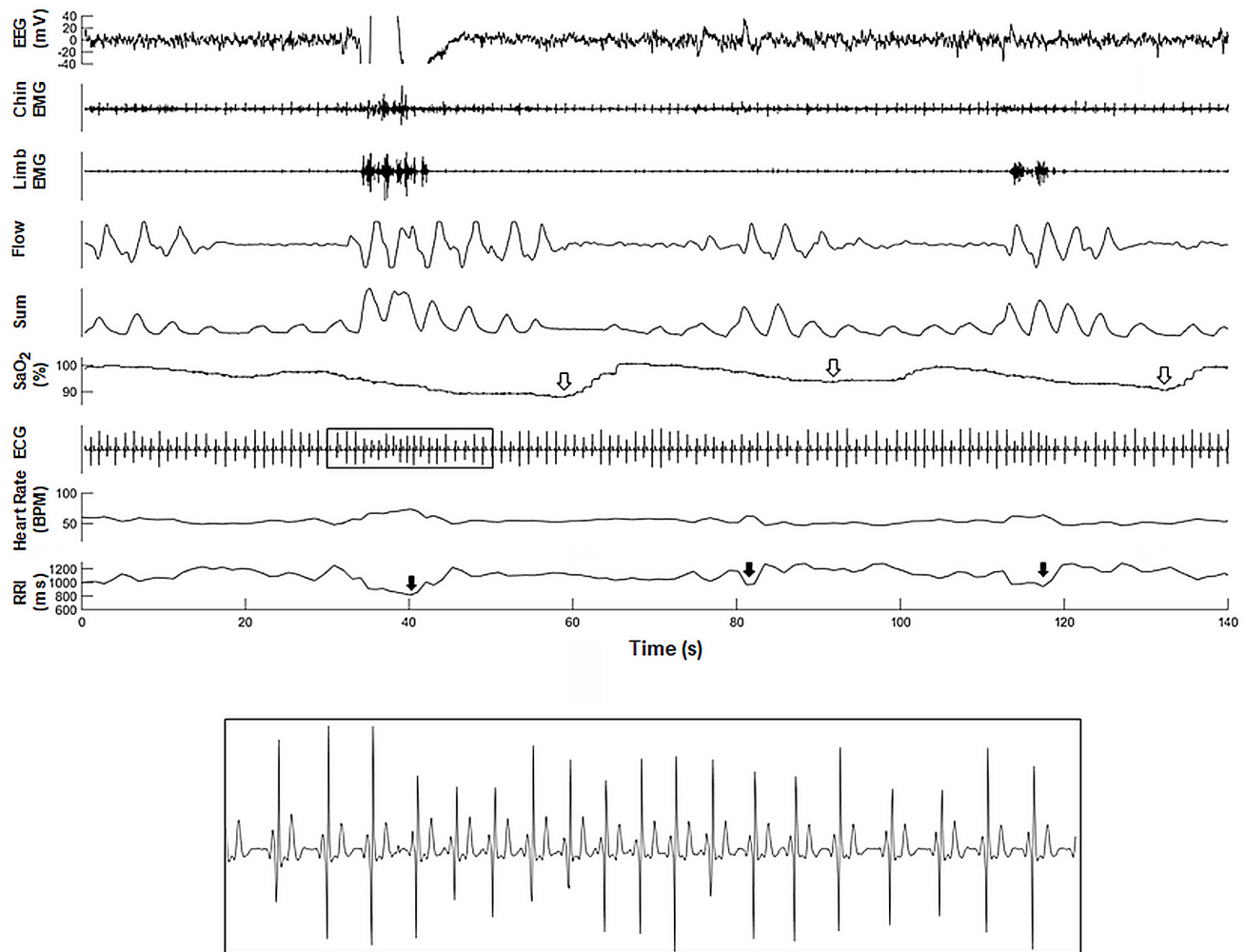


Figure 1 (A) A representative polygraph from one subject in the WSCS that illustrate changes in respective heart rate and computed RRI tracing during sleep. (B) A magnified segment of ECG illustrating RRI following respiratory event (apnoea). The RRI tracing was retro-graphed from the exported signal that calculates RRI from raw ECG recordings. Open arrows indicate oxygen desaturation following apnoea and closed arrows indicate RRI dip following apnoea. EEG, electroencephalogram; EMG, electromyogram;; RRI, R-R interval; WSCS, Wisconsin Sleep Cohort Study.

course of follow-up, the first reported event was used in this analysis.

Covariates

Participants underwent a baseline overnight 18-channel PSG (Grass model 78; Quincy, Massachusetts, USA) at the University of Wisconsin-Madison Clinical Research Unit using a standard protocol.¹⁵ The PSG recorded sleep state using electroencephalography, electrooculography and electromyography; and breathing, using respiratory inductance plethysmography (Respirace; Ambulatory Monitoring, Ardsley, New York, USA), nasal and oral airflow (ProTec thermocouples; Hendersonville, Tennessee, USA) and oxyhaemoglobin saturation, using pulse oximetry (Ohmeda Biox 3740, Englewood, Colorado, USA). Each 30s epoch of the polysomnographic recordings was scored for sleep stage and apnoea and hypopnoea events by trained technicians and reviewed using standard criteria.¹⁵ Apnoea was defined as the cessation of nasal and oral airflow for ≥ 10 s and hypopnoea as

a discernible reduction in breathing (sum of the chest and abdominal excursions) with a decrease in oxyhaemoglobin saturation of $\geq 4\%$. The apnoea-hypopnoea index was calculated as the mean number of apnoea and hypopnoea events per hour of sleep.

Statistical analysis

Cox proportional hazards regression was used to estimate adjusted HRs and 95% CIs for the association between RRDI and subsequent risk of an incident CVD event.¹⁶ Because of the strong dependence of CVD risk on age, Cox-regression models were based on age as the time scale, (the age when RRDI was measured and age at the event) allowing for left truncation (late entry).¹⁷ In addition to adjusting for age using this methodology, models 2 were adjusted for body mass index (BMI) and gender. Models 3 were adjusted for AHI 4% (as continuous and categorical variables (AHI < 5 , 5–15, or > 15 events/hour)). Subsequently, models 4 were adjusted for other factors: diabetes, hypertension, stroke, smoking, average

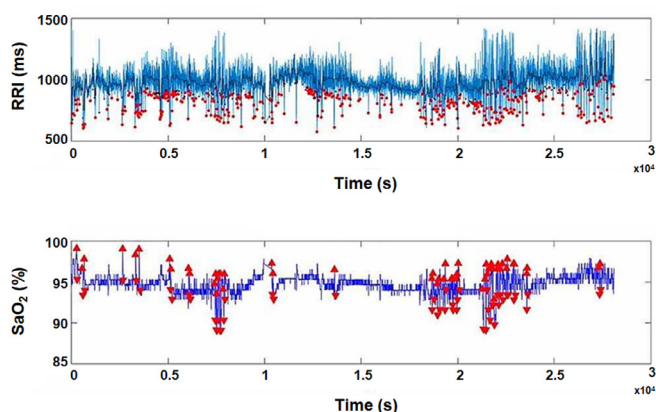


Figure 2 A representative computed data of RRI and oxygen saturation (SaO_2) from one individual during sleep. The red dots represent the RRI dips throughout the duration of the PSG recording (approximately 8 hours). The RRDI at 90% threshold for this participant was 54.5 dips/hour, the average heart rate was 61.1 bpm and the ODI (3%) was 2.3 de-saturations/hour (from the original PSG recording). The RRI tracing was retro-graphed from the exported signal that calculates RRI and SaO_2 from raw ECG and pulse oximetry recordings, respectively. ODI, oxygen desaturation index; PSG, polysomnography; RRI, R-R intervals; RRDI, RRI dips index; SaO_2 , oxygen saturation.

HR, % TST <90%, Kaplan-Meier techniques were used to compare survival across RRDI categories.¹⁸

RESULTS

Baseline characteristics

Table 1 presents the baseline characteristics of the eligible participants. A total of 1440 sleep studies were examined for inclusion in this study as depicted in **figure 3**. The final sample included 569 participants (one sleep study per participant) after excluding those on continuous positive airway pressure (CPAP) treatment, individuals who had a prior history of cardiovascular disease, use of beta blocker the night of the study or in other visits during the study, lack of follow-up or if they had events before PSG. RRDI (at 90% threshold) significantly correlated with the following sleep parameters: AHI 4%, periodic legs movements index (PLMI) and respiratory electroencephalogram arousals ($p < 0.001$) (**table 2**).

CVD incidence—association with total RRDI

Using Cox Proportional Hazards Model, continuous total RRDI (with 90% threshold for RRI dips) was significantly associated with new-onset CVD event(s) (HR, 1.24 per 10-unit increment in RRDI (95% CI 1.10 to 1.39), $p < 0.001$) which remained significant after adjustment for age, BMI and gender (model 1) and the addition of AHI 4% (model 3) (as depicted in **table 3**). Lower thresholds (80%, 70% and 60%) of RRDI correlated with total RRDI 90% but were less sensitive in predicting CVD (less than five individuals attained RRDI >20 dips/hour). The association between total RRDI at 90% threshold and the incidence of new-onset

Table 1 Baseline characteristics

Characteristics	Value
n	569
Age in years, mean (SD) range	58 (8) 39–79
Body mass index in kg/m^2 , mean (SD) range	31 (7) 18–66
Years to event/censor, mean (SD) range	8 (4) 0.1–15
Males, n (%)	300 (53)
Apnoea–hypopnoea Index, n (%)	
<5	253 (44)
5–15	168 (30)
>15	148 (26)
Diabetes, n (%)	32 (6)
Hypertension, n (%)	191 (34)
Stroke, n (%)	11 (2)
Antihypertensive medication use (excluding Beta Blockers or any chronotropic medication), n (%)	133 (24)
Smoking, n (%)	
Current	63 (11)
Past	213 (37)
Never	293 (52)
White race, n (%)	538 (97)
Number of alcoholic drinks per week, mean (SD) range	4 (5) 0–32
Total sleep time, minutes, mean (SD) range	368 (61) 30–514
Per cent stage 1 sleep, mean (SD)	10.6 (6.5)
Per cent stage 2 sleep, mean (SD)	65.0 (9.3)
Per cent stage 3, 4 sleep, mean (SD)	7.8 (8.0)
Per cent REM sleep, mean (SD)	16.6 (6.4)
Mean SaO_2 , mean (SD)	95.4 (1.8)
Mean desaturation, mean (SD)	4.5 (1.5)
Percentage of total sleep time below 90% saturation, mean (SD)	2.7 (11.2)

REM, rapid eye movement; SaO_2 , oxygen saturation.

CVD remained significant after the adjustment for AHI 4% (model 3) ($p < 0.001$). Total RRDI category 3 ($\geq 28.4/\text{hour}$ vs < 15.1 dips/hour) was associated with increased CVD hazards risk of 6.1 (95% CI 1.7 to 27.7, $p = 0.005$) and remained significant after adjustment for AHI 4% ($p = 0.003$). Continuous total RRDI 90% remained significant (HR, 1.22 (per 10-unit increment in RRDI (95% CI 1.08, 1.37), $p = 0.001$) after additionally adjusted for diabetes, hypertension, stroke, smoking, average heart rate and % total time with oxygen saturation less than 90% (model 4) (as depicted in **table 3**).

Figure 4 illustrates the changes in CVD incidence and hazard ratios for total RRDI less than 15.1 dips per hour (as reference), RRDI 15.1–28.4 dips/hour (second tertile) and for the third group (tertile) of individuals with RRDI equal

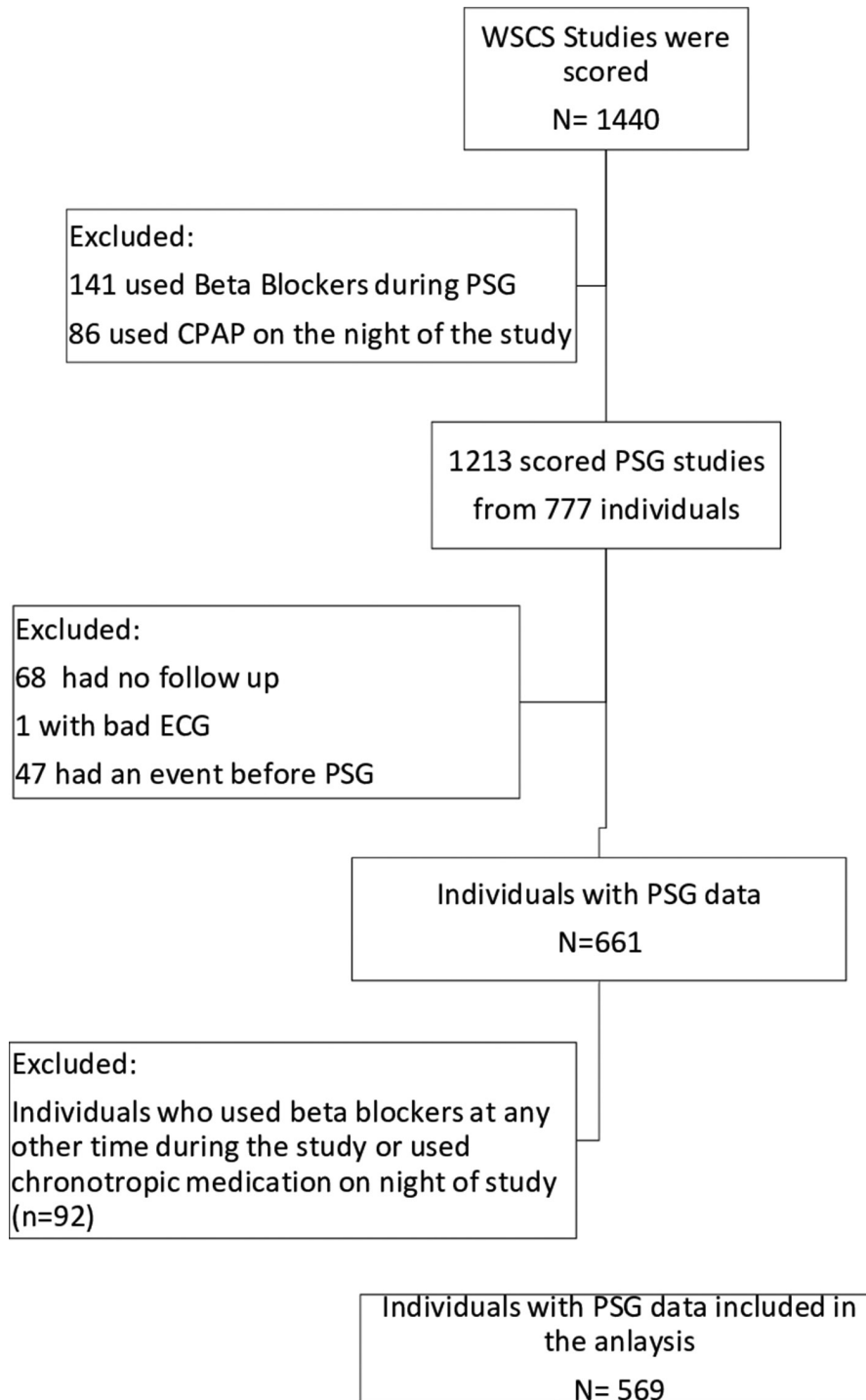


Figure 3 The WSCS study sample. CPAP, continuous positive airway pressure; PSG, polysomnography; WSCS, Wisconsin Sleep Cohort Study.

or more than 28.4 dips/hour. Kaplan-Meier survival curves (figure 5) illustrate decreased CVD event-free survival with increasing total RRDI category from RRDI less than 15.1 to RRDI equal or more than 28.4 dips/hour.

CVD incidence—association with sleep RRDI

Using Cox Proportional Hazards Model, continuous sleep RRDI 90% was significantly associated with new-onset CVD event(s) (HR, 1.21 per 10-unit increment in RRDI (95% CI 1.09 to 1.35), $p < 0.001$) which remained

significant after the model adjusted for age, BMI and gender (model 2) and the addition of AHI 4% (model 3) (as depicted in table 4). RRDI category 3 (≥ 23.5 /hour vs < 9.0 /hour) was associated with increased CVD hazards risk of 3.39 (95% CI 1.06 to 10.85, $p = 0.04$) and remained significant after adjustment for demographics and AHI 4% (model 4) ($p = 0.037$). The relationship between sleep RRDI categories and CVD events were predominantly in non-REM sleep as depicted in table 5.

Table 2 Pearson correlation for RRDl (at 90% threshold)

	Correlation coefficients	P value
AHI 4%	0.18	<0.0001
PLMI	0.19	<0.0001
Respiratory arousal index	0.24	<0.0001
Leg movement arousal index	0.23	<0.0001
Spontaneous arousal index	-0.09	0.127

AHI 4%, apnoea-hypopnoea index based on hypopnoea associated with 4% desaturation; PLMI, periodic legs movement index; RRDl, R-R interval dips index.

Continuous RRDl 90% during REM sleep was significant (HR, 1.19 per 10-unit increment in RRDl (95% CI 1.07 to 1.32), $p=0.001$) in the unadjusted model (model 1) and remained significant after the model adjusted for age, BMI and gender (model 2) and the addition of AHI 4% (model 3) (as depicted in table 6). However, sleep RRDl category 3 (≥ 24 /hour vs < 9.0 /hour) was only significant in the unadjusted model (model 1) with hazards risk of new CVD events of 2.92 ($p=0.04$).

Cardiovascular events were detected in 25 participants (4%), of the sample, over a follow-up interval of 15 years with mean age 59 years old (range 41–80). Cardiovascular events consisted of heart failure, heart attack, CVD procedure (before any events), or CVD death (table 7). No difference between mean age at the start for those who had CVD versus not ($p=0.58$).

Gender effect on the association of CVD incidence with total and sleep RRDl

Table 8 presents the baseline characteristics of men and women participants and associated total and sleep RRDl.

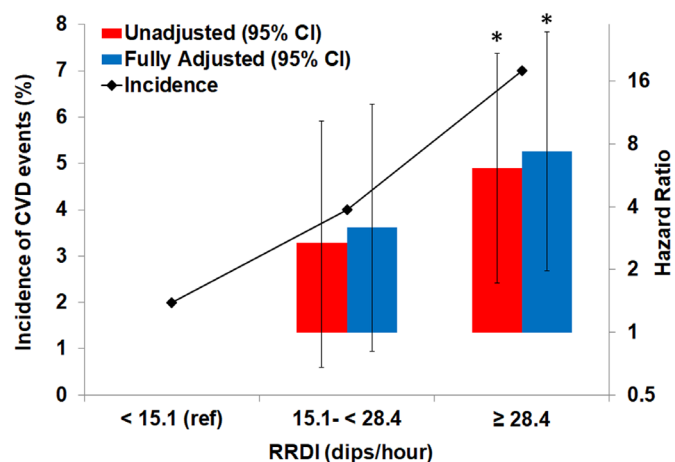


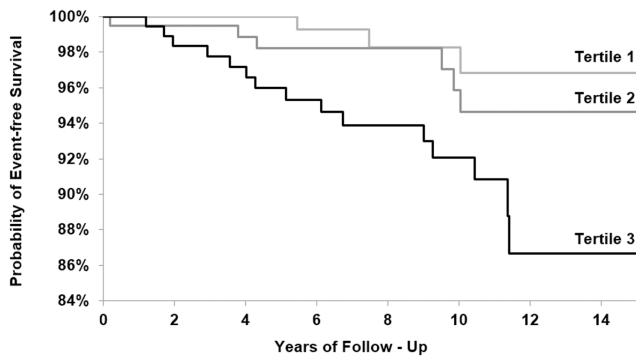
Figure 4 Incidence of composite CVD and hazard ratio across different total RRDl severity: Category 1 as a reference (RRDI <15.1), category 2 (RRDI 15.1-<28.4) and category 3 (RRDI ≥ 28.4) ($n=569$). (*) versus unadjusted model (1) RRDI <15.1 dips/hour, $p<0.01$; (***) versus adjusted model (3) RRDI <15.1 dips/hour, $p<0.01$. CVD, Cardiovascular disease; RRDl, R-R interval dips index.

While BMI was higher in women (32.0 ± 7.0 vs. 30.0 ± 5.0 kg/m², $p=0.0001$), men had higher AHI, total RRDl and sleep RRDl than women ($p<0.01$). Using Cox Proportional Hazards adjusted model (3) (for age, body mass index and AHI 4%), continuous total RRDl 90% was significantly associated with new-onset CVD event(s) in men (HR, 1.22 per 10-unit increment in RRDl (95% CI 1.06 to 1.40), $p<0.001$) but not in women (as depicted in Table 8). Likewise, continuous sleep RRDl 90% was significantly associated with new-onset CVD event(s) in men (HR, 1.19 per 10-unit increment in RRDl (95% CI 1.04 to 1.36), $p<0.05$) but not

Table 3 The adjusted time to event Cox Proportional Hazards Models for total RRDl predicting the incidence of CVD event

	CVD events	HR (95% CI)			
		n (%)	Unadjusted model (1)	Adjusted model (2)	Adjusted model (3)
Continuous RRDl (10-unit increment)	24/569 (4)	1.24 (1.10 to 1.39)	1.22 (1.08 to 1.38)	1.23 (1.11 to 1.38)	1.22 (1.08 to 1.37)
RRDI Category					
Tertile 1 (<15.1)	3/187 (2)	REF	REF	REF	REF
Tertile 2 (15.1-<28.4)	7/194 (4)	2.66 (0.68 to 10.34)	2.72 (0.70 to 10.59)	3.16 (0.81 to 12.40)	3.22 (0.80 to 12.93)
Tertile 3 (≥ 28.4)	14/188 (7)	6.11 (1.72 to 21.72)	5.87 (1.60 to 21.46)	7.40 (1.97 to 27.73)	8.99 (2.35 to 34.40)
P-trend		0.0024	0.0045	0.0017	0.0006

Model (1) is unadjusted. Model (2) is adjusted for age, sex and body mass index. Model (3) is additionally adjusted for age, sex, body mass index and AHI (4% criteria). Model (4) is additionally adjusted for diabetes, HTN, stroke and smoking, average HR and %TST<90%. AHI 4%, apnoea-hypopnoea index with hypopnoea scored if associated with at least 4% desaturation (events/hour); CVD, cardiovascular disease; HTN, hypertension; RRDl, R-R interval dips index (dips/hour); %TST<90, total sleep time spent less than 90% on oxygen saturation signal (%).



Log-Rank p=0.0139 for total RRDI

Not at Risk	0	2	4	6	8	10	12	14
Tertile 1	188	176	156	128	88	68	29	1
Tertile 2	193	182	160	134	106	79	35	2
Tertile 3	188	175	163	138	116	86	29	1

Figure 5 Kaplan-Meier estimates of the likelihood of survival according to total RRDI severity: Category 1 as a reference (RRDI <15.1), category 2 (RRDI 15.1–<28.4) and category 3 (RRDI ≥28.4) (n=569); log-rank test for differences in survival by RRDI category; survival was lower for category 3 compared with group 1 and 2. RRDI is a mean number of RRI dips/hour of total recording time of PSG. PSG, polysomnography; RRDI, R-R interval dips index.

in women. Using total RRDI threshold of 20 dips/hour or more from the adjusted model (model 3) was associated with increased CVD hazards risk of 4.34 in men only (95% CI 1.32 to 14.34, p = 0.016).

DISCUSSION

Our study prospectively examined the relationship of nocturnal beat-to-beat RRI changes, that is, brief accelerations in the heart rate, with long-term cardiovascular outcomes. The study revealed several important and

novel findings. First, increased frequency of heart rate accelerations (RRDI) during sleep study was associated with the development of cardiac events or mortality in a prospective large community-based cohort of individuals over a follow-up interval of 15 years, who had no known heart disease at the time of their sleep study. Second, the relationship between total RRDI and incidence of CVD remained significant after adjusting for demographics, SDB severity using AHI 4%, hypoxemia and other comorbidities. Third, the frequency of total RRDI was higher in men than in women and associated with CVD predominantly in men.

To our knowledge, this is the first community-based cohort that has shown an association between adverse cardiovascular outcomes and heart rate changes during sleep. Specifically, we found that the association between RRDI and incidence of new CVD events was independent of AHI (with a 4% desaturation threshold for hypopnoea scoring) and hypoxia. Prior studies assessed the relationship between AHI using different thresholds and adverse cardiovascular consequences. For example, in the Sleep Heart Health Study, Punjabi, et al. found an association of cardiovascular morbidity with SDB characterised by breathing events defined as having ≥4% desaturations, but not by SDB characterised by desaturations of less than 4%.¹⁹ More recently, it has been found that the desaturation hypoxic burden related to respiratory events, measured by the integration of the severity of the desaturation and its length, predicted CVD mortality.²⁰ Another study that examined heart rate variability during sleep found that SDB patients had shorter RRI and increased sympathetic burst frequency (49±4bursts/min) compared with control subjects.²¹ The authors speculated that abnormalities in heart rate and blood pressure variability might be implicated in the subsequent development of

Table 4 The adjusted time to event Cox Proportional Hazards Models for sleep RRDI predicting the incidence of CVD event

	CVD events n (%)	HR (95% CI) p value			
		Unadjusted model (1)	Adjusted model (2)	Adjusted model (3)	Adjusted model (4)
Continuous RRDI (10-unit increment)	24/569 (4)	1.21 (1.09 to 1.35) 0.0006	1.29 (1.06 to 1.33) 0.0037	1.20 (1.07 to 1.34) 0.0015	1.19 (1.06 to 1.33) 0.003
RRDI category					
Tertile 1 (<9.0)	4/187 (2)	REF	REF	REF	REF
Tertile 2 (9.0–<23.5)	9/194 (5)	2.61 (0.79 to 8.57) 0.1144	2.46 (0.75 to 8.11) 0.1383	2.66 (0.80 to 8.77) 0.1092	2.79 (0.83 to 9.36) 0.10
Tertile 3 (≥23.5)	11/188 (6)	3.39 (1.06 to 10.84) 0.0398	2.94 (0.91 to 9.56) 0.0729	3.61 (1.08 to 12.10) 0.0373	4.00 (1.17 to 13.68) 0.027
P-trend		0.0392	0.0768	0.0365	0.026

Model (1) is unadjusted. Model (2) is adjusted for age, sex and body mass index. Model (3) is additionally adjusted for age, sex, body mass index and AHI (4% criteria). Model (4) is additionally adjusted for diabetes, HTN, stroke, and smoking, average HR, and %TST<90%. AHI 4%, apnoea-hypopnoea index with hypopnoea scored if associated with at least 4% desaturation (events/hour); CVD, cardiovascular disease; HTN, hypertension; RRDI, R-R interval dips index (dips/hour); %TST<90, total sleep time spent less than 90% on oxygen saturation signal (%).

Table 5 The adjusted time to event Cox Proportional Hazards Models for RRDI during non-REM sleep predicting the incidence of CVD event

	CVD events	HR (95% CI) p value		
	n (%)	Unadjusted model (1)	Adjusted model (2)	Adjusted model (3)
Continuous RRDI (10-unit increment)	24/569 (4)	1.19 (1.08 to 1.33) 0.0009	1.17 (1.05 to 1.31) 0.0044	1.18 (1.07 to 1.32) 0.0019
RRDI category				
Tertile 1 (<8.5)	4/187 (2)	REF	REF	REF
Tertile 2 (8.5–<22.6)	9/194 (5)	2.69 (0.82 to 8.86) 0.1040	2.69 (0.82 to 8.82) 0.1023	2.85 (0.87 to 9.36) 0.0849
Tertile 3 (≥22.6)	11/188 (6)	3.40 (1.06 to 10.94) 0.0389	3.11 (0.96 to 10.06) 0.0577	3.92 (1.18 to 13.09) 0.0263
P-trend		0.0390	0.0612	0.0249

Model (1) is unadjusted. Model (2) is adjusted for age, sex and body mass index. Model (3) is additionally adjusted for age, sex, body mass index and AHI (4% criteria).

AHI 4%, apnoea–hypopnoea index with hypopnoea scored if associated with at least 4% desaturation (events/hour); CVD, cardiovascular disease; REM, rapid eye movement; RRDI, R-R interval dips index (dips/hour).

cardiovascular disease in patients with SDB. The present study confirmed this association between the frequency of heart rate accelerations (RRDI) and adverse cardiovascular consequence in a prospective large cohort of individuals who had no known heart disease at the time of their sleep study.

The mechanism of increased incidence of CVD and association with RRDI can be explained by an increased sympathetic tone and autonomic arousals. First, sleep disturbances like SDB, periodic limb movements, insufficient sleep are all associated with an increased risk of CVD. These sleep disorders are commonly associated with impaired autonomic nervous system leading to increased

sympathetic tone.²² Furthermore, sleep fragmentation due to autonomic or respiratory arousals from sleep increases the cardiac sympathetic tone activity resulting in a sudden elevation in vascular tone and heart rate generating a rise in arterial blood pressure.^{22 23} The increased sympathetic tone in patients with heart disease has been proposed as an intermediate outcome linking heart rate variability with increased mortality.²⁴ Resting heart rate also has been linked to CVD in patients with SDB and chronic obstructive pulmonary disease (COPD).^{21 25 26} Second, the augmented shear forces due to intermittent episodes of tachycardia secondary to respiratory events and the resultant mechanical shear forces may lead to

Table 6 The adjusted time to event Cox Proportional Hazards Models for RRDI during REM sleep predicting the incidence of CVD event

	CVD events	HR (95% CI) p value		
	n (%)	Unadjusted model (1)	Adjusted model (2)	Adjusted model (3)
Continuous RRDI (10-unit increment)	24/569 (4)	1.19 (1.07 to 1.32) 0.0013	1.17 (1.05 to 1.31) 0.0056	1.19 (1.07 to 1.33) 0.0016
RRDI category				
Tertile 1 (<9.0)	5/187 (3)	REF	REF	REF
Tertile 2 (9.0–<24.0)	7/194 (4)	1.34 (0.42 to 4.24) 0.6222	1.19 (0.37 to 3.78) 0.7732	1.24 (0.39 to 4.00) 0.7171
Tertile 3 (≥24.0)	12/188 (6)	2.92 (1.00 to 8.55) 0.0508	2.42 (0.80 to 7.29) 0.1173	2.69 (0.88 to 8.19) 0.0825
P-trend		0.0393	0.0936	0.0657

Model (1) is unadjusted. Model (2) is adjusted for age, sex and body mass index. Model (3) is additionally adjusted for age, sex, body mass index and AHI (4% criteria).

AHI 4%, apnoea–hypopnoea index with hypopnoea scored if associated with at least 4% desaturation (events/hour); CVD, cardiovascular events; REM, rapid eye movement; RRDI, R-R interval dips index (dips/hour).

Table 7 A summary of CVD events types

First event type*	CVD events types (n=24)
	n (%)
Myocardial infarction	9 (36)
Heart failure	4 (17)
Pacemaker	2 (8)
CAD/intervention	12 (48)
Bypass surgery	1 (4)
CVD death	5 (20)

*Individuals could have multiple type events (for example myocardial infarction and stent and coronary artery disease). If multiple events were reported over the course of follow-up, the first reported event was used in this analysis.

endothelial dysfunction.²⁷ This possibility is physiologically plausible particularly in male patients,²⁸ who may have significant endothelial dysfunction secondary to activation of several inflammatory pathways. Such pathophysiological changes in untreated SDB patients have been linked to nocturnal angina, myocyte necrosis leading to cardiomyopathy and cardiac remodelling.²⁹⁻³¹ Our findings corroborate these pathological changes particularly in the coronary vessels making the vast majority of CVD

events either related to coronary artery disease (CAD) (48%) or myocardial infarction (36%), predominantly in men. On the other hand, medications such as Beta-blockers attenuate the increase in heart rate related to respiratory events during sleep in patients with hypertension and untreated SDB.³² This modulation of cardiac responses in patients with SDB provides a mechanism by which Beta-blockers may decrease the risk of sudden cardiac death, particularly in patients with CVD.³³ Finally, hypoxic events can affect the autonomic cardiac response and generate significant RRI dips events.³⁴ Hypoxemia and RRI dips may represent different features of SDB-related stress, both of which may contribute to CVD morbidity and mortality through independent pathways. Our findings suggest a need to further identify the intermediate mechanisms that link RRI dips events to long-term outcomes.

This study has several strengths including its prospective design with longitudinal follow-up of participants, community-based including a diverse group of ages and morbidities from both genders, and the use of the gold-standard laboratory-based polysomnography for assessment of SDB. This study assessed the role of heart rate changes, a heritable and physiological phenotype, on CVD outcome. These findings can allow clinicians

Table 8 Adjusted time to event Cox Proportional Hazards Models for RRI Predicting Incidence of CVD Event Stratified by Gender for Continuous RRI and across Categories of Participants with RRI more than 20 dips/hour.

	Males (n=300)	Females (n=269)	P value
Age, mean (SD)	58 (8)	58 (8)	0.52
Body mass index, kg/m ² , mean (SD)	30 (5)	32 (7)	0.0001
AHI, mean (SD)	13 (16)	10 (12)	0.0045
RRDI (SLEEP), mean (SD)	26 (24)	18 (21)	<0.0001
RRDI (ALL), mean (SD)	30 (23)	22 (20)	<0.0001
RRDI (SLEEP)>20, n (%)	143 (48)	78 (29)	<0.0001
RRDI (ALL)>20, n (%)	179 (60)	105 (39)	<0.0001
	Adjusted model (3)* (95% CI)	Adjusted model (3) * (95% CI)	
	p value	p value	
Continuous RRI (SLEEP) (10-unit increment)	1.19 (1.04 to 1.36)	1.22 (0.96 to 1.54)	0.011
		0.109	
RRDI category			
<20	REF	REF	
>20	1.85 (0.67 to 5.07)	1.29 (0.22 to 7.47)	0.234
		0.779	
Continuous RRI (ALL) (10-unit increment)	1.22 (1.06 to 1.40)	1.25 (0.97 to 1.67)	0.006
		0.086	
RRDI category			
<20	REF	REF	
>20	4.34 (1.32 to 14.34)	2.03 (0.38 to 10.77)	0.016
		0.407	

*Model adjusted for age, body mass index and AHI 4% categories.

AHI 4%, apnoea-hypopnoea index with hypopnoea scored if associated with at least 4% desaturation (events/hour); RRI, R-R interval dips index (dips/hour).

to identify early on high-risk patients and implement an intervention to prevent cardiovascular disease and premature death. In addition, our study used a novel method of automatic detection of heart rate accelerations that can be translated into an executable program or a plug-in for sleep scoring software and can be used in any sleep study across the world. The study has some limitations. First, we used the self-reported diagnosis of CVD (including dates of diagnosis). However, there is evidence that self-reported CVD is very reliable and accurate as noted in the AusDiab cohort. Barr *et al* reported more than 99% of self-reported CVD events were correctly verified in the patients' medical records; only 0.2% of those denying any CVD event being recorded as having had an event on the medical record.³⁵ Second, we lack racial diversity in our study as 95% was reported as a white race. Therefore, the results may not be generalisable to other races. Third, the incidence of CVD in this population is relatively smaller than what was observed in other high cardiovascular risk population. This is might be due to the inclusion of only those who have no prior history of CVD. Finally, the study excluded participants who had pre-existing cardiac disease history (history of any CVD event as listed in table 7), were on CPAP treatment or were on beta-blocker and/or other chronotropic medications, which alter the cardiac autonomic responses, particularly heart rate bursts following respiratory events. Therefore, this study could not include all WSCS participants and might not be applicable to individuals with heart disease or if taking Beta-blocker or chronotropic medications. Likewise, this study might not be applicable to individuals with arrhythmia, frequent ectopic beats and in case of cardiac pacemakers.

Clinical perspectives

The association between heart rate changes and the cardiovascular outcome may have significant clinical implications. First, the association between increased incidence of cardiovascular events and the RRDI suggest that early detection of heart rate fluctuations during sleep could identify those who are at increased risk of future CVD events and inform primary preventions strategies. Second, several behavioural factors³⁶ and medical conditions, such as SDB³⁷ and COPD,²⁶ are associated with changes in resting heart rate, which increase the risk of cardiovascular diseases. Third, the attenuation of heart rate accelerations by Beta-blockers during sleep as recently shown in patients with SDB,³² indicate that Beta-blockers may play an important role in preventing CVD. However, large prospective clinical studies are needed to confirm this finding.

In summary, this study demonstrates that after adjusting for age, BMI, sex, AHI and other comorbidities, people with high RRDI during sleep study are at increased risk for incident CVD events. These results suggest that assessing the ECG of high-risk patients for RRDI during sleep may assist in predicting cardiovascular disease early on. Further research is needed to understand the

pathophysiology of heart rate bursts during sleep and whether the RRI dips provide markers of subclinical cardiac disease or whether their occurrence represents pathophysiological responses to respiratory events that increase the risk of cardiovascular morbidity.

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