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Nocturnal R-R Intervals Dips Index Predicts Cardiovascular Mortality and Morbidity in the Wisconsin Sleep Cohort study

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3 **Nocturnal R-R Intervals Dips Index Predicts Cardiovascular Mortality and**
4 **Morbidity in the Wisconsin Sleep Cohort study**
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53

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11
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15
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17
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23
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ABSTRACT

Rationale: Sleep-disordered breathing (SDB) is strongly linked to adverse cardiovascular outcomes (CVD). Whether heart rate changes measured by nocturnal R-R interval dips (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 analyzed 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 were estimated by Cox proportional hazards in the WSCS.

Results: The 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, 1.10-1.39], $P < 0.001$). After adjusting for demographic factors (age 58 ± 8 years old; 53% male; and BMI 31 ± 7 kg/m²), and apnea-hypopnea index, individuals with highest total nocturnal RRDI category (≥ 28 vs < 15 dips/h) had a significant hazard ratio for increased incidence of CVD and mortality of 7.4 [95% CI, 1.97-27.7], $P = 0.003$). Sleep RRDI was significantly

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3 associated with new-onset CVD event(s) (HR, 1.21 per 10-unit increment in
4
5 RRDl [95% CI, 1.09-1.35], $P < 0.001$) which remained significant after adjusting
6
7 for demographic factors, AHI 4%, hypoxemia and other comorbidities.
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10
11 **Conclusion:** Increased nocturnal RRDl predicts cardiovascular mortality and
12
13 morbidity, independent of the known effects of SDB on beat-to-beat variability.
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15 The frequency of RRDl is higher in men than in women, and is significantly
16
17 associated with new-onset CVD event(s) in men but not in women.
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25 **Keywords:** Heart rate, R-R interval, sleep-disordered breathing, cardiovascular
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27 disease.
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32 **Abbreviations:** BMI, body mass index; CVD, cardiovascular disease; ECG,
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34 electrocardiograph; HR, heart rate; PSG, polysomnography; RRI, R-R interval;
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36 RRDl, RRI dips index; SDB, Sleep-disordered breathing; SHHS, Sleep Heart
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38 Health Study; WSCS, Wisconsin Sleep Cohort Study.
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Article summary

Strengths and limitations of this study:

This study has the following strengths and limitations:

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

Introduction

Sleep-disordered breathing (SDB) is a disorder characterized by the occurrence of recurrent episodes of apnea and hypopnea, resulting in a cascade of physiological responses including hypoxemia, hypercapnia, intrathoracic pressure swings due to the inspiratory effort, activation of the sympathetic nervous system, and arousal from sleep¹. In clinical practice, SDB is defined by the measurement of apnea-hypopnea 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 night². 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 risk³. In addition, a recent study found that varying hypopnea definitions in the general population can affect the risk stratification of cardiovascular disease in patients with SDB⁴.

While recent reports included measurements of sleep fragmentation and respiratory event duration as a surrogate of arousal threshold,⁵ 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 phenotype⁶ independent of covariates, suggesting that

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3 genetic factors play an important role in controlling these cardiovascular risk
4 factors⁷. Therefore, R-R interval, a time domain measure of heart rate variability,
5 may reflect a physiological trait that predicts the risk of adverse cardiovascular
6 outcomes, otherwise missed by SDB severity classification using traditional AHI
7 and desaturation criteria⁸. However, the long-term effect of heart rate changes
8 during sleep on the cardiovascular outcome and mortality is unknown.
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12 The objectives of this study were to examine whether R-R interval (RRI) or heart
13 rate accelerations can serve as predictors of cardiovascular disease in the
14 Wisconsin Sleep Cohort study (WSCS), a prospective community cohort. We
15 hypothesized that increased nocturnal RRI dip index (RRDI) would be associated
16 with increased cardiovascular disease (CVD) or mortality independent of the
17 known effects of SDB on beat-to-beat variability. Results of this study have been
18 previously reported in the form of an abstract.⁹
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35 **Methods**

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38 Participants: We studied individuals from the WSCS. The protocols of the WSCS
39 were approved by the Health Sciences Institutional Review Board of the
40 University of Wisconsin-Madison. All participants provided written informed
41 consent. Sampling and data collection protocols of the WSCS have been
42 described previously¹⁰.
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51 Cohort Description: The WSCS comprises 1546 adult employees of state
52 agencies, ages 30-60 years old at the Cohort's inception, which underwent
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3 attended in-laboratory overnight polysomnography (PSG) and provided health-
4 related questionnaires approximately every four years. Data presented here were
5 collected from August of 2000 through August 2016 (the period when digital PSG
6 recording systems were in use by the WSCS). The most recent available PSG
7 study was used for analysis. WSCS participants were eligible to be included in
8 the study if they had full PSG with adequate ECG recording, not treated for SDB,
9 had no prior CVD event and did not use beta blockers or chronotropic drugs
10 (Table 3S; supplement) on the night of the sleep study or at any other point
11 during follow-up.
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25 Patient and Public Involvement: This study was a secondary analysis for
26 preexisting data from an established cohort of the Wisconsin study. Therefore,
27 participants were not involved in the design, recruitment, or conduct of this
28 study.
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35 Predictor: The main predictor variable is the hourly rate of R-R interval (RRI)
36 changes assessed over an entire night's sleep period. The recorded ECG signals
37 were retrieved from PSG to measure the RRI, which are time intervals between
38 successive pairs of QRS complexes, by using software for the detection of R
39 waves in LabChart 7 with heart rate variability Module (AD Instruments, Colorado
40 Springs, CO) (Fig.1). In this program, ECG signal was examined and retrieved to
41 a MatLab R2017a program (MathWorks, Natick, MA) developed and validated by
42 our group to obtain RRI signal for the entire night (Fig. 2)^{2, 11}. The RRI dips,
43 defined by a decreased RRI compared to the average RRI for the corresponding
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3 1-minute segment as a baseline, were collected. Given that 90% dips threshold
4 correlated previously with most respiratory events (apneic and non-apneic
5 respiratory events, defined below)², total RRI dip index (RRDI) was defined by
6 the number of RRI dips below the 90% baseline divided by the total PSG
7 recording time in hours (from light on to light out), regardless of wake or sleep
8 stages. Sleep RRDI for non-REM and REM stages combined were defined by
9 the number of RRI dips below the 90% baseline divided by the total sleep time in
10 hours (for both REM and non-REM sleep stages). Subsequently, sleep RRDI
11 was calculated for specific sleep stages for REM and non-REM, respectively. In
12 subgroup analysis, the gender differences in total and sleep RRDI were
13 compared between men and women.
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29 The person performing the analysis was blinded to the participant's demographic
30 information. The RRDI values were examined as a continuous variable and as a
31 categorical variable divided into tertiles [lower 25% (low), middle 50% (medium),
32 and upper 25% (high)].
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39 Outcome: Incident CVD events were death related to CVD, self-reported
40 physician-diagnosed heart attack, heart failure, or a CVD procedure (angioplasty,
41 stent, pacemaker, bypass, or defibrillation). Information on CVD events, including
42 the date of the event, was obtained on the overnight health questionnaire. Death
43 certificates and cause of mortality in the cohort were obtained by matching social
44 security numbers with two data sources: The National Death Index (NDI) and the
45 Wisconsin State Bureau of Health Information and Policy, Vial Records Section.
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55 All included participants in this study were able to complete the follow-up
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3 information. The censored analysis was used, and the data is censored at the
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5 last visit. If multiple events were reported over the course of follow-up, the first
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7 reported event was used in this analysis.
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12 Covariates: Participants underwent a baseline overnight 18-channel PSG
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14 (Grass model 78; Quincy, MA) at the University of Wisconsin-Madison Clinical
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16 Research Unit using a standard protocol¹². The PSG recorded sleep state using
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18 electroencephalography, electrooculography, and electromyography; and
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20 breathing, using respiratory inductance plethysmography (Respirace;
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22 Ambulatory Monitoring, Ardsley, NY), nasal and oral airflow (ProTec
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24 thermocouples; Hendersonville, TN) and oxyhemoglobin saturation, using pulse
25
26 oximetry (Ohmeda Biox 3740, Englewood, CO). Each 30-second epoch of the
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28 polysomnographic recordings was scored for sleep stage, and apnea and
29
30 hypopnea events by trained technicians and reviewed using standard criteria¹².
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32 Apnea was defined as cessation of nasal and oral airflow for ≥ 10 seconds and
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34 hypopnea as a discernible reduction in breathing (sum of the chest and
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36 abdominal excursions) with a decrease in oxyhemoglobin saturation of $\geq 4\%$.
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38 The apnea-hypopnea index was calculated as the mean number of apnea and
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40 hypopnea events per hour of sleep.
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51 Statistical analysis:
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3 Cox proportional hazards regression was used to estimate adjusted hazard
4 ratios and 95% CIs for the association between RRD1 and subsequent risk of an
5 incident CVD event¹⁴. Because of the strong dependence of CVD risk on age,
6
7 Cox-regression models were based on age as the time scale, (the age when
8 RRD1 was measured and age at the event) allowing for left truncation (late
9 entry)¹⁵. In addition to adjusting for age using this methodology, models were
10 adjusted for BMI and gender. Models were subsequently adjusted for AHI 4%
11 (as continuous and categorical variables [AHI<5, 5-15, or >15 events/hour]).
12 Subsequently, the model was adjusted for other factors: diabetes, hypertension,
13 stroke, smoking, average HR, % TST < 90%, Kaplan-Meier techniques were
14 used to compare survival across RRD1 categories¹⁶.
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30 **Results**

31 Baseline characteristics:

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35 Table 1 presents the baseline characteristics of the eligible participants. A total
36 of 1440 sleep studies were examined for inclusion in this study as depicted in
37 Figure 3. The final sample included 569 participants (one sleep study per
38 participant) after excluding those on CPAP treatment, individuals who had a
39 prior history of cardiovascular disease, use of beta blocker the night of the study
40 or in other visits during the study, lack of follow up or if they had events before
41 PSG. RRD1 (at 90% threshold) significantly correlated with the following sleep
42 parameters: AHI 4%, periodic legs movements index (PLMI) and respiratory
43 EEG arousals (P<0.001) (Table 2).
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CVD incidence – association with total RRDl:

Using Cox Proportional Hazards Model, continuous total RRDl (with 90% threshold for RRI dips) was significantly associated with new-onset CVD event(s) (HR, 1.24 per 10-unit increment in RRDl [95% CI, 1.10-1.39], $P < 0.001$) which remained significant after adjustment for age, BMI and gender (model 1) and the addition of AHI 4% (model 2) (as depicted in Table 3). Lower thresholds (80%, 70%, and 60%) of RRDl correlated with total RRDl 90% but were less sensitive in predicting CVD (less than 5 individuals attained RRDl >20 dips/h). The association between total RRDl at 90% threshold and the incidence of new-onset CVD remained significant after the adjustment for AHI 4% ($P < 0.001$). Total RRDl category 3 ($\geq 28.4/h$ vs < 15.1 dips/h) was associated with increased CVD hazards risk of 6.1 (95% CI, 1.7-27.7, $P = 0.005$) and remained significant after adjustment for AHI 4% ($P = 0.003$).

Figure 4 illustrates the changes in CVD incidence and hazard ratios for total RRDl less than 15.1 dips per hour (as reference), RRDl 15.1-28.4 dips per hour (second tertile) and for the third group (tertile) of individuals with RRDl equal or more than 28.4 dips per hour. Kaplan-Meier survival curves (Fig.5) illustrate decreased CVD event-free survival with increasing RRDl category from RRDl less than 15.1 to RRDl equal or more than 28.4 dips per hour. Continuous total RRDl 90% remained significant (HR, 1.22 (per 10-unit increment in RRDl [95% CI, 1.08, 1.37], $P = 0.001$) after additionally adjusted for diabetes, hypertension,

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3 stroke, smoking, average heart rate, and % total time with oxygen saturation
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5 less than 90% (as depicted in Table 1S; supplement).
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12 CVD incidence – association with sleep RRDl:
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15 Using Cox Proportional Hazards Model, continuous sleep RRDl 90% was
16 significantly associated with new-onset CVD event(s) (HR, 1.21 per 10-unit
17 increment in RRDl [95% CI, 1.09-1.35], $P < 0.001$) which remained significant
18 after the model adjusted for age, BMI and gender (model 1) and the addition of
19 AHI 4% (model 2) (as depicted in Table 4). RRDl category 3 ($\geq 23.5/h$ vs.
20 $< 9.0/h$) was associated with increased CVD hazards risk of 3.39 (95% CI, 1.06-
21 10.85, $P = 0.04$) and remained significant after adjustment for demographics
22 and AHI 4% ($P = 0.037$). The relationship between sleep RRDl categories and
23 CVD events were predominantly in non-REM sleep as depicted in Table 5.
24 Continuous RRDl 90% during REM sleep was significant (HR, 1.19 per 10-unit
25 increment in RRDl [95% CI, 1.07-1.32], $P = 0.001$) and remained significant
26 after the model adjusted for age, BMI and gender (model 1) and the addition of
27 AHI 4% (model 2) (as depicted in Table 6). However, sleep RRDl category 3
28 ($\geq 24/h$ vs. $< 9.0/h$) was only mildly significant in the unadjusted model with
29 hazards risk of new CVD events of 2.92 ($p=0.05$). Continuous sleep RRDl 90%
30 remained significant (HR, 1.19 per 10-unit increment in RRDl [95% CI, 1.06-
31 1.33], $P = 0.003$) after additionally adjusted for diabetes, hypertension, stroke,
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3 smoking, average heart rate, and % total sleep time with oxygen saturation less
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5 than 90% (as depicted in Table 2S; supplement).
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9 Cardiovascular events were detected in twenty-five participants (4%), of the
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11 sample, over a follow-up interval of 15 years with mean age 59 years old (range
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13 41- 80). Cardiovascular events consisted of heart failure, heart attack, CVD
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15 Procedure (before any events), or CVD Death (Table 7). No difference between
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17 mean age at the start for those who had CVD vs not (P=0.58).
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20 21 Gender effect on the association of CVD incidence with total and sleep RRDl: 22

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24 Table 8 presents the baseline characteristics of men and women participants and
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26 associated total and sleep RRDl. While BMI was higher in women (32.0
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28 ± 7.0 vs. 30.0 ± 5.0 kg/m², p=0.0001), men had higher AHI, total RRDl and sleep
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30 RRDl than women (P<0.01). Using Cox Proportional Hazards adjusted Model (for
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32 age, body mass index, and AHI 4%), continuous total RRDl 90% was
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34 significantly associated with new-onset CVD event(s) in men (HR, 1.22 per 10-
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36 unit increment in RRDl [95% CI, 1.06, 1.40], P < 0.001) but not in women (as
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38 depicted in Table 8). Likewise, continuous sleep RRDl 90% was significantly
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40 associated with new-onset CVD event(s) in men (HR, 1.19 per 10-unit increment
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42 in RRDl [95% CI, 1.04, 1.36], P < 0.05) but not in women. Using total RRDl
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44 threshold of 20 dips per hour or more was associated with increased CVD
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46 hazards risk of 4.34 in men only (95% CI, 1.32, 14.34, P = 0.016).
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Discussion

Our study prospectively examined the relationship of nocturnal beat-to-beat RR interval changes, i.e. 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 hypopnea 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 (SHHS), Punjabi, et al. (2008) found an association of cardiovascular morbidity with SDB characterized by breathing events defined as having $\geq 4\%$ de-saturations, but not by SDB characterized by de-saturations of $< 4\%$ ¹⁷. More recently, it has been

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2
3 found that the desaturation hypoxic burden related to respiratory events,
4 measured by the integration of the severity of the desaturation and its length,
5 predicted CVD mortality¹⁸. Another study that examined heart rate variability
6 during sleep found that SDB patients had shorter RRI and increased sympathetic
7 burst frequency (49±4 bursts/min) compared with control subjects¹⁹. The authors
8 speculated that abnormalities in heart rate and blood pressure variability might
9 be implicated in the subsequent development of cardiovascular disease in
10 patients with SDB. The present study confirmed this association between the
11 frequency of heart rate accelerations (RRDI) and adverse cardiovascular
12 consequence in a prospective large cohort of individuals who had no known
13 heart disease at the time of their sleep study.
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30 The mechanism of increased incidence of CVD and association with RRDI can
31 be explained by an increased sympathetic tone and autonomic arousals. First,
32 sleep disturbances like SDB, periodic limb movements, insufficient sleep are all
33 associated with an increased risk of CVD. These sleep disorders are commonly
34 associated with impaired autonomic nervous system leading to increased
35 sympathetic tone²⁰. Furthermore, sleep fragmentation due to autonomic or
36 respiratory arousals from sleep increases the cardiac sympathetic tone activity
37 resulting in a sudden elevation in vascular tone and heart rate generating a rise
38 in arterial blood pressure^{20, 21}. The increased sympathetic tone in patients with
39 heart disease has been proposed as an intermediate outcome linking heart rate
40 variability with increased mortality²². Resting heart rate also has been linked to
41 CVD in patients with SDB and COPD^{19, 23, 24}. Second, the augmented shear
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3 forces due to intermittent episodes of tachycardia secondary to respiratory
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5 events and the resultant mechanical shear forces may lead to endothelial
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7 dysfunction²⁵. This possibility is physiologically plausible particularly in male
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9 patients,²⁶ who may have significant endothelial dysfunction secondary to
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11 activation of several inflammatory pathways. Such pathophysiologic changes in
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13 untreated SDB patients have been linked to nocturnal angina, myocyte necrosis
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15 leading to cardiomyopathy, and cardiac remodeling²⁷⁻²⁹. Our findings corroborate
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17 these pathological changes particularly in the coronary vessels making the vast
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19 majority of CVD events either related to CAD (48%) or myocardial infarction
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21 (36%), predominantly in men. On the other hand, medications such as Beta-
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23 blockers attenuate the increase in heart rate related to respiratory events during
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25 sleep in patients with hypertension and untreated SDB³⁰. This modulation of
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27 cardiac responses in patients with SDB provides a mechanism by which Beta-
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29 blockers may decrease the risk of sudden cardiac death, particularly in patients
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31 with CVD³¹. Finally, hypoxic events can affect the autonomic cardiac response
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33 and generate significant RRI dips events³². Hypoxemia and RRI dips may
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35 represent different features of SDB-related stress, both of which may contribute
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37 to CVD morbidity and mortality through independent pathways. Our findings
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39 suggest a need to further identify the intermediate mechanisms that link RRI dips
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41 events to long-term outcomes.
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50 This study has several strengths including its prospective design with longitudinal
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52 follow-up of participants, community-based including a diverse group of ages and
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54 morbidities from both genders, and the use of the gold-standard laboratory-based
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3 polysomnography for assessment of SDB. This study assessed the role of heart
4 rate changes, a heritable and physiological phenotype, on CVD outcome. These
5 findings can allow clinicians to identify early on high-risk patients and implement
6 an intervention to prevent cardiovascular disease and premature death. In
7 addition, our study used a novel method of automatic detection of heart rate
8 accelerations that can be translated into an executable program or a plug-in for
9 sleep scoring software and can be used in any sleep study across the world. The
10 study has some limitations. First, we used the self-reported diagnosis of CVD
11 (including dates of diagnosis). However, there is evidence that self-reported CVD
12 is very reliable and accurate as noted in the AusDiab cohort. Barr et al. reported
13 more than 99% of self-reported CVD events were correctly verified in the
14 patients' medical records; only 0.2% of those denying any CVD event being
15 recorded as having had an event on the medical record³³. Second, we lack racial
16 diversity in our study as 95% was reported as a white race. Therefore, the results
17 may not be generalizable to other races. Third, the incidence of CVD in this
18 population is relatively smaller than what was observed in other high
19 cardiovascular risk population. This is might be due to the inclusion of only those
20 who have no prior history of CVD. Finally, the study excluded participants who
21 had preexisting cardiac disease history (history of any CVD event as listed in
22 table 7), were on CPAP treatment or were on beta-blocker and/or other
23 chronotropic medications, which alter the cardiac autonomic responses,
24 particularly heart rate bursts following respiratory events. Therefore, this study
25 could not include all WSCS participants and may not be applicable to individuals
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3 with heart disease or if taking Beta-blocker or chronotropic medications.

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5 Likewise, this study may not be applicable to individuals with arrhythmia, frequent
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8 ectopic beats and in case of cardiac pacemakers.
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10 11 12 13 14 **Clinical Perspectives:**

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18 The association between heart rate changes and the cardiovascular outcome
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20 may have significant clinical implications. First, the association between
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22 increased incidence of cardiovascular events and the RRI dips index suggest
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24 that early detection of heart rate fluctuations during sleep could identify those
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26 who are at increased risk of future CVD events and inform primary preventions
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28 strategies. Second, several behavioral factors³⁴ and medical conditions, such as
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30 SDB³⁵ and COPD²⁴, are associated with changes in resting heart rate, which
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32 increase the risk of cardiovascular diseases. Third, the attenuation of heart rate
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34 accelerations by Beta-blockers during sleep as recently shown in patients with
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36 SDB,³⁰ indicate that Beta-blockers may play an important role in preventing CVD.
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41 However, large prospective clinical studies are needed to confirm this finding.
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45 In summary, this study demonstrates that after adjusting for age, BMI, sex, AHI,
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47 and other comorbidities, people with high RRI dips index during sleep study are
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49 at increased risk for incident CVD events. These results suggest that assessing
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51 the ECG of high-risk patients for RRDI during sleep may assist in predicting
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53 cardiovascular disease early on. Further research is needed to understand the
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3 pathophysiology of heart rate bursts during sleep and whether the RRI dips
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5 provide markers of subclinical cardiac disease or whether their occurrence
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7 represents pathophysiological responses to respiratory events that increase the
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9 risk of cardiovascular morbidity.
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For peer review only

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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)
Apnea-Hypopnea 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
Percent Stage 1 Sleep, mean (sd)	10.6 (6.5)
Percent Stage 2 Sleep, mean (sd)	65.0 (9.3)
Percent Stage 3,4 Sleep, mean (sd)	7.8 (8.0)
Percent 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)

Table 2: Pearson Correlation for RRDl (at 90% threshold):

	Correlation Coefficients	P value
AHI 4%	0.18	<.0001
PLMI	0.19	<.0001
Respiratory arousal index	0.24	<.0001
Leg movement arousal index	0.23	<.0001
Spontaneous arousal index	-0.09	0.127

Abbreviations: AHI 4%= apnea-hypopnea index based on hypopnea associated with 4 % desaturation; PLMI=periodic legs movement index; RRDl= R-R interval dips index.

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

	CVD	Hazard Ratio (95% CI)		
	Events	p-value		
	N (%)	Unadjusted Model	Minimally Adjusted Model*	Fully Adjusted Model**
Continuous RREDI (10-unit increment)	24/569 (4)	1.24 (1.10, 1.39) 0.0003	1.22 (1.08, 1.38) 0.0018	1.23 (1.11, 1.38) 0.0007
RREDI Category				
Tertile 1 (< 15.1)	3/187 (2)	REF	REF	REF
Tertile 2 (15.1-< 28.4)	7/194 (4)	2.66 (0.68, 10.34) 0.1586	2.72 (0.70, 10.59) 0.1481	3.16 (0.81, 12.40) 0.099
Tertile 3 (≥ 28.4)	14/188 (7)	6.11 (1.72, 21.72) 0.0052	5.87 (1.60, 21.46) 0.0075	7.40 (1.97, 27.73) 0.003
p-trend		0.0024	0.0045	0.0017

* Adjusted for age, sex, and body mass index.

** Adjusted for age, sex, body mass index, and apnea-hypopnea index

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

	CVD Events	Hazard Ratio (95% CI)		
			p-value	
	N (%)	Unadjusted Model	Minimally Adjusted Model*	Fully Adjusted Model**
Continuous RRDl (10-unit increment)	24/569 (4)	1.21 (1.09, 1.35) 0.0006	1.29 (1.06, 1.33) 0.0037	1.20 (1.07, 1.34) 0.0015
RRDI Category				
Tertile 1 (< 9.0)	4/187 (2)	REF	REF	REF
Tertile 2 (9.0- < 23.5)	9/194 (5)	2.61 (0.79, 8.57) 0.1144	2.46 (0.75, 8.11) 0.1383	2.66 (0.80, 8.77) 0.1092
Tertile 3 (≥ 23.5)	11/188 (6)	3.39 (1.06, 10.84) 0.0398	2.94 (0.91, 9.56) 0.0729	3.61 (1.08, 12.10) 0.0373
p-trend		0.0392	0.0768	0.0365

* Adjusted for age, sex, and body mass index.

** Adjusted for age, sex, body mass index, and apnea-hypopnea index

Table 5: The adjusted time to event Cox Proportional Hazards Models for NREM RRDI predicting the incidence of CVD event.

	CVD Events	Hazard Ratio (95% CI)		
		p-value		
	N (%)	Unadjusted Model	Minimally Adjusted Model*	Fully Adjusted Model**
Continuous RRDI (10-unit increment)	24/569 (4)	1.19 (1.08, 1.33) 0.0009	1.17 (1.05, 1.31) 0.0044	1.18 (1.07, 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, 8.86) 0.1040	2.69 (0.82, 8.82) 0.1023	2.85 (0.87, 9.36) 0.0849
Tertile 3 (≥ 22.6)	11/188 (6)	3.40 (1.06, 10.94) 0.0389	3.11 (0.96, 10.06) 0.0577	3.92 (1.18, 13.09) 0.0263
p-trend		0.0390	0.0612	0.0249

* Adjusted for age, sex, and body mass index.

** Adjusted for age, sex, body mass index, and apnea-hypopnea index

Table 6: The adjusted time to event Cox Proportional Hazards Models for REM RRDl predicting the incidence of CVD event.

	CVD Events	Hazard Ratio (95% CI)		
			p-value	
	N (%)	Unadjusted Model	Minimally Adjusted Model*	Fully Adjusted Model**
Continuous RRDl (10-unit increment)	24/569 (4)	1.19 (1.07, 1.32)	1.17 (1.05, 1.31)	1.19 (1.07, 1.33)
		0.0013	0.0056	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, 4.24)	1.19 (0.37, 3.78)	1.24 (0.39, 4.00)
		0.6222	0.7732	0.7171
Tertile 3 (≥ 24.0)	12/188 (6)	2.92 (1.00, 8.55)	2.42 (0.80, 7.29)	2.69 (0.88, 8.19)
		0.0508	0.1173	0.0825
p-trend		0.0393	0.0936	0.0657

* Adjusted for age, sex, and body mass index.

** Adjusted for age, sex, body mass index, and apnea-hypopnea index

Table 7: A summary of CVD events types.

	CVD events types (n=24)
First Event Type*	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.

Table 8: Adjusted time to event Cox Proportional Hazards Models for RRDl Predicting Incidence of CVD Event Stratified by Gender for Continuous RRDl and across Categories of Participants with RRDl more than 20 dips per 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* (95% CI) p-value	Adjusted Model * (95% CI) p-value	
Continuous RRDl (SLEEP) (10-unit increment)	1.19 (1.04, 1.36) 0.011	1.22 (0.96, 1.54) 0.109	
RRDI Category			
<20	REF	REF	
>20	1.85 (0.67, 5.07) 0.234	1.29 (0.22, 7.47) 0.779	
	Adjusted Model* (95% CI) p-value	Adjusted Model * (95% CI) p-value	
Continuous RRDl (ALL) (10-unit increment)	1.22 (1.06, 1.40) 0.006	1.25 (0.97, 1.67) 0.086	
RRDI Category			
<20	REF	REF	
>20	4.34 (1.32, 14.34) 0.016	2.03 (0.38, 10.77) 0.407	

Abbreviations: AHI 4%, apnea-hypopnea index with hypopnea scored if associated with at least 4 % denaturation (events/hour); HR, hazard ratio; RRDl, R-R interval dips index (dips/hour). * Model Adjusted for age, body mass index, and AHI 4% categories.

Figure Legends

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

Figure 2: A representative computed data of RRI and oxygen saturation (S_aO_2) from one individual during sleep. The red dots represent the RRI dips throughout the duration of the PSG recording (approximately 8 hours). The RRI dips index (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 S_aO_2 from raw ECG and pulse oxymetry recordings, respectively. Abbreviations: ECG, electrocardiogram; ODI, oxygen desaturation index; RRI, R-R intervals; S_aO_2 , oxygen saturation.

Figure 3: The Wisconsin Sleep Cohort Study (WSCS) sample. ECG= electrocardiography; PSG= polysomnography; CPAP= continuous positive airway pressure.

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3 **Figure 4:** Incidence of composite CVD and hazard ratios across different total
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5 RRDl severity: Category 1 as a reference (RRDI < 15.1), category 2 (RRDI 15.1-
6 <28.4), and category 3 (RRDI ≥ 40) (n = 569). CVD=cardiovascular disease;
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8 RRDl=R-R interval dips index. (*) versus unadjusted RRDl <15.1 dips per hour,
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10 P<0.01 ; (**) versus adjusted RRDl <15.1 dips per hour, P<0.01.
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16 **Figure 5:** Kaplan-Meier estimates of the likelihood of survival according to RRDl
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18 severity: Category 1 as a reference (RRDI < 15.1), category 2 (RRDI 15.1- <
19 28.4), and category 3 (RRDI ≥ 28.4) (n = 569); log-rank test for differences in
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21 survival by RRDl category; Survival was lower for category 3 compared to group
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23 1 and 2. RRDl is a mean number of RRI dips/hr of total recording time of PSG.
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25 RRDl=R-R interval dips index.
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Supplements Materials:

Analysis supplement:

Table 1S: Adjusted Time to Event Cox Proportional Hazards Models for RRDI Predicting Incidence of Composite CVD Event for Continuous RRDI and across Categories of **All RRDI**

	N	Model 1 Adjusted for Age, BMI, Gender, AHI categories Adjusted HR* (95% CI) P-value	Model 2 Additionally adjusted for Diabetes, HTN, Stroke, and Smoking Adjusted HR* (95% CI) P-value	Model 3 Additionally adjusted for Average HR, % TST < 90% Adjusted HR ** (95% CI) P-value
Continuous RRDI (10-unit increment)	24/569 (4)	1.23 (1.109, 1.38) 0.0007	1.21 (1.08, 1.37) 0.0014	1.22 (1.08, 1.37) 0.0012
RRDI Category				
Tertile 1 (< 15.1)	3/187 (2)	REF	REF	REF
Tertile 2 (15.1- < 28.4)	7/194 (4)	3.16 (0.81, 12.40) 0.099	3.22 (0.82, 12.64) 0.094	3.22 (0.80, 12.93) 0.10
Tertile 3 (≥ 28.4)	14/188 (7)	7.40 (1.97, 27.73) 0.003	7.48 (1.98, 28.25) 0.003	8.99 (2.35, 34.40) 0.001
p-trend		0.0017	0.0017	0.0006

Abbreviations: AHI 4%, apnea-hypopnea index with hypopnea scored if associated with at least 4 % denaturation (events/hour); HR, hazard ratio; RRDI,

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3 R-R interval dips index (dips/hour); HTN, hypertension; % TST<90, total sleep
4 time spent less than 90% on oxygen saturation signal (%). * Model 1 Adjusted for
5 age, body mass index, and AHI 4% categories. * Model 2 adjusted for age, body
6 mass index, AHI 4% categories, Diabetes, HTN, stroke, and smoking. ** Model 3
7 additionally adjusted for average heart rate, and % TST < 90%.
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Table 2S: Adjusted Time to Event Cox Proportional Hazards Models for RRDl Predicting Incidence of Composite CVD Event for Continuous RRDl and across Categories of **sleep RRDl**

	N	Model 1 Adjusted for Age, BMI, Gender, AHI categories	Model 2 Additionally adjusted for Diabetes, HTN, Stroke, and Smoking	Model 3 Additionally adjusted for Average HR, % TST < 90%
		Adjusted HR* (95% CI) P-value	Adjusted HR* (95% CI) P-value	Adjusted HR ** (95% CI) P-value
Continuous RRDl (10-unit increment)	24/569 (4)	1.20 (1.07, 1.34) 0.0015	1.19 (1.06, 1.33) 0.003	1.19 (1.06, 1.33) 0.003
RRDl Category				
Tertile 1 (< 9.0)	4/187 (2)	REF	REF	REF
Tertile 2 (9.0- < 23.5)	9/194 (5)	2.66 (0.80, 8.77) 0.1092	2.70 (0.81, 8.98) 0.10	2.79 (0.83, 9.36) 0.10
Tertile 3 (≥ 23.5)	11/188 (6)	3.61 (1.08, 12.10) 0.0373	3.60 (1.07, 12.06) 0.038	4.00 (1.17, 13.68) 0.027
p-trend		0.0365	0.038	0.026

Abbreviations: AHI 4%, apnea-hypopnea index with hypopnea scored if associated with at least 4 % denaturation (events/hour); HR, hazard ratio; RRDl, R-R interval dips index (dips/hour); HTN, hypertension; % TST<90, total sleep time spent less than 90% on oxygen saturation signal (%). * Model 1 Adjusted for

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3 age, body mass index, and AHI 4% categories. * Model 2 adjusted for age, body
4 mass index, AHI 4% categories, Diabetes, HTN, stroke, and smoking. ** Model 3
5 additionally adjusted for average heart rate, and % TST < 90%.
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Table 3S. Medications were taken by individuals excluded due to the potential effect on heart rate.

Medication name (Brand)
Metoprolol tartrate (Lopressor, nebivolol)
Enalapril maleate (Vasotec)
Nadolol (Corgard)
Atenolol (Tenormin, Zebeta)
Betaxolol hydrochloride (Kerlone)
Acebutolol hydrochloride (Sectral)
Clonidine (Catapres, other alpha adrenergic agonist agents)
Atenolol & Chlorthalidone (Tenoretic)
Metoprolol succinate (Toprol XL)
Diazac (dup see 604)
Labetalol Hydrochloride (Normodyne, Tradate)
Betachron (Propranolol)
Ziac (Hydrochlorothiazide / Bisoprolol combo)
Carvediol (Coreg, Cartrol)
Pindolol (Visken)
Diltiazem HCL (Cardizem, Dilacor,

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Taztia XT, Tiazac)
Amiodarone HCl (Cordarone, tikosyn)

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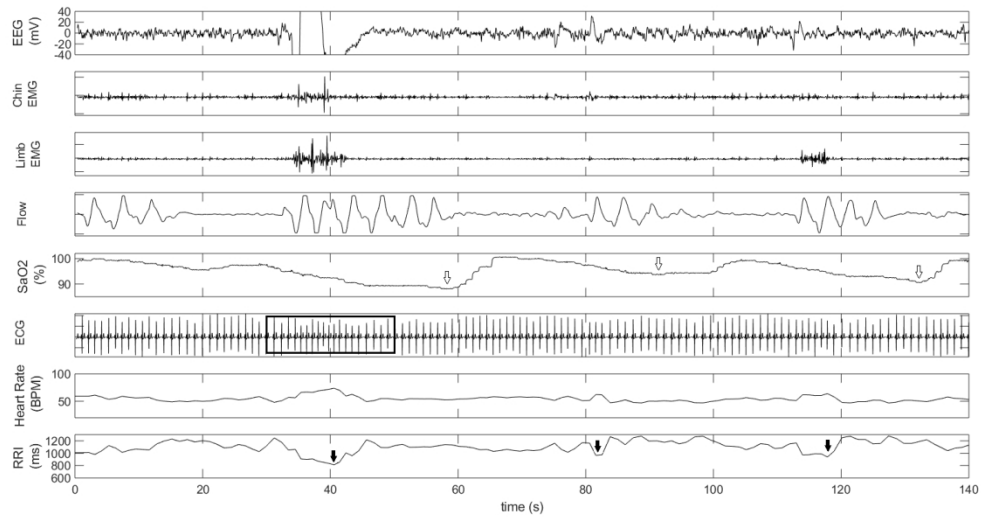


Figure 1-A

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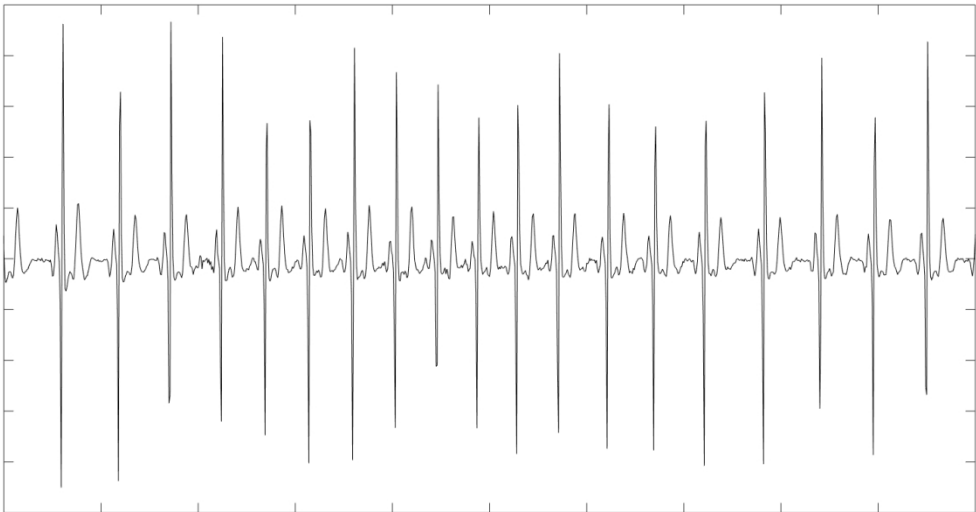


Figure 1-B

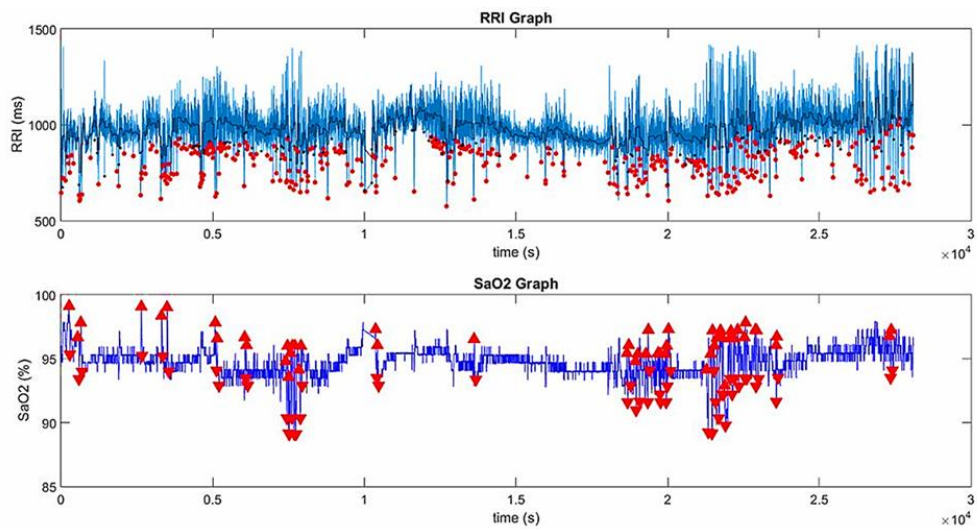


Figure 2

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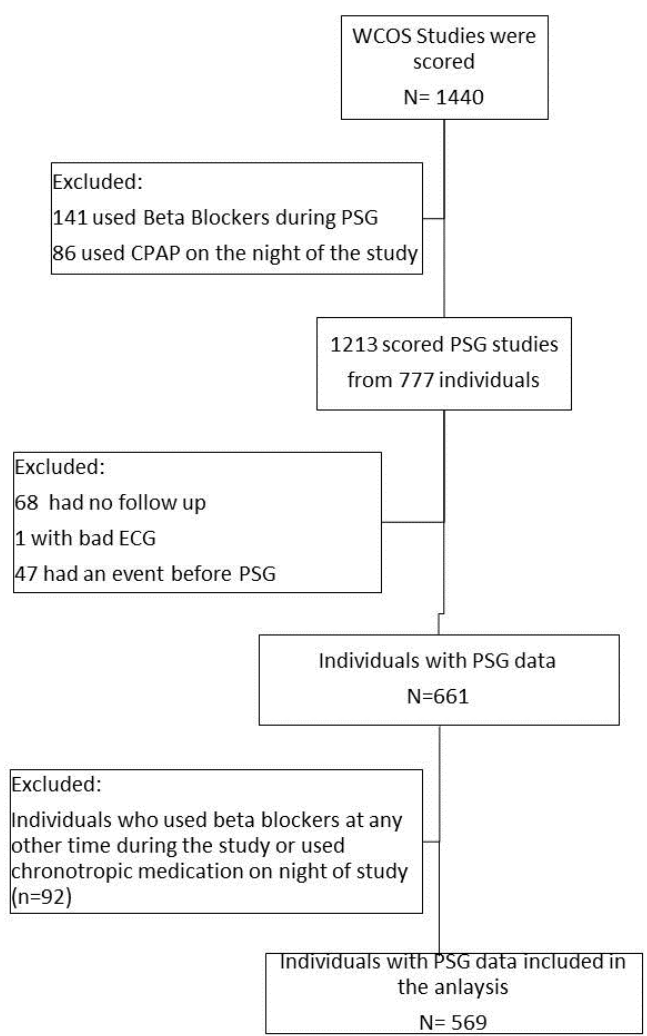


Figure 3

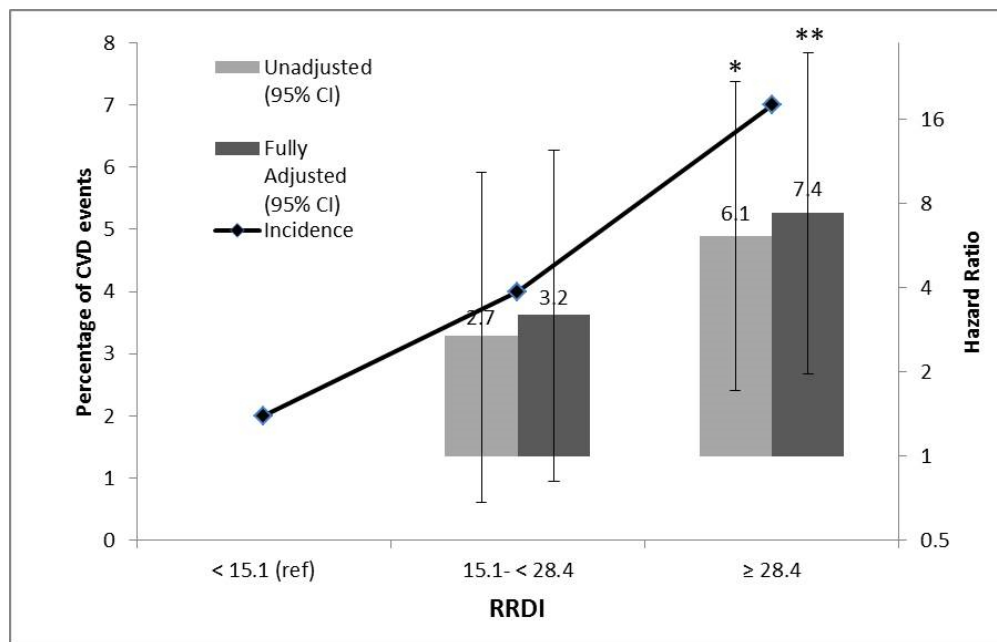
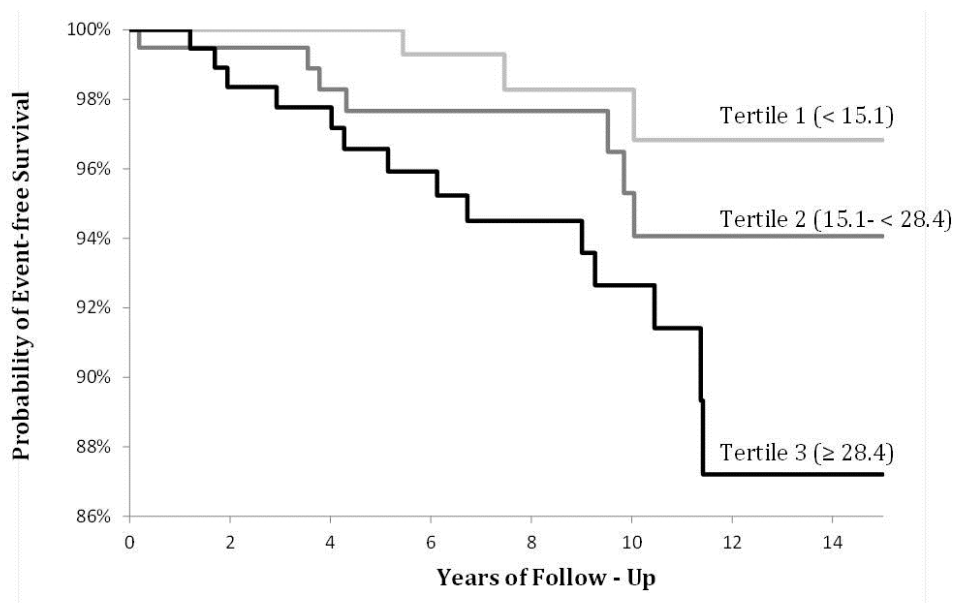


Figure 4

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No at Risk p	0	2	4	6	8	10	12	14
Tertile 1	187	175	155	128	88	68	29	1
Tertile 2	194	182	160	134	106	79	35	2
Tertile 3	188	175	163	138	116	86	29	1

Figure 5

BMJ Open

Longitudinal Effect of Nocturnal R-R Intervals Changes on Cardiovascular Outcome in a Community-Based Cohort

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-030559.R1
Article Type:	Research
Date Submitted by the Author:	28-May-2019
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Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	SLEEP MEDICINE, cardiovascular disease, Heart rate, Cardiac Epidemiology < CARDIOLOGY, EPIDEMIOLOGY

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Manuscripts

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3 **Longitudinal Effect of Nocturnal R-R Intervals Changes on Cardiovascular**
4 **Outcome in a Community-Based Cohort**
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37
38 **Contributorship statement:**
39

40 Conception and design: A. Sankari, M. S. Badr, and P. E. Peppard.
41

42 The acquisition, analysis, or interpretation of data: A. Sankari, M. S. Badr, P. E.
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49 Statistical analysis: L. A. Ravelo, E. W. Hagen, and P. E. Peppard.
50

51 Obtained funding: A. Sankari and P. E. Peppard.
52

53
54 Study supervision: A. Sankari and P. E. Peppard.
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3 **Conflict of interest:** All authors declare no conflict of interest.
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8 **Disclosure Statement:** The following authors A. Sankari, M.S Badr, and S.
9
10 Maresh and Wayne State University have a pending Patent #US62395634,
11
12 entitled "The Detection of Sleep Disordered Breathing Using Cardiac Autonomic
13
14 Responses", application number#15/706097 for Utility/Design using an
15
16 application data sheet (37 CFR 1.54), Date Filed: September 15, 2017. The
17
18 content is solely the responsibility of the authors and does not necessarily
19
20 represent the official views of the Department of Veterans Affairs, National
21
22 Institute of Health or Wayne State University.
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28 **Data Availability:**

29
30 Data may be obtained from a third party and are not publicly available. Upon
31
32 approval from the University of Wisconsin IRB and the PI of Wisconsin Sleep
33
34 Cohort study (Dr Paul Peppard) access to de-identified data may be provided.
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39

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Development of the VA Office of Research and Development from the (U.S.)
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ABSTRACT

Rationale: Sleep-disordered breathing (SDB) is strongly linked to adverse cardiovascular outcomes (CVD). Whether heart rate changes measured by nocturnal R-R interval dips (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 analyzed 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 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, 1.10-1.39], $P < 0.001$). After adjusting for demographic factors (age 58 ± 8 years old; 53% male; and BMI 31 ± 7 kg/m²), and apnea-hypopnea index (AHI 4%), individuals with highest total nocturnal RRDI category (≥ 28 vs < 15 dips/h) had a significant hazard ratio for increased incidence of CVD and mortality of 7.4 [95% CI, 1.97-27.7], $P = 0.003$). Sleep RRDI was significantly associated with new-onset CVD event(s) (HR, 1.21 per

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3 10-unit increment in RRDl [95% CI, 1.09-1.35], $P < 0.001$) which remained
4 significant after adjusting for demographic factors, AHI 4%, hypoxemia, and other
5 comorbidities.
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11 **Conclusion:** Increased nocturnal RRDl predicts cardiovascular mortality and
12 morbidity, independent of the known effects of SDB on beat-to-beat variability.
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14 The frequency of RRDl is higher in men than in women, and is significantly
15 associated with new-onset CVD event(s) in men but not in women.
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25 **Keywords:** Heart rate, R-R interval, sleep-disordered breathing, cardiovascular
26 disease.
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32 **Abbreviations:** AHI, apnea-hypopnea index, BMI, body mass index; CVD,
33 cardiovascular disease; ECG, electrocardiograph; HR, heart rate; PSG,
34 polysomnography; RRI, R-R interval; RRDl, RRI dips index; SDB, Sleep-
35 disordered breathing; SHHS, Sleep Heart Health Study; WSCS, Wisconsin Sleep
36 Cohort Study.
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Article summary

Strengths and limitations of this study:

This study has the following strengths and limitations:

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

Introduction

Sleep-disordered breathing (SDB) is a disorder characterized by the occurrence of recurrent episodes of apnea and hypopnea, resulting in a cascade of physiological responses including hypoxemia, hypercapnia, intrathoracic pressure swings due to the inspiratory effort, activation of the sympathetic nervous system, and arousal from sleep¹. In clinical practice, SDB is defined by the measurement of apnea-hypopnea 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 night². 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 risk³. In addition, a recent study found that varying hypopnea definitions in the general population can affect the risk stratification of cardiovascular disease in patients with SDB^{4, 5}.

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

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3 genetic factors play an important role in controlling these cardiovascular risk
4 factors⁸. Therefore, R-R interval, a time domain measure of heart rate variability,
5 may reflect a physiological trait that predicts the risk of adverse cardiovascular
6 outcomes, otherwise missed by SDB severity classification using traditional AHI
7 and desaturation criteria⁹. However, the long-term effect of heart rate changes
8 during sleep on the cardiovascular outcome and mortality is unknown.
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18 The objectives of this study were to examine whether R-R interval (RRI) or heart
19 rate accelerations can serve as predictors of cardiovascular disease in the
20 Wisconsin Sleep Cohort study (WSCS), a prospective community cohort. We
21 hypothesized that increased nocturnal RRI dip index (RRDI) would be associated
22 with increased cardiovascular disease (CVD) or mortality independent of the
23 known effects of SDB on beat-to-beat variability. Results of this study have been
24 previously reported in the form of an abstract^{10, 11}.
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35 **Methods**

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38 Participants: We studied individuals from the WSCS. The protocols of the WSCS
39 were approved by the Health Sciences Institutional Review Board of the
40 University of Wisconsin-Madison. All participants provided written informed
41 consent. Sampling and data collection protocols of the WSCS have been
42 described previously¹².
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51 Cohort Description: The WSCS comprises 1546 adult employees of state
52 agencies, ages 30-60 years old at the Cohort's inception, which underwent
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3 attended in-laboratory overnight polysomnography (PSG) and provided health-
4 related questionnaires approximately every four years. Data presented here were
5 collected from August of 2000 through August 2016 (the period when digital PSG
6 recording systems were in use by the WSCS). The most recent available PSG
7 study was used for analysis. WSCS participants were eligible to be included in
8 the study if they had full PSG with adequate ECG recording, not treated for SDB,
9 had no prior CVD event and did not use beta blockers or chronotropic drugs
10 (Table 1S; supplement) on the night of the sleep study or at any other point
11 during follow-up.
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25 Patient and Public Involvement: This study was a secondary analysis for
26 preexisting data from an established cohort of the Wisconsin study. Therefore,
27 participants were not involved in the design, recruitment, or conduct of this
28 study.
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35 Predictor: The main predictor variable is the hourly rate of R-R interval (RRI)
36 changes assessed over an entire night's sleep period. The recorded ECG signals
37 were retrieved from PSG to measure the RRI, which are time intervals between
38 successive pairs of QRS complexes, by using software for the detection of R
39 waves in LabChart 7 with heart rate variability Module (AD Instruments, Colorado
40 Springs, CO) (Fig.1). In this program, ECG signal was examined and retrieved to
41 a MatLab R2017a program (MathWorks, Natick, MA) developed and validated by
42 our group to obtain RRI signal for the entire night (Fig. 2)^{13, 14}. The RRI dips,
43 defined by a decreased RRI compared to the average RRI for the corresponding
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3 1-minute segment as a baseline, were collected. Given that 90% dips threshold
4 correlated previously with most respiratory events (apneic and non-apneic
5 respiratory events, defined below)¹⁴, total RRI dip index (RRDI) was defined by
6 the number of RRI dips below the 90% baseline divided by the total PSG
7 recording time in hours (from light on to light out), regardless of wake or sleep
8 stages. Sleep RRDI for non-REM and REM stages combined were defined by
9 the number of RRI dips below the 90% baseline divided by the total sleep time in
10 hours (for both REM and non-REM sleep stages). Subsequently, sleep RRDI
11 was calculated for specific sleep stages for REM and non-REM, respectively. In
12 subgroup analysis, the gender differences in total and sleep RRDI were
13 compared between men and women.
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29 The person performing the analysis was blinded to the participant's demographic
30 information. The RRDI values were examined as a continuous variable and as a
31 categorical variable divided into tertiles [lower 25% (low), middle 50% (medium),
32 and upper 25% (high)].
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39 Outcome: Incident CVD events were death related to CVD, self-reported
40 physician-diagnosed heart attack, heart failure, or a CVD procedure (angioplasty,
41 stent, pacemaker, bypass, or defibrillation). Information on CVD events, including
42 the date of the event, was obtained on the overnight health questionnaire. Death
43 certificates and cause of mortality in the cohort were obtained by matching social
44 security numbers with two data sources: The National Death Index (NDI) and the
45 Wisconsin State Bureau of Health Information and Policy, Vial Records Section.
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55 All included participants in this study were able to complete the follow-up
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3 information. The censored analysis was used, and the data is censored at the
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5 last visit. If multiple events were reported over the course of follow-up, the first
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7 reported event was used in this analysis.
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12 Covariates: Participants underwent a baseline overnight 18-channel PSG
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14 (Grass model 78; Quincy, MA) at the University of Wisconsin-Madison Clinical
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16 Research Unit using a standard protocol¹⁵. The PSG recorded sleep state using
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18 electroencephalography, electrooculography, and electromyography; and
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20 breathing, using respiratory inductance plethysmography (Respitrace;
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22 Ambulatory Monitoring, Ardsley, NY), nasal and oral airflow (ProTec
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24 thermocouples; Hendersonville, TN) and oxyhemoglobin saturation, using pulse
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26 oximetry (Ohmeda Biox 3740, Englewood, CO). Each 30-second epoch of the
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28 polysomnographic recordings was scored for sleep stage, and apnea and
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30 hypopnea events by trained technicians and reviewed using standard criteria¹⁵.
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32 Apnea was defined as the cessation of nasal and oral airflow for ≥ 10 seconds
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34 and hypopnea as a discernible reduction in breathing (sum of the chest and
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36 abdominal excursions) with a decrease in oxyhemoglobin saturation of $\geq 4\%$.
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38 The apnea-hypopnea index was calculated as the mean number of apnea and
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40 hypopnea events per hour of sleep.
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48 Statistical analysis:
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52 Cox proportional hazards regression was used to estimate adjusted hazard
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54 ratios and 95% CIs for the association between RRDl and subsequent risk of an
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3 incident CVD event¹⁶. Because of the strong dependence of CVD risk on age,
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incident CVD event¹⁶. Because of the strong dependence of CVD risk on age, Cox-regression models were based on age as the time scale, (the age when RRDl 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 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 HR, % TST < 90%, Kaplan-Meier techniques were used to compare survival across RRDl categories¹⁸.

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 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. RRDl (at 90% threshold) significantly correlated with the following sleep parameters: AHI 4%, periodic legs movements index (PLMI) and respiratory EEG arousals (P<0.001) (Table 2).

CVD incidence – association with total RRDl:

Using Cox Proportional Hazards Model, continuous total RRDl (with 90% threshold for RRI dips) was significantly associated with new-onset CVD event(s) (HR, 1.24 per 10-unit increment in RRDl [95% CI, 1.10-1.39], $P < 0.001$) 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 RRDl correlated with total RRDl 90% but were less sensitive in predicting CVD (less than 5 individuals attained RRDl >20 dips/h). The association between total RRDl at 90% threshold and the incidence of new-onset CVD remained significant after the adjustment for AHI 4% (model 3) ($P < 0.001$). Total RRDl category 3 ($\geq 28.4/h$ vs < 15.1 dips/h) was associated with increased CVD hazards risk of 6.1 (95% CI, 1.7-27.7, $P = 0.005$) and remained significant after adjustment for AHI 4% ($P = 0.003$). Continuous total RRDl 90% remained significant (HR, 1.22 (per 10-unit increment in RRDl [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 RRDl less than 15.1 dips per hour (as reference), RRDl 15.1-28.4 dips per hour (second tertile) and for the third group (tertile) of individuals with RRDl equal or more than 28.4 dips per hour. Kaplan-Meier survival curves (Fig.5) illustrate

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3 decreased CVD event-free survival with increasing total RRDl category from
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5 RRDl less than 15.1 to RRDl equal or more than 28.4 dips per hour.
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12 CVD incidence – association with sleep RRDl:
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15 Using Cox Proportional Hazards Model, continuous sleep RRDl 90% was
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17 significantly associated with new-onset CVD event(s) (HR, 1.21 per 10-unit
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19 increment in RRDl [95% CI, 1.09-1.35], $P < 0.001$) which remained significant
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21 after the model adjusted for age, BMI and gender (model 2) and the addition of
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23 AHI 4% (model 3) (as depicted in Table 4). RRDl category 3 ($\geq 23.5/h$ vs.
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25 $< 9.0/h$) was associated with increased CVD hazards risk of 3.39 (95% CI, 1.06-
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27 10.85, $P = 0.04$) and remained significant after adjustment for demographics
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29 and AHI 4% (model 4) ($P = 0.037$). The relationship between sleep RRDl
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31 categories and CVD events were predominantly in non-REM sleep as depicted
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33 in Table 5. Continuous RRDl 90% during REM sleep was significant (HR, 1.19
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35 per 10-unit increment in RRDl [95% CI, 1.07-1.32], $P = 0.001$) in the unadjusted
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37 model (model 1) and remained significant after the model adjusted for age, BMI
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39 and gender (model 2) and the addition of AHI 4% (model 3) (as depicted in
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41 Table 6). However, sleep RRDl category 3 ($\geq 24/h$ vs. $< 9.0/h$) was only
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43 significant in the unadjusted model (model 1) with hazards risk of new CVD
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45 events of 2.92 ($p=0.04$).
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53 Cardiovascular events were detected in twenty-five participants (4%), of the
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55 sample, over a follow-up interval of 15 years with mean age 59 years old (range
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3 41- 80). Cardiovascular events consisted of heart failure, heart attack, CVD
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5 Procedure (before any events), or CVD Death (Table 7). No difference between
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7 mean age at the start for those who had CVD vs not (P=0.58).
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11 Gender effect on the association of CVD incidence with total and sleep RRDl:
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14 Table 8 presents the baseline characteristics of men and women participants and
15 associated total and sleep RRDl. While BMI was higher in women (32.0
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17 ± 7.0 vs. 30.0 ± 5.0 kg/m², P=0.0001), men had higher AHI, total RRDl and sleep
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19 RRDl than women (P<0.01). Using Cox Proportional Hazards adjusted model (3)
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21 (for age, body mass index, and AHI 4%), continuous total RRDl 90% was
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23 significantly associated with new-onset CVD event(s) in men (HR, 1.22 per 10-
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25 unit increment in RRDl [95% CI, 1.06, 1.40], P < 0.001) but not in women (as
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27 depicted in Table 8). Likewise, continuous sleep RRDl 90% was significantly
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29 associated with new-onset CVD event(s) in men (HR, 1.19 per 10-unit increment
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31 in RRDl [95% CI, 1.04, 1.36], P < 0.05) but not in women. Using total RRDl
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33 threshold of 20 dips per hour or more from the adjusted model (model 3) was
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35 associated with increased CVD hazards risk of 4.34 in men only (95% CI, 1.32,
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37 14.34, P = 0.016).
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Discussion

Our study prospectively examined the relationship of nocturnal beat-to-beat RR interval changes, i.e. 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 hypopnea 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 (SHHS), Punjabi, et al. (2008) found an association of cardiovascular morbidity with SDB characterized by breathing events defined as having $\geq 4\%$ de-saturations, but not by SDB characterized by de-saturations of less than 4%¹⁹. More recently, it has

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2
3 been found that the desaturation hypoxic burden related to respiratory events,
4 measured by the integration of the severity of the desaturation and its length,
5 predicted CVD mortality²⁰. Another study that examined heart rate variability
6 during sleep found that SDB patients had shorter RRI and increased sympathetic
7 burst frequency (49±4 bursts/min) compared with control subjects ²¹. The authors
8 speculated that abnormalities in heart rate and blood pressure variability might
9 be implicated in the subsequent development of cardiovascular disease in
10 patients with SDB. The present study confirmed this association between the
11 frequency of heart rate accelerations (RRDI) and adverse cardiovascular
12 consequence in a prospective large cohort of individuals who had no known
13 heart disease at the time of their sleep study.
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30 The mechanism of increased incidence of CVD and association with RRDI can
31 be explained by an increased sympathetic tone and autonomic arousals. First,
32 sleep disturbances like SDB, periodic limb movements, insufficient sleep are all
33 associated with an increased risk of CVD. These sleep disorders are commonly
34 associated with impaired autonomic nervous system leading to increased
35 sympathetic tone²². Furthermore, sleep fragmentation due to autonomic or
36 respiratory arousals from sleep increases the cardiac sympathetic tone activity
37 resulting in a sudden elevation in vascular tone and heart rate generating a rise
38 in arterial blood pressure ^{22, 23}. The increased sympathetic tone in patients with
39 heart disease has been proposed as an intermediate outcome linking heart rate
40 variability with increased mortality ²⁴. Resting heart rate also has been linked to
41 CVD in patients with SDB and COPD ^{21, 25, 26}. Second, the augmented shear
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3 forces due to intermittent episodes of tachycardia secondary to respiratory
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5 events and the resultant mechanical shear forces may lead to endothelial
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7 dysfunction²⁷. This possibility is physiologically plausible particularly in male
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9 patients,²⁸ who may have significant endothelial dysfunction secondary to
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11 activation of several inflammatory pathways. Such pathophysiologic changes in
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13 untreated SDB patients have been linked to nocturnal angina, myocyte necrosis
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15 leading to cardiomyopathy, and cardiac remodeling²⁹⁻³¹. Our findings corroborate
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17 these pathological changes particularly in the coronary vessels making the vast
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19 majority of CVD events either related to CAD (48%) or myocardial infarction
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21 (36%), predominantly in men. On the other hand, medications such as Beta-
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23 blockers attenuate the increase in heart rate related to respiratory events during
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25 sleep in patients with hypertension and untreated SDB³². This modulation of
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27 cardiac responses in patients with SDB provides a mechanism by which Beta-
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29 blockers may decrease the risk of sudden cardiac death, particularly in patients
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31 with CVD³³. Finally, hypoxic events can affect the autonomic cardiac response
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33 and generate significant RRI dips events³⁴. Hypoxemia and RRI dips may
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35 represent different features of SDB-related stress, both of which may contribute
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37 to CVD morbidity and mortality through independent pathways. Our findings
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39 suggest a need to further identify the intermediate mechanisms that link RRI dips
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41 events to long-term outcomes.
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50 This study has several strengths including its prospective design with longitudinal
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52 follow-up of participants, community-based including a diverse group of ages and
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54 morbidities from both genders, and the use of the gold-standard laboratory-based
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3 polysomnography for assessment of SDB. This study assessed the role of heart
4 rate changes, a heritable and physiological phenotype, on CVD outcome. These
5 findings can allow clinicians to identify early on high-risk patients and implement
6 an intervention to prevent cardiovascular disease and premature death. In
7 addition, our study used a novel method of automatic detection of heart rate
8 accelerations that can be translated into an executable program or a plug-in for
9 sleep scoring software and can be used in any sleep study across the world. The
10 study has some limitations. First, we used the self-reported diagnosis of CVD
11 (including dates of diagnosis). However, there is evidence that self-reported CVD
12 is very reliable and accurate as noted in the AusDiab cohort. Barr et al. reported
13 more than 99% of self-reported CVD events were correctly verified in the
14 patients' medical records; only 0.2% of those denying any CVD event being
15 recorded as having had an event on the medical record ³⁵. Second, we lack racial
16 diversity in our study as 95% was reported as a white race. Therefore, the results
17 may not be generalizable to other races. Third, the incidence of CVD in this
18 population is relatively smaller than what was observed in other high
19 cardiovascular risk population. This is might be due to the inclusion of only those
20 who have no prior history of CVD. Finally, the study excluded participants who
21 had preexisting cardiac disease history (history of any CVD event as listed in
22 table 7), were on CPAP treatment or were on beta-blocker and/or other
23 chronotropic medications, which alter the cardiac autonomic responses,
24 particularly heart rate bursts following respiratory events. Therefore, this study
25 could not include all WSCS participants and might not be applicable to
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3 individuals with heart disease or if taking Beta-blocker or chronotropic
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5 medications. Likewise, this study might not be applicable to individuals with
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7 arrhythmia, frequent ectopic beats and in case of cardiac pacemakers.
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14 **Clinical Perspectives:**

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18 The association between heart rate changes and the cardiovascular outcome
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20 may have significant clinical implications. First, the association between
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22 increased incidence of cardiovascular events and the RRI dips index suggest
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24 that early detection of heart rate fluctuations during sleep could identify those
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26 who are at increased risk of future CVD events and inform primary preventions
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28 strategies. Second, several behavioral factors³⁶ and medical conditions, such as
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30 SDB³⁷ and COPD²⁶, are associated with changes in resting heart rate, which
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32 increase the risk of cardiovascular diseases. Third, the attenuation of heart rate
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34 accelerations by Beta-blockers during sleep as recently shown in patients with
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36 SDB,³² indicate that Beta-blockers may play an important role in preventing CVD.
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39 However, large prospective clinical studies are needed to confirm this finding.
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45 In summary, this study demonstrates that after adjusting for age, BMI, sex, AHI,
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47 and other comorbidities, people with high RRI dips index during sleep study are
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49 at increased risk for incident CVD events. These results suggest that assessing
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51 the ECG of high-risk patients for RRDI during sleep may assist in predicting
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53 cardiovascular disease early on. Further research is needed to understand the
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3 pathophysiology of heart rate bursts during sleep and whether the RRI dips
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5 provide markers of subclinical cardiac disease or whether their occurrence
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7 represents pathophysiological responses to respiratory events that increase the
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9 risk of cardiovascular morbidity.
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For peer review only

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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)
Apnea-Hypopnea 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
Percent Stage 1 Sleep, mean (sd)	10.6 (6.5)
Percent Stage 2 Sleep, mean (sd)	65.0 (9.3)
Percent Stage 3,4 Sleep, mean (sd)	7.8 (8.0)
Percent 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)

Table 2: Pearson Correlation for RRDI (at 90% threshold):

	Correlation Coefficients	P value
AHI 4%	0.18	<.0001
PLMI	0.19	<.0001
Respiratory arousal index	0.24	<.0001
Leg movement arousal index	0.23	<.0001
Spontaneous arousal index	-0.09	0.127

Abbreviations: AHI 4%= apnea-hypopnea index based on hypopnea associated with 4 % desaturation; PLMI=periodic legs movement index; RRDI= R-R interval dips index.

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

	CVD	Hazard Ratio (95% CI)			
	Events	p-value			
	N (%)	Unadjusted Model (1)	Adjusted Model (2)	Adjusted Model (3)	Adjusted Model (4)
Continuous RRDl (10-unit increment)	24/569 (4)	1.24 (1.10, 1.39) 0.0003	1.22 (1.08, 1.38) 0.0018	1.23 (1.11, 1.38) 0.0007	1.22 (1.08, 1.37) 0.0012
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, 10.34) 0.1586	2.72 (0.70, 10.59) 0.1481	3.16 (0.81, 12.40) 0.099	3.22 (0.80, 12.93) 0.10
Tertile 3 (≥ 28.4)	14/188 (7)	6.11 (1.72, 21.72) 0.0052	5.87 (1.60, 21.46) 0.0075	7.40 (1.97, 27.73) 0.003	8.99 (2.35, 34.40) 0.001
p-trend		0.0024	0.0045	0.0017	0.0006

Abbreviations: AHI 4%, apnea-hypopnea index with hypopnea scored if associated with at least 4 % desaturation (events/hour); HR, hazard ratio; RRDl, R-R interval dips index (dips/hour); HTN, hypertension; % TST<90, total sleep time spent less than 90% on oxygen saturation signal (%). 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%.

Table 4: The adjusted time to event Cox Proportional Hazards Models for **sleep RRDl** predicting the incidence of CVD event.

	CVD Events	Hazard Ratio (95% CI)			
		p-value			
	N (%)	Unadjusted Model (1)	Adjusted Model (2)	Adjusted Model (3)	Adjusted Model (4)
Continuous RRDI (10-unit increment)	24/569 (4)	1.21 (1.09, 1.35) 0.0006	1.29 (1.06, 1.33) 0.0037	1.20 (1.07, 1.34) 0.0015	1.19 (1.06, 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, 8.57) 0.1144	2.46 (0.75, 8.11) 0.1383	2.66 (0.80, 8.77) 0.1092	2.79 (0.83, 9.36) 0.10
Tertile 3 (≥ 23.5)	11/188 (6)	3.39 (1.06, 10.84) 0.0398	2.94 (0.91, 9.56) 0.0729	3.61 (1.08, 12.10) 0.0373	4.00 (1.17, 13.68) 0.027
p-trend		0.0392	0.0768	0.0365	0.026

Abbreviations: AHI 4%, apnea-hypopnea index with hypopnea scored if associated with at least 4 % desaturation (events/hour); HR, hazard ratio; RRDl, R-R interval dips index (dips/hour); HTN, hypertension; % TST<90, total sleep time spent less than 90% on oxygen saturation signal (%). 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%.

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	Hazard Ratio (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, 1.33) 0.0009	1.17 (1.05, 1.31) 0.0044	1.18 (1.07, 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, 8.86) 0.1040	2.69 (0.82, 8.82) 0.1023	2.85 (0.87, 9.36) 0.0849
Tertile 3 (≥ 22.6)	11/188 (6)	3.40 (1.06, 10.94) 0.0389	3.11 (0.96, 10.06) 0.0577	3.92 (1.18, 13.09) 0.0263
p-trend		0.0390	0.0612	0.0249

Abbreviations: AHI 4%, apnea-hypopnea index with hypopnea scored if associated with at least 4 % desaturation (events/hour); HR, hazard ratio; RRDI, R-R interval dips index (dips/hour). 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).

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

	CVD Events	Hazard Ratio (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, 1.32)	1.17 (1.05, 1.31)	1.19 (1.07, 1.33)
		0.0013	0.0056	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, 4.24)	1.19 (0.37, 3.78)	1.24 (0.39, 4.00)
		0.6222	0.7732	0.7171
Tertile 3 (≥ 24.0)	12/188 (6)	2.92 (1.00, 8.55)	2.42 (0.80, 7.29)	2.69 (0.88, 8.19)
		0.0508	0.1173	0.0825
p-trend		0.0393	0.0936	0.0657

Abbreviations: AHI 4%, apnea-hypopnea index with hypopnea scored if associated with at least 4 % desaturation (events/hour); HR, hazard ratio; RRDI, R-R interval dips index (dips/hour). 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).

Table 7: A summary of CVD events types.

	CVD events types (n=24)
First Event Type*	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.

Table 8: Adjusted time to event Cox Proportional Hazards Models for RRDI Predicting Incidence of CVD Event Stratified by Gender for Continuous RRDI and across Categories of Participants with RRDI more than 20 dips per 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) p-value	Adjusted Model (3) * (95% CI) p-value	
Continuous RRDI (SLEEP) (10-unit increment)	1.19 (1.04, 1.36) 0.011	1.22 (0.96, 1.54) 0.109	
RRDI Category			
<20	REF	REF	
>20	1.85 (0.67, 5.07) 0.234	1.29 (0.22, 7.47) 0.779	
	Adjusted Model (3)* (95% CI) p-value	Adjusted Model (3) * (95% CI) p-value	
Continuous RRDI (ALL) (10-unit increment)	1.22 (1.06, 1.40) 0.006	1.25 (0.97, 1.67) 0.086	
RRDI Category			
<20	REF	REF	
>20	4.34 (1.32, 14.34) 0.016	2.03 (0.38, 10.77) 0.407	

Abbreviations: AHI 4%, apnea-hypopnea index with hypopnea scored if associated with at least 4 % desaturation (events/hour); HR, hazard ratio; RRDI,

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3 R-R interval dips index (dips/hour). * Model adjusted for age, body mass index,
4 and AHI 4% categories.
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6 **Figure Legends**

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9 **Figure 1:** (A) A representative polygraph from one subject in the Wisconsin Sleep
10 Cohort Study (WSCS) that illustrate changes in respective heart rate and
11 computed R-R-intervals (RRI) tracing during sleep. (B) A magnified segment of
12 ECG illustrating RRI following respiratory event (apnea). The RRI tracing was
13 retro-graphed from the exported signal that calculates RRI from raw ECG
14 recordings. Abbreviations: EEG= electroencephalogram; EMG= electromyogram;
15 ECG=electrocardiogram; RRI=R-R interval. Open arrows indicate oxygen
16 desaturation following apnea and closed arrows indicate RRI dip following apnea.
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29 **Figure 2:** A representative computed data of RRI and oxygen saturation (S_aO_2)
30 from one individual during sleep. The red dots represent the RRI dips throughout
31 the duration of the PSG recording (approximately 8 hours). The RRI dips index
32 (RRDI) at 90% threshold for this participant was 54.5 dips /hour, the average heart
33 rate was 61.1 BPM, and the ODI (3%) was 2.3 de-saturations/hour (from the
34 original PSG recording). The RRI tracing was retro-graphed from the exported
35 signal that calculates RRI and S_aO_2 from raw ECG and pulse oximetry recordings,
36 respectively. Abbreviations: ECG, electrocardiogram; ODI, oxygen desaturation
37 index; RRI, R-R intervals; S_aO_2 , oxygen saturation.
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51 **Figure 3:** The Wisconsin Sleep Cohort Study (WSCS) sample. ECG=
52 electrocardiography; PSG= polysomnography; CPAP= continuous positive airway
53 pressure.
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3 **Figure 4:** Incidence of composite CVD and hazard ratios across different total
4 RRDl severity: Category 1 as a reference (RRDI < 15.1), category 2 (RRDI 15.1-
5 <28.4), and category 3 (RRDI ≥ 28.4) (n = 569). CVD=Cardiovascular disease;
6 RRDl=R-R interval dips index. (*) versus unadjusted model (1) RRDl <15.1 dips
7 per hour, P<0.01 ; (**) versus adjusted model (3) RRDl <15.1 dips per hour,
8 P<0.01.
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18 **Figure 5:** Kaplan-Meier estimates of the likelihood of survival according to total
19 RRDl severity: Category 1 as a reference (RRDI < 15.1), category 2 (RRDI 15.1-
20 < 28.4), and category 3 (RRDI ≥ 28.4) (n = 569); log-rank test for differences in
21 survival by RRDl category; Survival was lower for category 3 compared to group 1
22 and 2. RRDl is a mean number of RRI dips/hr of total recording time of PSG.
23 RRDl=R-R interval dips index.
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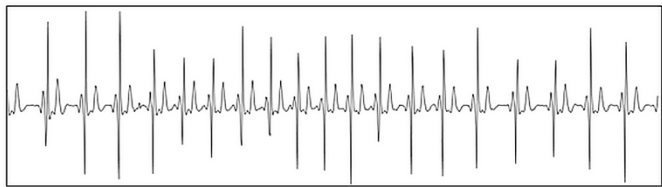
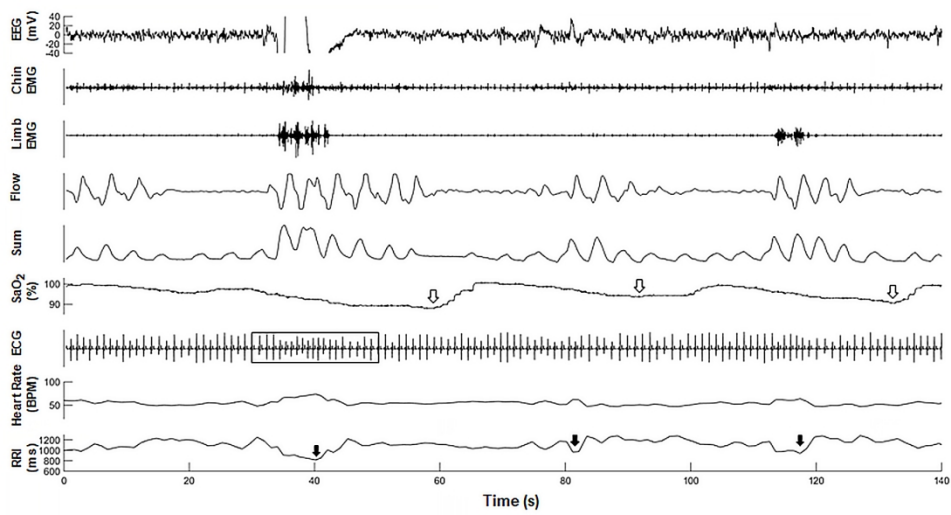


Figure 1

199x150mm (300 x 300 DPI)

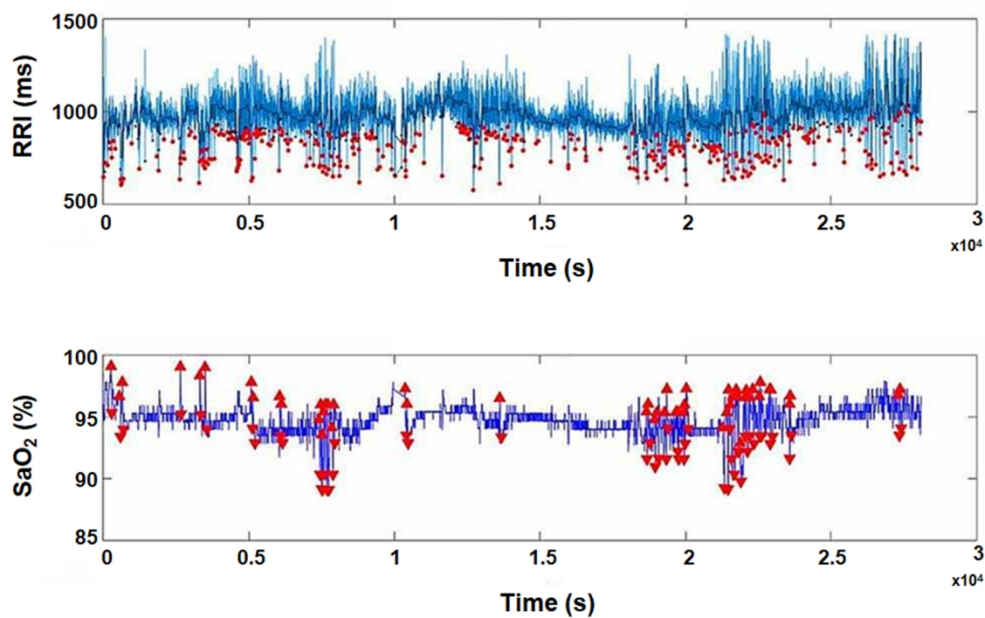


Figure 2

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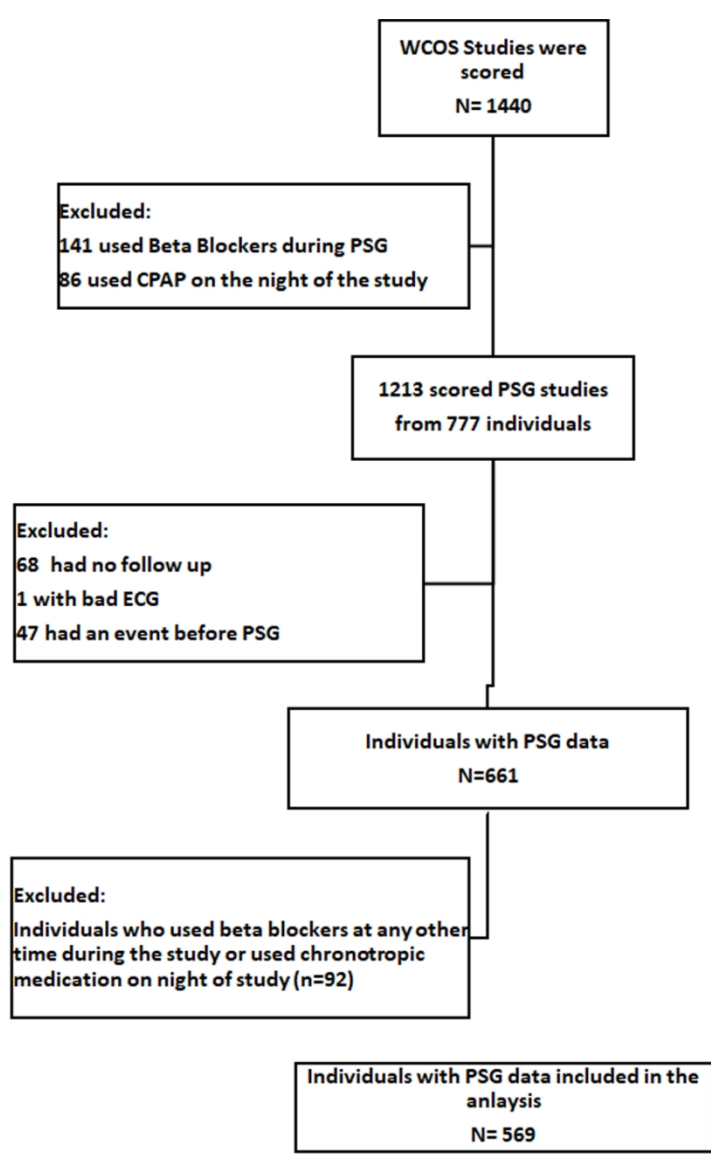


Figure 3

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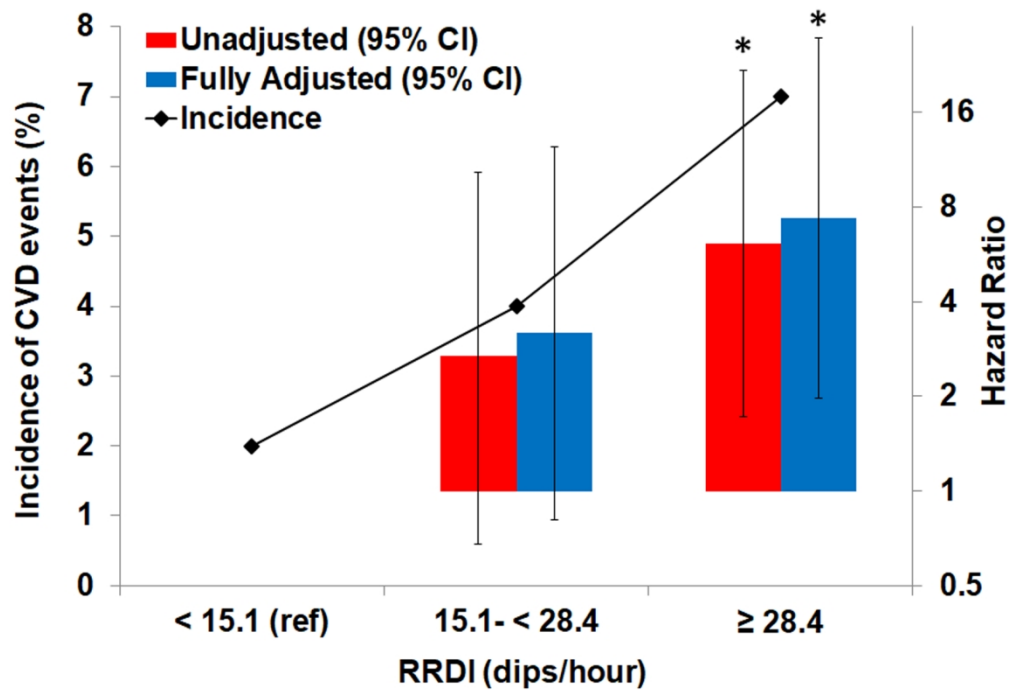
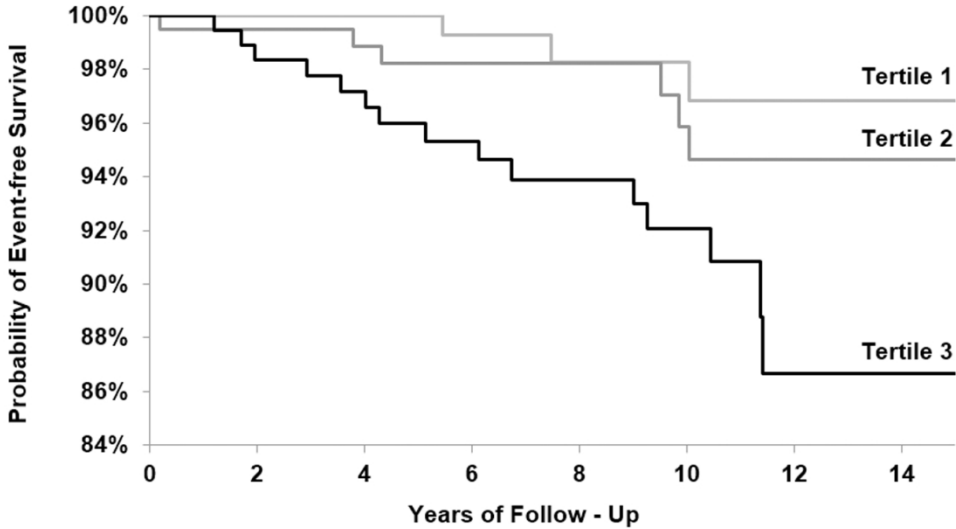


Figure 4

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Log-Rank p=0.0139 for total RRDl

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

119x90mm (300 x 300 DPI)

Supplements Materials:

Analysis supplement:

Table 1S. Medications were taken by individuals excluded due to the potential effect on heart rate.

Medication name (Brand)
Metoprolol tartrate (Lopressor, nebivolol)
Enalapril maleate (Vasotec)
Nadolol (Corgard)
Atenolol (Tenormin, Zebeta)
Betaxolol hydrochloride (Kerlone)
Acebutolol hydrochloride (Sectral)
Clonidine (Catapres, other alpha adrenergic agonist agents)
Atenolol & Chlorthalidone (Tenoretic)
Metoprolol succinate (Toprol XL)
Diazac
Labetalol Hydrochloride (Normodyne, Tradate)
Betachron (Propranolol)
Ziac (Hydrochlorothiazide / Bisoprolol combo)
Carvediol (Coreg, Cartrol)
Pindolol (Visken)
Diltiazem HCL (Cardizem, Dilacor, Taztia XT, Tiazac)
Amiodarone HCl (Cordarone, tikosyn)

STROBE Statement—checklist of items that should be included in reports of observational studies

	<i>Item No.</i>	<i>Recommendation</i>	<i>Page No.</i>	<i>Relevant text from manuscript</i>
Title and abstract	1		1-3	Longitudinal Effect of Nocturnal R-R Intervals Changes on Cardiovascular Outcome in a Community-Based Cohort
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5	While recent reports included measurements of sleep fragmentation and respiratory event duration as a surrogate of arousal threshold, ⁵ 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 phenotype ⁶ independent of covariates, suggesting that genetic factors play an important role in controlling these cardiovascular risk factors ⁷ . Therefore, R-R interval, 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 ⁸ . However, the long-term effect of heart rate changes during sleep on the cardiovascular outcome and mortality is unknown.
Objectives	3	State specific objectives, including any prespecified hypotheses	6	The objectives of this study were to examine whether R-R interval (RRI) or heart rate accelerations can serve as predictors of cardiovascular disease in the Wisconsin Sleep Cohort study (WCS), a prospective community cohort. We hypothesized 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.
Methods				
Study design	4	Present key elements of study	7	Cohort study design

		<i>design early in the paper</i>		
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8	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 four 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.
Participants	6	(a) Cohort study— Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8	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 (Table 3S; supplement) on the night of the sleep study or at any other point during follow-up.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8	The main predictor variable is the hourly rate of R-R interval (RRI) changes assessed over an entire night's sleep period. The recorded ECG signals were retrieved from PSG to measure the RRI
Data sources/ measurement	8*	For each variable of interest, give sources of data and	7	We studied individuals from the WSCS.

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details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group

Bias	9	Describe any efforts to address potential sources of bias	9	The person performing the analysis was blinded to the participant's demographic information.
Study size	10	Explain how the study size was arrived at	11	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 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.

Continued on next page

variables

variables were handled in

the analyses. If

applicable, describe

which groupings were

chosen and why

Statistical methods

12

(a) Describe all statistical methods, including those used to control for confounding

11

Cox proportional hazards regression was used to estimate adjusted hazard ratios and 95% CIs for the association between RRDI and subsequent risk of an incident CVD event. In addition to adjusting for age using this methodology, models were adjusted for BMI and gender. Models were subsequently adjusted for AHI 4% (as continuous and categorical variables [AHI < 5, 5-15, or > 15 events/hour]). Subsequently, the model was adjusted for other factors: diabetes, hypertension, stroke, smoking, average HR, % TST < 90%, Kaplan-Meier techniques were used to compare survival across RRDI categories

(b) Describe any methods used to examine subgroups and interactions

NA

(c) Explain how missing data were addressed

There was no missing data

(d) Cohort study—If applicable, explain how loss to follow-up was addressed

All eligible participants had follow up.

(e) Describe any sensitivity analyses

12

Lower thresholds (80%, 70%, and 60%) of RRDI correlated with total RRDI 90% but were less sensitive in predicting CVD (less than 5 individuals attained RRDI > 20 dips/h). The association between total RRDI at 90% threshold and the incidence of new-onset CVD remained significant after the adjustment for AHI 4% (P < 0.001)

Results**Participants**

13*

(a) Report numbers of individuals at each stage of study—eg numbers

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Figure 3: The Wisconsin Sleep Cohort Study (WSCS) sample

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potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed

(b) Give reasons for non-participation at each stage

(c) Consider use of a flow diagram

Descriptive data

14*

(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders

Table 1

(b) Indicate number of participants with missing data for each variable of interest

NA

(c) Cohort study—Summarise follow-up time (eg, average and total amount)

14 *a follow-up interval of 15 years*

Outcome data

15*

Cohort study—Report numbers of outcome events or summary measures over time

14

Cardiovascular events were detected in twenty-five 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).

Case-control study—Report numbers in each exposure category, or summary measures of

 exposure

Cross-sectional study—

Report numbers of

outcome events or

 summary measures

Main results

16

(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included

12

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, 1.10-1.39], $P < 0.001$) which remained significant after adjustment for age, BMI and gender (model 1) and the addition of AHI 4% (model 2) (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 5 individuals attained RRDI >20 dips/h). The association between total RRDI at 90% threshold and the incidence of new-onset CVD remained significant after the adjustment for AHI 4% ($P < 0.001$). Total RRDI category 3 ($\geq 28.4/h$ vs < 15.1 dips/h) was associated with increased CVD hazards risk of 6.1 (95% CI, 1.7-27.7, $P = 0.005$) and remained significant after adjustment for AHI 4% ($P = 0.003$).

(b) Report category boundaries when continuous variables were categorized

12

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 per hour (second tertile) and for the third group (tertile) of individuals with RRDI equal or more than 28.4 dips per hour.

(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Continued on next page

<i>Other analyses</i>	17	<i>Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses</i>	14	Table 8 presents the baseline characteristics of men and women participants and associated total and sleep RRDI.
Discussion				
<i>Key results</i>	18	<i>Summarise key results with reference to study objectives</i>	15	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.
<i>Limitations</i>	19	<i>Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias</i>	18-19	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 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

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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 record³³. Second, we lack racial diversity in our study as 95% was reported as a white race. Therefore, the results may not be generalizable 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 preexisting 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 may not be applicable to individuals with heart disease or if taking Beta-blocker or chronotropic medications. Likewise, this study may not be applicable to individuals with arrhythmia, frequent ectopic beats and in case of cardiac pacemakers.

<i>Interpretation</i>	20	<i>Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence</i>	19	this study could not include all WSCS participants and may not be applicable to individuals with heart disease or if taking Beta-blocker or chronotropic medications.
<i>Generalisability</i>	21	<i>Discuss the generalisability (external validity) of the study results</i>	19	this study may not be applicable to individuals with arrhythmia, frequent ectopic beats and in case of cardiac pacemakers.
<i>Other information</i>				
<i>Funding</i>	22	<i>Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based</i>	2	This secondary analysis is supported by the National Heart, Lung, and Blood Institute (R21HL140447). The Wisconsin Sleep Cohort Study was supported by the National Heart, Lung, and Blood Institute (R01HL62252), National Institute on Aging (R01AG036838), and the National Center for Research Resources (UL1RR025011) at the US NIH. Author (Sankari) is supported by Career Development Award # IK2CX000547 from the Clinical Science Research & Development Service of the VA Office of Research and Development of the VA Office of Research and Development from the (U.S.) Department of Veterans Affairs and by Cardiovascular Research Institute [CVR].