BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (http://bmjopen.bmj.com).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-030559
Article Type:	Research
Date Submitted by the Author:	20-Mar-2019
Complete List of Authors:	Sankari, Abdulghani; Wayne State University School of Medicine, ; Wayne State University-Detroit Finn, Laurel; University of Wisconsin, Population Health Sciences Maresh, Scott; Wayne State University School of Medicine Aljundi, Nawar; Wayne State University School of Medicine Alsabri, Bander; Wayne State University School of Medicine Fawaz, Serene; Wayne State University School of Medicine Hamdon, Mulham; Wayne State University School of Medicine Al-kubaisi, Ghazwan Hagen, Erika; University of Wisconsin, Population Health Sciences Badr, Safwan; Wayne State University School of Medicine, Division of Pulmonary Critical Care and Sleep Medicine, Department of Internal Medicine; Peppard, Paul; University of Wisconsin, Population Health Sciences
Keywords:	SLEEP MEDICINE, cardiovascular disease, Heart rate, Cardiac Epidemiology < CARDIOLOGY, EPIDEMIOLOGY

SCHOLARONE™
Manuscripts

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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

Abdulghani Sankari[1], Laurel A. Finn[2], Scott A. Maresh[1], Nawar Aljundi[1], Bander Alsabri[1], Serene Fawaz[1], Mulham S. Hamdon[1], Ghazwan Al-Kubaisi[1], Erika. W. Hagen[2], M.S. Badr[1], Paul E. Peppard[2]

(1) Sleep Research Laboratory, John D. Dingell Veterans Affairs Medical Center, Wayne State University School of Medicine, Detroit, MI

(2) Population Health Sciences, the University of Wisconsin School of Medicine and Public Health, Madison, WI

Address correspondence to Abdulghani Sankari, M.D., Ph.D., Division of Pulmonary, Critical Care, and Sleep Medicine, 3990 John R, 3-Hudson, Detroit, MI 48201; Tel: (313) 745-6033; Fax: (313) 745-8725; Email:asankari@wayne.edu

Words count: 7237

Contributorship statement:

Conception and design: Sankari, Badr, and Peppard.

The acquisition, analysis, or interpretation of data: Sankari, Badr, Finn, Maresh, Aljundi, Alsabri, Fawaz, Hamdon, Al-Kubaisi, Peppard.

Statistical analysis: Finn, Hagen, and Peppard.

Obtained funding: Sankari and Peppard.

Study supervision: Sankari and Peppard.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Conflict of interest: All authors declare no conflict of interest.

Disclosure Statement: The following authors A. Sankari, M.S Badr, and S. Maresh and Wayne State University have a pending Patent #US62395634, entitled "The Detection of Sleep Disordered Breathing Using Cardiac Autonomic Responses", application number#15/706097 for Utility/Design using an application data sheet (37 CFR 1.54), Date Filed: September 15, 2017. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Department of Veterans Affairs, National Institute of Health or Wayne State University.

Funding support: 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 [CVRI].

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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/m^2), 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

For peer review only

associated with new-onset CVD event(s) (HR, 1.21 per 10-unit increment in RRDI [95% CI, 1.09-1.35], P < 0.001) which remained significant after adjusting for demographic factors, AHI 4%, hypoxemia and other comorbidties.

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

Keywords: Heart rate, R-R interval, sleep-disordered breathing, cardiovascular disease.

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; ECG, electrocardiograph; HR, heart rate; PSG, polysomnography; RRI, R-R interval; RRDI, RRI dips index; SDB, Sleep-disordered breathing; SHHS, Sleep Heart Health Study; WSCS, Wisconsin Sleep Cohort Study.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 sleep [1]. 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 night[2]. Indeed, recent evidence showed that sub-type of excessively sleepy patients with moderate to severe SDB have significantly increased the risk for prevalent and incident cardiovascular events indicating the central role of sleep disruption in increased CVD risk[3]. In addition, a recent study found that varying hypopnea definitions in the general population can affect the risk stratification of cardiovascular disease in patients with SDB[4].

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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 crietria[8]. However, the long-term effect of heart rate changes during sleep on the cardiovascular outcome and mortality is unknown.

The objectives of this study were to examine whether R-R interval (RRI) or heart rate accelerations can serve as predictors of cardiovascular disease in the Wisconsin Sleep Cohort study (WSCS), 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. Results of this study have been previously reported in the form of an abstract.[9]

Methods

Participants: We studied individuals from the WSCS. The protocols of the WSCS were approved by the Health Sciences Institutional Review Board of the University of Wisconsin-Madison. All participants provided written informed consent. Sampling and data collection protocols of the WSCS have been described previously [10].

Cohort Description: The WSCS comprises 1546 adult employees of state agencies, ages 30-60 years old at the Cohort's inception, which underwent

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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. 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.

Patient and Public Involvement: This study was a secondary analysis for preexisting data from an established cohort of the Wisconsin study. Therefore, participants were not involved in the design, recruitment, or conduct of this study.

Predictor: The main predictor variable is the hourly rate of 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, which are time intervals between successive pairs of QRS complexes, by using software for the detection of R waves in LabChart 7 with heart rate variability Module (AD Instruments, Colorado Springs, CO) (Fig.1). In this program, ECG signal was examined and retrieved to a MatLab R2017a program (MathWorks, Natick, MA) developed and validated by our group to obtain RRI signal for the entire night (Fig. 2)[2, 11]. The RRI dips, defined by a decreased RRI compared to the average RRI for the corresponding

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

1-minute segment as a baseline, were collected. Given that 90% dips threshold correlated previously with most respiratory events (apneic and non-apneic respiratory events, defined below)[2], total RRI dip index (RRDI) was defined by the number of RRI dips below the 90% baseline divided by the total PSG recording time in hours (from light on to light out), regardless of wake or sleep stages. Sleep RRDI for non-REM and REM stages combined were defined by the number of RRI dips below the 90% baseline divided by the total sleep time in hours (for both REM and non-REM sleep stages). Subsequently, sleep RRDI was calculated for specific sleep stages for REM and non-REM, respectively. In subgroup analysis, the gender differences in total and sleep RRDI were compared between men and women.

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

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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

information. The censored analysis was used, and the data is censored at the last visit. If multiple events were reported over the course of follow-up, the first reported event was used in this analysis.

Covariates: Participants underwent a baseline overnight 18-channel PSG (Grass model 78; Quincy, MA) at the University of Wisconsin-Madison Clinical Research Unit using a standard protocol [12]. The PSG recorded sleep state using electroencephalography, electrooculography, and electromyography; and breathing, using respiratory inductance plethysmography (Respitrace; Ambulatory Monitoring, Ardsley, NY), nasal and oral airflow (ProTec thermocouples; Hendersonville, TN) and oxyhemoglobin saturation, using pulse oximetry (Ohmeda Biox 3740, Englewood, CO). Each 30-second epoch of the polysomnographic recordings was scored for sleep stage, and apnea and hypopnea events by trained technicians and reviewed using standard criteria[12]. Apnea was defined as cessation of nasal and oral airflow for ≥10 seconds and hypopnea as a discernible reduction in breathing (sum of the chest and abdominal excursions) with a decrease in oxyhemoglobin saturation of ≥ 4%. The apnea-hypopnea index was calculated as the mean number of apnea and hypopnea events per hour of sleep.

Statistical analysis:

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 [14]. Because of the strong dependence of CVD risk on age, Cox-regression models were based on age as the time scale, (the age when RRDI was measured and age at the event) allowing for left truncation (late entry) [15]. 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[16].

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. RRDI (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).

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

CVD incidence – association with total RRDI:

Using Cox Proportional Hazards Model, continuous total RRDI (with 90% threshold for RRI dips) was significantly associated with new-onset CVD event(s) (HR, 1.24 per 10-unit increment in RRDI [95% CI, 1.10-1.39], 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 (≥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 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. Kaplan-Meier survival curves (Fig.5) illustrate decreased CVD event-free survival with increasing RRDI category from RRDI less than 15.1 to RRDI equal or more than 28.4 dips per hour. Continuous total RRDI 90% remained significant (HR, 1.22 (per 10-unit increment in RRDI [95% CI, 1.08, 1.37], P = 0.001) after additionally adjusted for diabetes, hypertension,

For peer review only

stroke, smoking, average heart rate, and % total time with oxygen saturation less than 90% (as depicted in Table 1S; supplement).

CVD incidence – association with sleep RRDI:

Using Cox Proportional Hazards Model, continuous sleep RRDI 90% was significantly associated with new-onset CVD event(s) (HR, 1.21 per 10-unit increment in RRDI [95% CI, 1.09-1.35], P < 0.001) which remained significant after the model adjusted for age, BMI and gender (model 1) and the addition of AHI 4% (model 2) (as depicted in Table 4). RRDI category 3 (≥23.5/h vs. <9.0/h) was associated with increased CVD hazards risk of 3.39 (95% CI, 1.06-10.85, P = 0.04) and remained significant after adjustment for demographics and AHI 4% (P = 0.037). The relationship between sleep RRDI categories and CVD events were predominantly in non-REM sleep as depicted in Table 5. Continuous RRDI 90% during REM sleep was significant (HR, 1.19 per 10-unit increment in RRDI [95% CI, 1.07-1.32], P = 0.001) and remained significant after the model adjusted for age, BMI and gender (model 1) and the addition of AHI 4% (model 2) (as depicted in Table 6). However, sleep RRDI category 3 (≥24/h vs. <9.0/h) was only mildly significant in the unadjusted model with hazards risk of new CVD events of 2.92 (p=0.05). Continuous sleep RRDI 90% remained significant (HR, 1.19 per 10-unit increment in RRDI [95% CI, 1.06-1.33], P = 0.003) after additionally adjusted for diabetes, hypertension, stroke,

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

smoking, average heart rate, and % total sleep time with oxygen saturation less than 90% (as depicted in Table 2S; supplement).

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). No difference between mean age at the start for those who had CVD vs not (P=0.58).

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

Table 8 presents the baseline characteristics of men and women participants and associated total and sleep RRDI. While BMI was higher in women (32.0 ± 7.0 *vs.* 30.0 ± 5.0 kg/m^2, p=0.0001), men had higher AHI, total RRDI and sleep RRDI than women (P<0.01). Using Cox Proportional Hazards adjusted Model (for age, body mass index, and AHI 4%), continuous total RRDI 90% was significantly associated with new-onset CVD event(s) in men (HR, 1.22 per 10-unit increment in RRDI [95% CI, 1.06, 1.40], P < 0.001) but not in women (as depicted in Table 8). Likewise, continuous sleep RRDI 90% was significantly associated with new-onset CVD event(s) in men (HR, 1.19 per 10-unit increment in RRDI [95% CI, 1.04, 1.36], P < 0.05) but not in women. Using total RRDI threshold of 20 dips per hour or more was associated with increased CVD hazards risk of 4.34 in men only (95% CI, 1.32, 14.34, P = 0.016).

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 ≥4% de-saturations, but not by SDB characterized by de-saturations of <4%[17]. More recently, it has been

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

found that the desaturation hypoxic burden related to respiratory events, measured by the integration of the severity of the desaturation and its length, predicted CVD mortality[18]. Another study that examined heart rate variability during sleep found that SDB patients had shorter RRI and increased sympathetic burst frequency (49±4 bursts/min) compared with control subjects [19]. The authors speculated that abnormalities in heart rate and blood pressure variability might be implicated in the subsequent development of cardiovascular disease in patients with SDB. The present study confirmed this association between the frequency of heart rate accelerations (RRDI) and adverse cardiovascular consequence in a prospective large cohort of individuals who had no known heart disease at the time of their sleep study.

The mechanism of increased incidence of CVD and association with RRDI can be explained by an increased sympathetic tone and autonomic arousals. First, sleep disturbances like SDB, periodic limb movements, insufficient sleep are all associated with an increased risk of CVD. These sleep disorders are commonly associated with impaired autonomic nervous system leading to increased sympathetic tone[20]. Furthermore, sleep fragmentation due to autonomic or respiratory arousals from sleep increases the cardiac sympathetic tone activity resulting in a sudden elevation in vascular tone and heart rate generating a rise in arterial blood pressure [20, 21]. The increased sympathetic tone in patients with heart disease has been proposed as an intermediate outcome linking heart rate variability with increased mortality [22]. Resting heart rate also has been linked to CVD in patients with SDB and COPD [19, 23, 24]. Second, the augmented shear

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

forces due to intermittent episodes of tachycardia secondary to respiratory events and the resultant mechanical shear forces may lead to endothelial dysfunction [25]. This possibility is physiologically plausible particularly in male patients,[26] who may have significant endothelial dysfunction secondary to activation of several inflammatory pathways. Such pathophysiologic changes in untreated SDB patients have been linked to nocturnal angina, myocyte necrosis leading to cardiomyopathy, and cardiac remodeling [27-29]. Our findings corroborate these pathological changes particularly in the coronary vessels making the vast majority of CVD events either related to CAD (48%) or myocardial infarction (36%), predominantly in men. On the other hand, medications such as Beta-blockers attenuate the increase in heart rate related to respiratory events during sleep in patients with hypertension and untreated SDB [30]. This modulation of cardiac responses in patients with SDB provides a mechanism by which Beta-blockers may decrease the risk of sudden cardiac death, particularly in patients with CVD [31]. Finally, hypoxic events can affect the autonomic cardiac response and generate significant RRI dips events [32]. Hypoxemia and RRI dips may represent different features of SDB-related stress, both of which may contribute to CVD morbidity and mortality through independent pathways. Our findings suggest a need to further identify the intermediate mechanisms that link RRI dips events to long-term outcomes.

This study has several strengths including its prospective design with longitudinal follow-up of participants, community-based including a diverse group of ages and morbidities from both genders, and the use of the gold-standard laboratory-based

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 accelerations that can be translated into an executable program or a plug-in for sleep scoring software and can be used in any sleep study across the world. The study has some limitations. First, we used the self-reported diagnosis of CVD (including dates of diagnosis). However, there is evidence that self-reported CVD is very reliable and accurate as noted in the AusDiab cohort. Barr et al. reported more than 99% of self-reported CVD events were correctly verified in the patients' medical records; only 0.2% of those denying any CVD event being recorded as having had an event on the medical record [33]. 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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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.

Clinical Perspectives:

The association between heart rate changes and the cardiovascular outcome may have significant clinical implications. First, the association between increased incidence of cardiovascular events and the RRI dips index suggest that early detection of heart rate fluctuations during sleep could identify those who are at increased risk of future CVD events and inform primary preventions strategies. Second, several behavioral factors[34] and medical conditions, such as SDB[35] and COPD[24], are associated with changes in resting heart rate, which increase the risk of cardiovascular diseases. Third, the attenuation of heart rate accelerations by Beta-blockers during sleep as recently shown in patients with SDB,[30] indicate that Beta-blockers may play an important role in preventing CVD. However, large prospective clinical studies are needed to confirm this finding.

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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Acknowledgments

The authors would like to thank Amanda Rasmuson for her assistance throughout the study. The authors would like also to thank Dr. Aiden Abidov and Naresh Punjabi for their valuable advice.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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)

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 SaO2, 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)

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 3: The adjusted time to event Cox Proportional Hazards Models for total 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)	**1.24 (1.10, 1.39) 0.0003**	**1.22 (1.08, 1.38) 0.0018**	**1.23 (1.11, 1.38) 0.0007**
RRDI 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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 4: The adjusted time to event Cox Proportional Hazards Models for sleep 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)	**1.21 (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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 6: The adjusted time to event Cox Proportional Hazards Models for REM 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 (1.07, 1.32) 0.0013**	**1.17 (1.05, 1.31) 0.0056**	**1.19 (1.07, 1.33) 0.0016**
RRDI Category				
Tertile 1 (< 9.0)	5/187 (3)	REF	REF	REF
Tertile 2 (9.0- < 24.0)	7/194 (4)	1.34 (0.42, 4.24) 0.6222	1.19 (0.37, 3.78) 0.7732	1.24 (0.39, 4.00) 0.7171
Tertile 3 (≥ 24.0)	12/188 (6)	2.92 (1.00, 8.55) 0.0508	2.42 (0.80, 7.29) 0.1173	2.69 (0.88, 8.19) 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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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* (95% CI) p-value	**Adjusted Model * (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* (95% CI) p-value	**Adjusted Model * (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 % denaturation (events/hour); HR, hazard ratio; RRDI, R-R interval dips index (dips/hour). * Model Adjusted for age, body mass index, and AHI 4% categories.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Figure 4: Incidence of composite CVD and hazard ratios across different total RRDI severity: Category 1 as a reference (RRDI < 15.1), category 2 (RRDI 15.1- <28.4), and category 3 (RRDI ≥ 40) (n = 569). CVD=cardiovascular disease; RRDI=R-R interval dips index. (*) versus unadjusted RRDI <15.1 dips per hour, P<0.01 ; (**) versus adjusted RRDI <15.1 dips per hour, P<0.01.

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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

References

1. Somers V. American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology. American Heart Association Stroke Council. American Heart Association Council on Cardiovascular Nursing. American College of Cardiology Foundation. Sleep apnea and cardiovascular disease: an American Heart Association/American College Of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council On Cardiovascular Nursing. In collaboration with the National Heart, Lung, and Blood Institute National Center on Sleep Disorders Research (National Institutes of Health). *Circulation*. 2008;118:1080-1111.
2. Sankari A, Pranathiageswaran S, Maresh S, Hosni AM and Badr MS. Characteristics and Consequences of Non-apneic Respiratory Events during Sleep. *Sleep*. 2016.
3. Mazzotti DR, Keenan BT, Lim DC, Gottlieb DJ, Kim J and Pack AI. Symptom Subtypes of Obstructive Sleep Apnea Predict Incidence of Cardiovascular Outcomes. *American Journal of Respiratory and Critical Care Medicine*. 2019.
4. Won C, Qin L, Selim B and Yaggi H. Varying Hypopnea Definitions Affect Obstructive Sleep Apnea Severity Classification and Association With Cardiovascular Disease. *Journal of clinical sleep medicine: JCSM: official publication of the American Academy of Sleep Medicine*. 2018.
5. Butler MP, Emch JT, Rueschman M, Sands SA, Shea SA, Wellman A and Redline S. Apnea-Hypopnea Event Duration Predicts Mortality in Men and Women in the Sleep Heart Health Study. *American Journal of Respiratory and Critical Care Medicine*. 0:null.
6. Singh JP, Larson MG, O'Donnell CJ, Tsuji H, Evans JC and Levy D. Heritability of heart rate variability: the Framingham Heart Study. *Circulation*. 1999;99:2251-2254.
7. Fava C, Burri P, Almgren P, Arcaro G, Groop L, Hulthén UL and Melander O. Dipping and Variability of Blood Pressure and Heart Rate at Night Are Heritable Traits*. *American Journal of Hypertension*. 2005;18:1402-1407.
8. Zinchuk AV, Jeon S, Koo BB, Yan X, Bravata DM, Qin L, Selim BJ, Strohl KP, Redeker NS and Concato J. Polysomnographic phenotypes and their cardiovascular implications in obstructive sleep apnoea. *Thorax*. 2018;73:472-480.
9. Finn LA, Maresh S, Hamdon MS, Al-kubaisi G, Badr M, Peppard PE and Sankari A. A New Marker Of Cardiovascular Disease In Patients With Sleep-Disordered Breathing: Results From The Wisconsin Sleep Cohort Study *A80-A ARE HSTS OBSOLETE? NOVEL DIAGNOSTICS IN SDB*: American Thoracic Society; 2016: A2523-A2523.
10. Young T, Palta M, Dempsey J, Skatrud J, Weber S and Badr S. The Occurrence of Sleep-Disordered Breathing among Middle-Aged Adults. *New England Journal of Medicine*. 1993;328:1230-1235.
11. Sankari A, Badr MS and Maresh S. Detection of sleep disordered breathing using cardiac autonomic responses. 2018.
12. Flemons W, Buysse D, Redline S, Oack A, Strohl K, Wheatley J, Young T, Douglas N, Levy P and McNicolas W. Sleep-related breathing disorders in adults. *Sleep*. 1999;22:667-689.
13. Berry R, Brooks R, Gamaldo C, Harding S, Marcus C and Vaughn B. The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications, version 2.0. *Darien, Illinois: American Academy of Sleep Medicine*. 2012:47.
14. Cox PR. *Life Tables*: Wiley Online Library; 1972.
15. Kom EL, Graubard BI and Midthune D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. *American journal of epidemiology*. 1997;145:72-80.

For Peer Review Only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

16. Kaplan EL and Meier P. Nonparametric estimation from incomplete observations. *Journal of the American statistical association*. 1958;53:457-481.

17. Punjabi NM, Newman AB, Young TB, Resnick HE and Sanders MH. Sleep-disordered breathing and cardiovascular disease: an outcome-based definition of hypopneas. *American journal of respiratory and critical care medicine*. 2008;177:1150-1155.

18. Azarbarzin A, Sands SA, Stone KL, Taranto-Montemurro L, Messineo L, Terrill PI, Ancoli-Israel S, Ensrud K, Purcell S and White DP. The hypoxic burden of sleep apnoea predicts cardiovascular disease-related mortality: the Osteoporotic Fractures in Men Study and the Sleep Heart Health Study. *European heart journal*. 2018.

19. Narkiewicz K, Montano N, Cogliati C, Van De Borne PJ, Dyken ME and Somers VK. Altered cardiovascular variability in obstructive sleep apnea. *Circulation*. 1998;98:1071-1077.

20. Tamisier R, Weiss JW and Pépin JL. Sleep biology updates: hemodynamic and autonomic control in sleep disorders. *Metabolism*. 2018.

21. Morgan BJ, Crabtree DC, Puleo DS, Badr MS, Toiber F and Skatrud JB. Neurocirculatory consequences of abrupt change in sleep state in humans. *Journal of Applied Physiology*. 1996;80:1627-1636.

22. Tsuji H, Larson MG, Venditti FJ, Manders ES, Evans JC, Feldman CL and Levy D. Impact of reduced heart rate variability on risk for cardiac events The Framingham Heart Study. *Circulation*. 1996;94:2850-2855.

23. Wolf J, Lewicka J and Narkiewicz K. Obstructive sleep apnea: An update on mechanisms and cardiovascular consequences. *Nutrition, Metabolism and Cardiovascular Diseases*. 2007;17:233-240.

24. Jensen MT, Marott JL, Lange P, Vestbo J, Schnohr P, Nielsen OW, Jensen JS and Jensen GB. Resting heart rate is a predictor of mortality in COPD. *European Respiratory Journal*. 2013;42:341-349.

25. Fisher AB, Chien S, Barakat AI and Nerem RM. Endothelial cellular response to altered shear stress. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2001;281:L529-L533.

26. Alkatib S, Sankri-Tarbichi AG and Badr MS. The impact of obesity on cardiac dysfunction in patients with sleep-disordered breathing. *Sleep and Breathing*. 2014;18:137-142.

27. Dincer HE and O'Neill W. Deleterious effects of sleep-disordered breathing on the heart and vascular system. *Respiration; international review of thoracic diseases*. 2006;73:124-30.

28. Franklin K, Sahlin C, Nilsson J and Näslund U. Sleep apnoea and nocturnal angina. *The Lancet*. 1995;345:1085-1087.

29. Kuniyoshi FHS, Garcia-Touchard A, Gami AS, Romero-Corral A, van der Walt C, Pusalavidyasagar S, Kara T, Caples SM, Pressman GS and Vasquez EC. Day–night variation of acute myocardial infarction in obstructive sleep apnea. *Journal of the American College of Cardiology*. 2008;52:343-346.

30. Wolf J, Drozdowski J, Czechowicz K, Winklewski PJ, Jassem E, Kara T, Somers VK and Narkiewicz K. Effect of beta-blocker therapy on heart rate response in patients with hypertension and newly diagnosed untreated obstructive sleep apnea syndrome. *International Journal of Cardiology*. 2016;202:67-72.

31. Gottlieb SS, McCarter RJ and Vogel RA. Effect of Beta-Blockade on Mortality among High-Risk and Low-Risk Patients after Myocardial Infarction. *New England Journal of Medicine*. 1998;339:489-497.

32. Sankari A, Bascom AT, Riehani A and Badr MS. Tetraplegia is associated with enhanced peripheral chemoreflex sensitivity and ventilatory long-term facilitation. *J Appl Physiol (1985)*. 2015;119:1183-93.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

33. Barr E, Tonkin A, Welborn T and Shaw J. Validity of self-reported cardiovascular disease events in comparison to medical record adjudication and a statewide hospital morbidity database: the AusDiab study. *Internal medicine journal*. 2009;39:49-53.
34. Ohira T, Roux AVD, Prineas RJ, Kizilbash MA, Carnethon MR and Folsom AR. Associations of psychosocial factors with heart rate and its short-term variability: multi-ethnic study of atherosclerosis. *Psychosomatic medicine*. 2008;70:141-146.
35. Kawano Y, Tamura A, Watanabe T and Kadota J. Influence of the severity of obstructive sleep apnea on heart rate. *Journal of Cardiology*. 2010;56:27-34.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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,

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 age, body mass index, and AHI 4% categories. * Model 2 adjusted for age, body mass index, AHI 4% categories, Diabetes, HTN, stroke, and smoking. ** Model 3 additionally adjusted for average heart rate, and % TST < 90%.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 2S: Adjusted Time to Event Cox Proportional Hazards Models for RRDI Predicting Incidence of Composite CVD Event for Continuous RRDI and across Categories of **sleep 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)	**1.20 (1.07, 1.34)** **0.0015**	**1.19 (1.06, 1.33)** **0.003**	**1.19 (1.06, 1.33)** **0.003**
RRDI 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; RRDI, 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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

age, body mass index, and AHI 4% categories. * Model 2 adjusted for age, body mass index, AHI 4% categories, Diabetes, HTN, stroke, and smoking. ** Model 3 additionally adjusted for average heart rate, and % TST < 90%.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 (Hydrocholorothiazide / Bisoprolol combo)
Carvediol (Coreg, Cartrol)
Pindolol (Visken)
Diltiazem HCL (Cardizem, Dilacor,

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

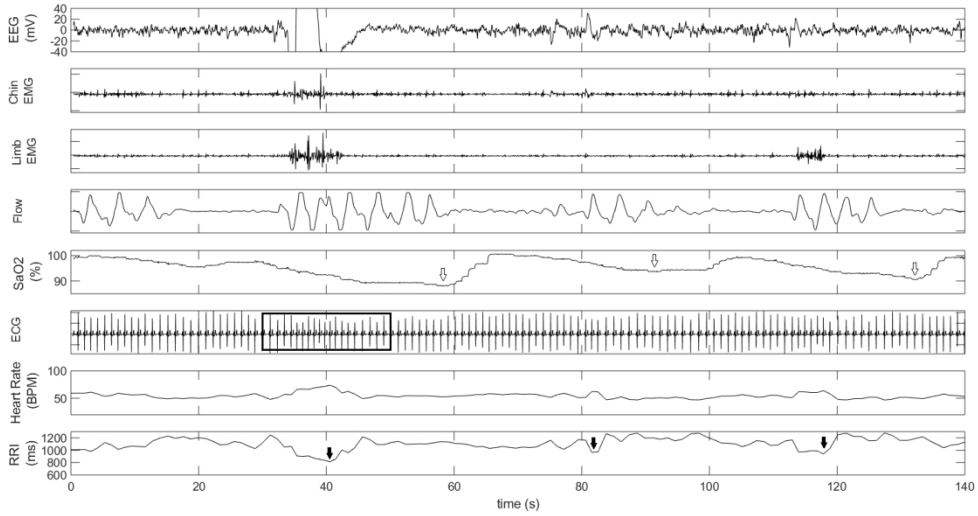


Figure 1-A

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

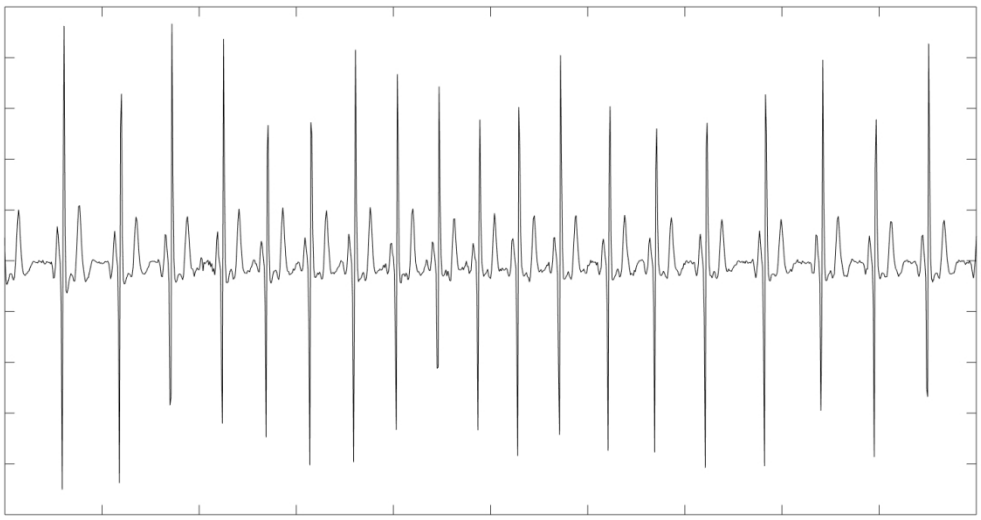


Figure 1-B

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

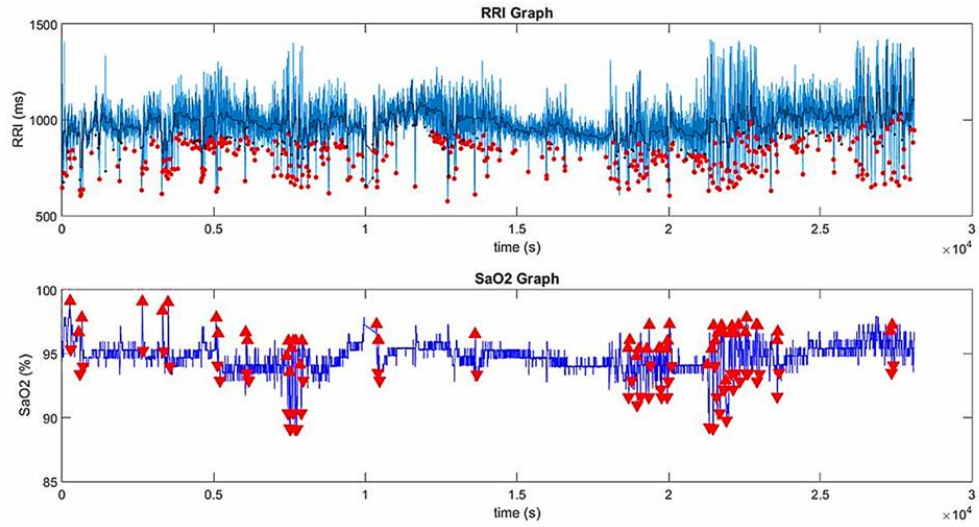


Figure 2

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

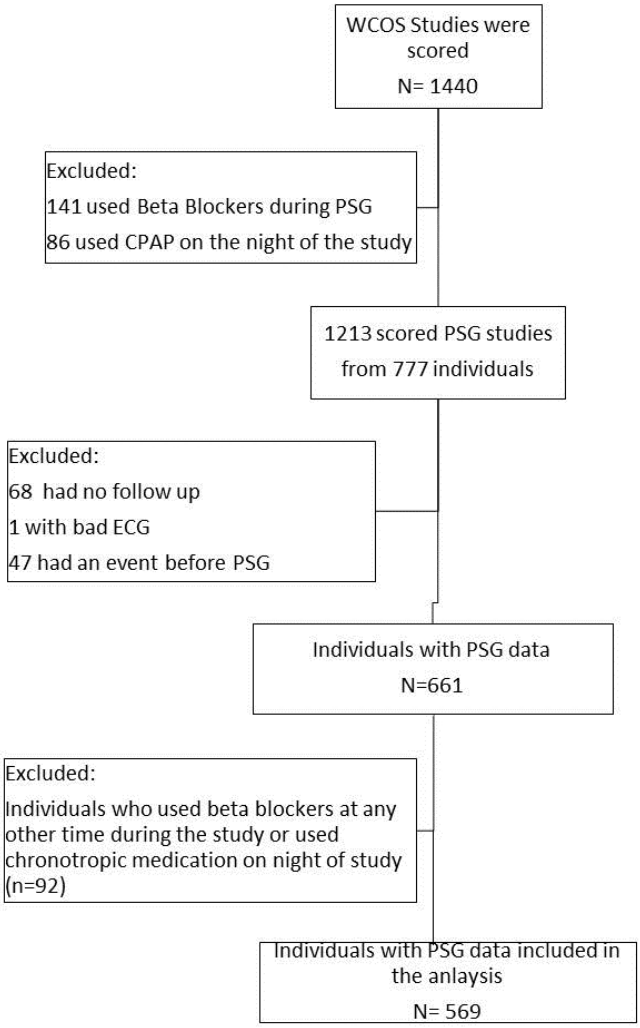


Figure 3

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

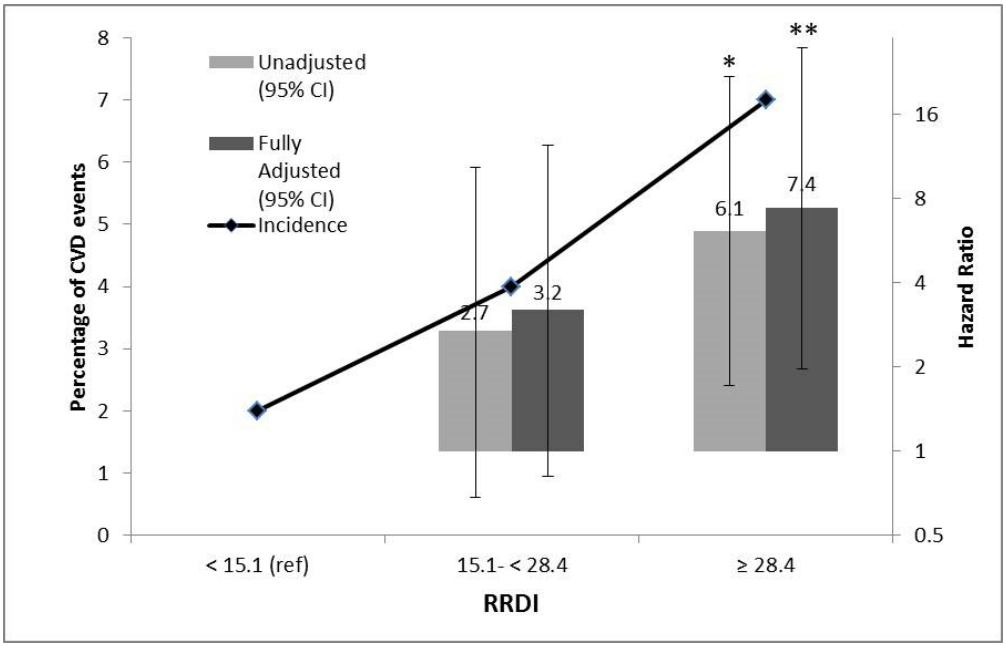
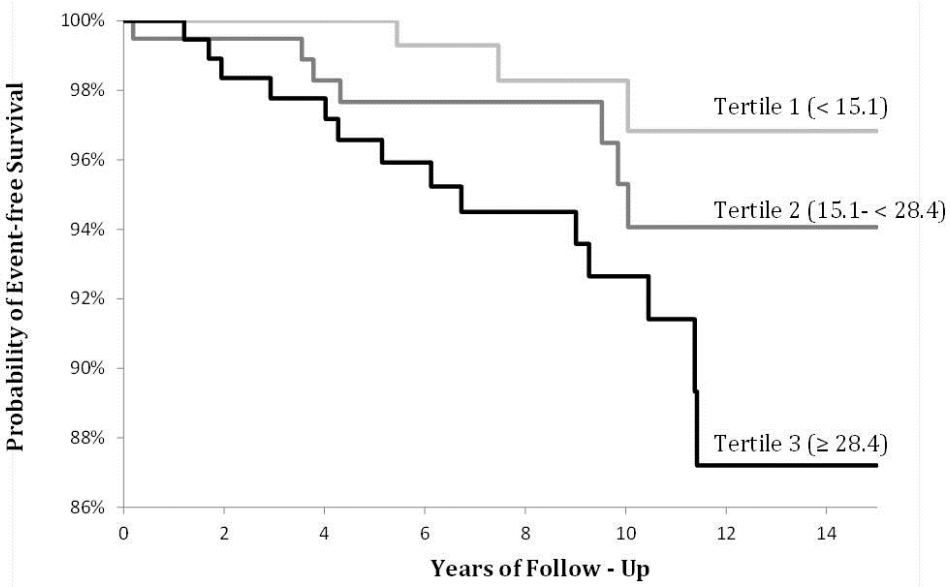


Figure 4

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.



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: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-030559.R1
Article Type:	Research
Date Submitted by the Author:	28-May-2019
Complete List of Authors:	Sankari, Abdulghani; Wayne State University School of Medicine, ; Wayne State University-Detroit Ravelo, Laurel; University of Wisconsin, Population Health Sciences Maresh, Scott; Wayne State University School of Medicine Aljundi, Nawar; Wayne State University School of Medicine Alsabri, Bander; Wayne State University School of Medicine Fawaz, Serene; Wayne State University School of Medicine Hamdon, Mulham; Wayne State University School of Medicine Al-kubaisi, Ghazwan ; Wayne State University School of Medicine Detroit, MI, USA Hagen, Erika; University of Wisconsin, Population Health Sciences Badr, Safwan; Wayne State University School of Medicine, Division of Pulmonary Critical Care and Sleep Medicine, Department of Internal Medicine; Peppard, Paul; University of Wisconsin, Population Health Sciences
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	SLEEP MEDICINE, cardiovascular disease, Heart rate, Cardiac Epidemiology < CARDIOLOGY, EPIDEMIOLOGY

SCHOLARONE™
Manuscripts

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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

Abdulghani Sankari[1], Laurel A. Ravelo[2], Scott A. Maresh[1], Nawar Aljundi[1], Bander Alsabri[1], Serene Fawaz[1], Mulham S. Hamdon[1], Ghazwan Al-Kubaisi[1], Erika. W. Hagen[2], M.S. Badr[1], Paul E. Peppard[2]

(1) Sleep Research Laboratory, John D. Dingell Veterans Affairs Medical Center, Wayne State University School of Medicine, Detroit, MI

(2) Population Health Sciences, the University of Wisconsin School of Medicine and Public Health, Madison, WI

Address correspondence to Abdulghani Sankari, M.D., Ph.D., Division of Pulmonary, Critical Care, and Sleep Medicine, 3990 John R, 3-Hudson, Detroit, MI 48201; Tel: (313) 745-6033; Fax: (313) 745-8725; Email:asankari@wayne.edu

Words count: 7237

Contributorship statement:

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

The acquisition, analysis, or interpretation of data: A. Sankari, M. S. Badr, P. E. Peppard, L. A. Ravelo, S. A. Maresh, N. Aljundi, B. Alsabri, S. Fawaz, M. S. Hamdon, G. Al-Kubaisi.

Statistical analysis: L. A. Ravelo, E. W. Hagen, and P. E. Peppard.

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

Study supervision: A. Sankari and P. E. Peppard.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Conflict of interest: All authors declare no conflict of interest.

Disclosure Statement: The following authors A. Sankari, M.S Badr, and S. Maresh and Wayne State University have a pending Patent #US62395634, entitled "The Detection of Sleep Disordered Breathing Using Cardiac Autonomic Responses", application number#15/706097 for Utility/Design using an application data sheet (37 CFR 1.54), Date Filed: September 15, 2017. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Department of Veterans Affairs, National Institute of Health or Wayne State University.

Data Availability:

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

Funding support: 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

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Development of the VA Office of Research and Development from the (U.S.) Department of Veterans Affairs and by Cardiovascular Research Institute [CVRI].

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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/m^2), 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

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

10-unit increment in RRDI [95% CI, 1.09-1.35], P < 0.001) which remained significant after adjusting for demographic factors, AHI 4%, hypoxemia, and other comorbidities.

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

Keywords: Heart rate, R-R interval, sleep-disordered breathing, cardiovascular disease.

Abbreviations: AHI, apnea-hypopnea index, BMI, body mass index; CVD, cardiovascular disease; ECG, electrocardiograph; HR, heart rate; PSG, polysomnography; RRI, R-R interval; RRDI, RRI dips index; SDB, Sleep-disordered breathing; SHHS, Sleep Heart Health Study; WSCS, Wisconsin Sleep Cohort Study.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

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.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 sleep [1]. 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 night[2]. Indeed, recent evidence showed that sub-type of excessively sleepy patients with moderate to severe SDB have significantly increased the risk for prevalent and incident cardiovascular events indicating the central role of sleep disruption in increased CVD risk[3]. In addition, a recent study found that varying 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 threshold [6] 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 [7] independent of covariates, suggesting that

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

genetic factors play an important role in controlling these cardiovascular risk factors[8]. 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 crietria[9]. However, the long-term effect of heart rate changes during sleep on the cardiovascular outcome and mortality is unknown.

The objectives of this study were to examine whether R-R interval (RRI) or heart rate accelerations can serve as predictors of cardiovascular disease in the Wisconsin Sleep Cohort study (WSCS), 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. Results of this study have been previously reported in the form of an abstract [10, 11].

Methods

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

Cohort Description: The WSCS comprises 1546 adult employees of state agencies, ages 30-60 years old at the Cohort's inception, which underwent

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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. 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 1S; supplement) on the night of the sleep study or at any other point during follow-up.

Patient and Public Involvement: This study was a secondary analysis for preexisting data from an established cohort of the Wisconsin study. Therefore, participants were not involved in the design, recruitment, or conduct of this study.

Predictor: The main predictor variable is the hourly rate of 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, which are time intervals between successive pairs of QRS complexes, by using software for the detection of R waves in LabChart 7 with heart rate variability Module (AD Instruments, Colorado Springs, CO) (Fig.1). In this program, ECG signal was examined and retrieved to a MatLab R2017a program (MathWorks, Natick, MA) developed and validated by our group to obtain RRI signal for the entire night (Fig. 2)[13, 14]. The RRI dips, defined by a decreased RRI compared to the average RRI for the corresponding

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

1-minute segment as a baseline, were collected. Given that 90% dips threshold correlated previously with most respiratory events (apneic and non-apneic respiratory events, defined below)[14], total RRI dip index (RRDI) was defined by the number of RRI dips below the 90% baseline divided by the total PSG recording time in hours (from light on to light out), regardless of wake or sleep stages. Sleep RRDI for non-REM and REM stages combined were defined by the number of RRI dips below the 90% baseline divided by the total sleep time in hours (for both REM and non-REM sleep stages). Subsequently, sleep RRDI was calculated for specific sleep stages for REM and non-REM, respectively. In subgroup analysis, the gender differences in total and sleep RRDI were compared between men and women.

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

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

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

information. The censored analysis was used, and the data is censored at the last visit. If multiple events were reported over the course of follow-up, the first reported event was used in this analysis.

Covariates: Participants underwent a baseline overnight 18-channel PSG (Grass model 78; Quincy, MA) at the University of Wisconsin-Madison Clinical Research Unit using a standard protocol [15]. The PSG recorded sleep state using electroencephalography, electrooculography, and electromyography; and breathing, using respiratory inductance plethysmography (Respitrace; Ambulatory Monitoring, Ardsley, NY), nasal and oral airflow (ProTec thermocouples; Hendersonville, TN) and oxyhemoglobin saturation, using pulse oximetry (Ohmeda Biox 3740, Englewood, CO). Each 30-second epoch of the polysomnographic recordings was scored for sleep stage, and apnea and hypopnea events by trained technicians and reviewed using standard criteria[15]. Apnea was defined as the cessation of nasal and oral airflow for ≥10 seconds and hypopnea as a discernible reduction in breathing (sum of the chest and abdominal excursions) with a decrease in oxyhemoglobin saturation of ≥ 4%. The apnea-hypopnea index was calculated as the mean number of apnea and hypopnea events per hour of sleep.

Statistical analysis:

Cox proportional hazards regression was used to estimate adjusted hazard ratios and 95% CIs for the association between RRDI and subsequent risk of an

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

incident CVD event [16]. Because of the strong dependence of CVD risk on age, Cox-regression models were based on age as the time scale, (the age when RRDI was measured and age at the event) allowing for left truncation (late entry) [17]. In addition to adjusting for age using this methodology, models 2 were adjusted for 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 RRDI categories[18].

Results

Baseline characteristics:

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

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

CVD incidence – association with total RRDI:

Using Cox Proportional Hazards Model, continuous total RRDI (with 90% threshold for RRI dips) was significantly associated with new-onset CVD event(s) (HR, 1.24 per 10-unit increment in RRDI [95% CI, 1.10-1.39], P < 0.001) which remained significant after adjustment for age, BMI and gender (model 1) and the addition of AHI 4% (model 3) (as depicted in Table 3). Lower thresholds (80%, 70%, and 60%) of RRDI correlated with total RRDI 90% but were less sensitive in predicting CVD (less than 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% (model 3) (P < 0.001). Total RRDI category 3 (≥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 RRDI 90% remained significant (HR, 1.22 (per 10-unit increment in RRDI [95% CI, 1.08, 1.37], P = 0.001) after additionally adjusted for diabetes, hypertension, stroke, smoking, average heart rate, and % total time with oxygen saturation less than 90% (model 4) (as depicted in Table 3).

Figure 4 illustrates the changes in CVD incidence and hazard ratios for total RRDI less than 15.1 dips per hour (as reference), RRDI 15.1-28.4 dips per hour (second tertile) and for the third group (tertile) of individuals with RRDI equal or more than 28.4 dips per hour. Kaplan-Meier survival curves (Fig.5) illustrate

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

decreased CVD event-free survival with increasing total RRDI category from RRDI less than 15.1 to RRDI equal or more than 28.4 dips per hour.

CVD incidence – association with sleep RRDI:

Using Cox Proportional Hazards Model, continuous sleep RRDI 90% was significantly associated with new-onset CVD event(s) (HR, 1.21 per 10-unit increment in RRDI [95% CI, 1.09-1.35], P < 0.001) which remained significant after the model adjusted for age, BMI and gender (model 2) and the addition of AHI 4% (model 3) (as depicted in Table 4). RRDI category 3 (≥23.5/h vs. <9.0/h) was associated with increased CVD hazards risk of 3.39 (95% CI, 1.06-10.85, P = 0.04) and remained significant after adjustment for demographics and AHI 4% (model 4) (P = 0.037). The relationship between sleep RRDI categories and CVD events were predominantly in non-REM sleep as depicted in Table 5. Continuous RRDI 90% during REM sleep was significant (HR, 1.19 per 10-unit increment in RRDI [95% CI, 1.07-1.32], P = 0.001) in the unadjusted model (model 1) and remained significant after the model adjusted for age, BMI and gender (model 2) and the addition of AHI 4% (model 3) (as depicted in Table 6). However, sleep RRDI category 3 (≥24/h vs. <9.0/h) was only significant in the unadjusted model (model 1) with hazards risk of new CVD events of 2.92 (p=0.04).

Cardiovascular events were detected in twenty-five participants (4%), of the sample, over a follow-up interval of 15 years with mean age 59 years old (range

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

41- 80). Cardiovascular events consisted of heart failure, heart attack, CVD Procedure (before any events), or CVD Death (Table 7). No difference between mean age at the start for those who had CVD vs not (P=0.58).

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

Table 8 presents the baseline characteristics of men and women participants and associated total and sleep RRDI. While BMI was higher in women (32.0 ± 7.0 $vs.$ 30.0 ± 5.0 kg/m^2, P=0.0001), men had higher AHI, total RRDI and sleep RRDI than women (P<0.01). Using Cox Proportional Hazards adjusted model (3) (for age, body mass index, and AHI 4%), continuous total RRDI 90% was significantly associated with new-onset CVD event(s) in men (HR, 1.22 per 10-unit increment in RRDI [95% CI, 1.06, 1.40], P < 0.001) but not in women (as depicted in Table 8). Likewise, continuous sleep RRDI 90% was significantly associated with new-onset CVD event(s) in men (HR, 1.19 per 10-unit increment in RRDI [95% CI, 1.04, 1.36], P < 0.05) but not in women. Using total RRDI threshold of 20 dips per hour or more from the adjusted model (model 3) was associated with increased CVD hazards risk of 4.34 in men only (95% CI, 1.32, 14.34, P = 0.016).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 ≥4% de-saturations, but not by SDB characterized by de-saturations of less than 4% [19]. More recently, it has

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

been found that the desaturation hypoxic burden related to respiratory events, measured by the integration of the severity of the desaturation and its length, predicted CVD mortality[20]. Another study that examined heart rate variability during sleep found that SDB patients had shorter RRI and increased sympathetic burst frequency (49±4 bursts/min) compared with control subjects [21]. The authors speculated that abnormalities in heart rate and blood pressure variability might be implicated in the subsequent development of cardiovascular disease in patients with SDB. The present study confirmed this association between the frequency of heart rate accelerations (RRDI) and adverse cardiovascular consequence in a prospective large cohort of individuals who had no known heart disease at the time of their sleep study.

The mechanism of increased incidence of CVD and association with RRDI can be explained by an increased sympathetic tone and autonomic arousals. First, sleep disturbances like SDB, periodic limb movements, insufficient sleep are all associated with an increased risk of CVD. These sleep disorders are commonly associated with impaired autonomic nervous system leading to increased sympathetic tone[22]. Furthermore, sleep fragmentation due to autonomic or respiratory arousals from sleep increases the cardiac sympathetic tone activity resulting in a sudden elevation in vascular tone and heart rate generating a rise in arterial blood pressure [22, 23]. The increased sympathetic tone in patients with heart disease has been proposed as an intermediate outcome linking heart rate variability with increased mortality [24]. Resting heart rate also has been linked to CVD in patients with SDB and COPD [21, 25, 26]. Second, the augmented shear

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

forces due to intermittent episodes of tachycardia secondary to respiratory events and the resultant mechanical shear forces may lead to endothelial dysfunction [27]. This possibility is physiologically plausible particularly in male patients,[28] who may have significant endothelial dysfunction secondary to activation of several inflammatory pathways. Such pathophysiologic changes in untreated SDB patients have been linked to nocturnal angina, myocyte necrosis leading to cardiomyopathy, and cardiac remodeling [29-31]. Our findings corroborate these pathological changes particularly in the coronary vessels making the vast majority of CVD events either related to CAD (48%) or myocardial infarction (36%), predominantly in men. On the other hand, medications such as Beta-blockers attenuate the increase in heart rate related to respiratory events during sleep in patients with hypertension and untreated SDB [32]. This modulation of cardiac responses in patients with SDB provides a mechanism by which Beta-blockers may decrease the risk of sudden cardiac death, particularly in patients with CVD [33]. Finally, hypoxic events can affect the autonomic cardiac response and generate significant RRI dips events [34]. Hypoxemia and RRI dips may represent different features of SDB-related stress, both of which may contribute to CVD morbidity and mortality through independent pathways. Our findings suggest a need to further identify the intermediate mechanisms that link RRI dips events to long-term outcomes.

This study has several strengths including its prospective design with longitudinal follow-up of participants, community-based including a diverse group of ages and morbidities from both genders, and the use of the gold-standard laboratory-based

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 accelerations that can be translated into an executable program or a plug-in for sleep scoring software and can be used in any sleep study across the world. The study has some limitations. First, we used the self-reported diagnosis of CVD (including dates of diagnosis). However, there is evidence that self-reported CVD is very reliable and accurate as noted in the AusDiab cohort. Barr et al. reported more than 99% of self-reported CVD events were correctly verified in the patients' medical records; only 0.2% of those denying any CVD event being recorded as having had an event on the medical record [35]. Second, we lack racial diversity in our study as 95% was reported as a white race. Therefore, the results may not be 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 might not be applicable to

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

individuals with heart disease or if taking Beta-blocker or chronotropic medications. Likewise, this study might not be applicable to individuals with arrhythmia, frequent ectopic beats and in case of cardiac pacemakers.

Clinical Perspectives:

The association between heart rate changes and the cardiovascular outcome may have significant clinical implications. First, the association between increased incidence of cardiovascular events and the RRI dips index suggest that early detection of heart rate fluctuations during sleep could identify those who are at increased risk of future CVD events and inform primary preventions strategies. Second, several behavioral factors[36] and medical conditions, such as SDB[37] and COPD[26], are associated with changes in resting heart rate, which increase the risk of cardiovascular diseases. Third, the attenuation of heart rate accelerations by Beta-blockers during sleep as recently shown in patients with SDB,[32] indicate that Beta-blockers may play an important role in preventing CVD. However, large prospective clinical studies are needed to confirm this finding.

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

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Acknowledgments

The authors would like to thank Amanda Rasmuson for her assistance throughout the study. The authors would like also to thank Dr. Aiden Abidov and Naresh Punjabi for their valuable advice.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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)

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 SaO2, 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)

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 3: The adjusted time to event Cox Proportional Hazards Models for **total RRDI** 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)	**1.24 (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; RRDI, 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%.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Table 4: The adjusted time to event Cox Proportional Hazards Models for **sleep RRDI** 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; RRDI, 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%.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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) 0.0013**	**1.17 (1.05, 1.31) 0.0056**	**1.19 (1.07, 1.33) 0.0016**
RRDI Category				
Tertile 1 (< 9.0)	5/187 (3)	REF	REF	REF
Tertile 2 (9.0- < 24.0)	7/194 (4)	1.34 (0.42, 4.24) 0.6222	1.19 (0.37, 3.78) 0.7732	1.24 (0.39, 4.00) 0.7171
Tertile 3 (≥ 24.0)	12/188 (6)	2.92 (1.00, 8.55) 0.0508	2.42 (0.80, 7.29) 0.1173	2.69 (0.88, 8.19) 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).

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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,

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 oximetry 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.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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

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

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

References

1. Somers V. American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology. American Heart Association Stroke Council. American Heart Association Council on Cardiovascular Nursing. American College of Cardiology Foundation. Sleep apnea and cardiovascular disease: an American Heart Association/American College Of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council On Cardiovascular Nursing. In collaboration with the National Heart, Lung, and Blood Institute National Center on Sleep Disorders Research (National Institutes of Health). *Circulation*. 2008;118:1080-1111.

2. Sankari A and Badr MS. Diagnosis of Sleep Disordered Breathing in Patients With Chronic Spinal Cord Injury. *Arch Phys Med Rehabil*. 2016;97:176-7.

3. Mazzotti DR, Keenan BT, Lim DC, Gottlieb DJ, Kim J and Pack AI. Symptom Subtypes of Obstructive Sleep Apnea Predict Incidence of Cardiovascular Outcomes. *American Journal of Respiratory and Critical Care Medicine*. 2019.

4. Won C, Qin L, Selim B and Yaggi H. Varying Hypopnea Definitions Affect Obstructive Sleep Apnea Severity Classification and Association With Cardiovascular Disease. *Journal of clinical sleep medicine: JCSM: official publication of the American Academy of Sleep Medicine*. 2018.

5. Mansukhani MP, Kolla BP, Wang Z and Morgenthaler TI. Effect of Varying Definitions of Hypopnea on the Diagnosis and Clinical Outcomes of Sleep-Disordered Breathing: A Systematic Review and Meta-Analysis. *Journal of Clinical Sleep Medicine*. 2019;15:687-696.

6. Butler MP, Emch JT, Rueschman M, Sands SA, Shea SA, Wellman A and Redline S. Apnea-Hypopnea Event Duration Predicts Mortality in Men and Women in the Sleep Heart Health Study. *American journal of respiratory and critical care medicine*. 2019;199:903-912.

7. Singh JP, Larson MG, O'Donnell CJ, Tsuji H, Evans JC and Levy D. Heritability of heart rate variability: the Framingham Heart Study. *Circulation*. 1999;99:2251-2254.

8. Fava C, Burri P, Almgren P, Arcaro G, Groop L, Hulthén UL and Melander O. Dipping and Variability of Blood Pressure and Heart Rate at Night Are Heritable Traits*. *American Journal of Hypertension*. 2005;18:1402-1407.

9. Zinchuk AV, Jeon S, Koo BB, Yan X, Bravata DM, Qin L, Selim BJ, Strohl KP, Redeker NS and Concato J. Polysomnographic phenotypes and their cardiovascular implications in obstructive sleep apnoea. *Thorax*. 2018;73:472-480.

10. Finn LA, Maresh S, Hamdon MS, Al-kubaisi G, Badr M, Peppard PE and Sankari A. A New Marker Of Cardiovascular Disease In Patients With Sleep-Disordered Breathing: Results From The Wisconsin Sleep Cohort Study *A80-A ARE HSTS OBSOLETE? NOVEL DIAGNOSTICS IN SDB*: American Thoracic Society; 2016: A2523-A2523.

11. Sankari A, Maresh S, Finn L, Aljundi N, Bander Alsabri B, Serene Fawaz S, Hamdon M, Hagen E, Badr M and Peppard P. Association of Nocturnal RR Intervals Changes and Cardiovascular Outcome in a Large Prospective Community-Based Cohort *D109 SRN: OUTCOMES AND IMPACT OF SLEEP AND RESPIRATORY DISORDERS*: American Thoracic Society; 2019: A7273-A7273.

12. Young T, Palta M, Dempsey J, Skatrud J, Weber S and Badr S. The Occurrence of Sleep-Disordered Breathing among Middle-Aged Adults. *New England Journal of Medicine*. 1993;328:1230-1235.

13. Sankari A, Badr MS and Maresh S. Detection of sleep disordered breathing using cardiac autonomic responses. 2018.

14. Sankari A, Pranathiageswaran S, Maresh S, Hosni AM and Badr MS. Characteristics and Consequences of Non-apneic Respiratory Events During Sleep. *Sleep*. 2016;40.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

15. Flemons W, Buysse D, Redline S, Oack A, Strohl K, Wheatley J, Young T, Douglas N, Levy P and McNicolas W. Sleep-related breathing disorders in adults. *Sleep*. 1999;22:667-689.
16. Cox PR. *Life Tables*: Wiley Online Library; 1972.
17. Kom EL, Graubard BI and Midthune D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. *American journal of epidemiology*. 1997;145:72-80.
18. Kaplan EL and Meier P. Nonparametric estimation from incomplete observations. *Journal of the American statistical association*. 1958;53:457-481.
19. Punjabi NM, Newman AB, Young TB, Resnick HE and Sanders MH. Sleep-disordered breathing and cardiovascular disease: an outcome-based definition of hypopneas. *American journal of respiratory and critical care medicine*. 2008;177:1150-1155.
20. Azarbarzin A, Sands SA, Stone KL, Taranto-Montemurro L, Messineo L, Terrill PI, Ancoli-Israel S, Ensrud K, Purcell S and White DP. The hypoxic burden of sleep apnoea predicts cardiovascular disease-related mortality: the Osteoporotic Fractures in Men Study and the Sleep Heart Health Study. *European heart journal*. 2018.
21. Narkiewicz K, Montano N, Cogliati C, Van De Borne PJ, Dyken ME and Somers VK. Altered cardiovascular variability in obstructive sleep apnea. *Circulation*. 1998;98:1071-1077.
22. Tamisier R, Weiss JW and Pépin JL. Sleep biology updates: hemodynamic and autonomic control in sleep disorders. *Metabolism*. 2018.
23. Morgan BJ, Crabtree DC, Puleo DS, Badr MS, Toiber F and Skatrud JB. Neurocirculatory consequences of abrupt change in sleep state in humans. *Journal of Applied Physiology*. 1996;80:1627-1636.
24. Tsuji H, Larson MG, Venditti FJ, Manders ES, Evans JC, Feldman CL and Levy D. Impact of reduced heart rate variability on risk for cardiac events The Framingham Heart Study. *Circulation*. 1996;94:2850-2855.
25. Wolf J, Lewicka J and Narkiewicz K. Obstructive sleep apnea: An update on mechanisms and cardiovascular consequences. *Nutrition, Metabolism and Cardiovascular Diseases*. 2007;17:233-240.
26. Jensen MT, Marott JL, Lange P, Vestbo J, Schnohr P, Nielsen OW, Jensen JS and Jensen GB. Resting heart rate is a predictor of mortality in COPD. *European Respiratory Journal*. 2013;42:341-349.
27. Fisher AB, Chien S, Barakat AI and Nerem RM. Endothelial cellular response to altered shear stress. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2001;281:L529-L533.
28. Alkatib S, Sankri-Tarbichi AG and Badr MS. The impact of obesity on cardiac dysfunction in patients with sleep-disordered breathing. *Sleep and Breathing*. 2014;18:137-142.
29. Dincer HE and O'Neill W. Deleterious effects of sleep-disordered breathing on the heart and vascular system. *Respiration; international review of thoracic diseases*. 2006;73:124-30.
30. Franklin K, Sahlin C, Nilsson J and Näslund U. Sleep apnoea and nocturnal angina. *The Lancet*. 1995;345:1085-1087.
31. Kuniyoshi FHS, Garcia-Touchard A, Gami AS, Romero-Corral A, van der Walt C, Pusalavidyasagar S, Kara T, Caples SM, Pressman GS and Vasquez EC. Day–night variation of acute myocardial infarction in obstructive sleep apnea. *Journal of the American College of Cardiology*. 2008;52:343-346.
32. Wolf J, Drozdowski J, Czechowicz K, Winklewski PJ, Jassem E, Kara T, Somers VK and Narkiewicz K. Effect of beta-blocker therapy on heart rate response in patients with hypertension and newly diagnosed untreated obstructive sleep apnea syndrome. *International Journal of Cardiology*. 2016;202:67-72.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

33. Gottlieb SS, McCarter RJ and Vogel RA. Effect of Beta-Blockade on Mortality among High-Risk and Low-Risk Patients after Myocardial Infarction. *New England Journal of Medicine*. 1998;339:489-497.

34. Sankari A, Bascom AT, Riehani A and Badr MS. Tetraplegia is associated with enhanced peripheral chemoreflex sensitivity and ventilatory long-term facilitation. *J Appl Physiol (1985)*. 2015;119:1183-93.

35. Barr E, Tonkin A, Welborn T and Shaw J. Validity of self-reported cardiovascular disease events in comparison to medical record adjudication and a statewide hospital morbidity database: the AusDiab study. *Internal medicine journal*. 2009;39:49-53.

36. Ohira T, Roux AVD, Prineas RJ, Kizilbash MA, Carnethon MR and Folsom AR. Associations of psychosocial factors with heart rate and its short-term variability: multi-ethnic study of atherosclerosis. *Psychosomatic medicine*. 2008;70:141-146.

37. Kawano Y, Tamura A, Watanabe T and Kadota J. Influence of the severity of obstructive sleep apnea on heart rate. *Journal of Cardiology*. 2010;56:27-34.

For peer review only

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

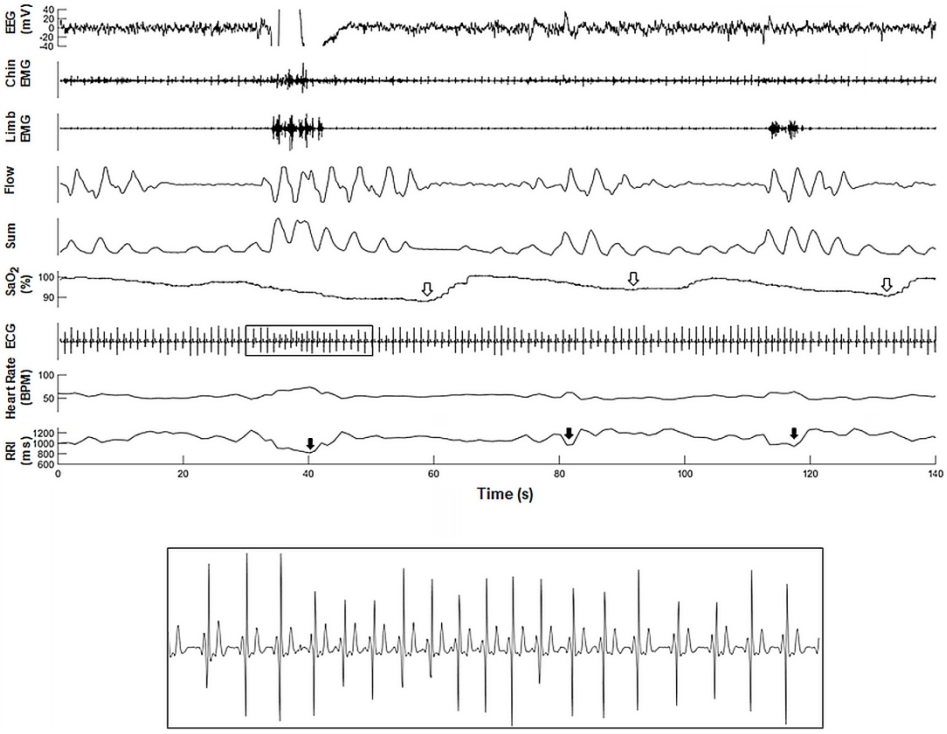


Figure 1

199x150mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

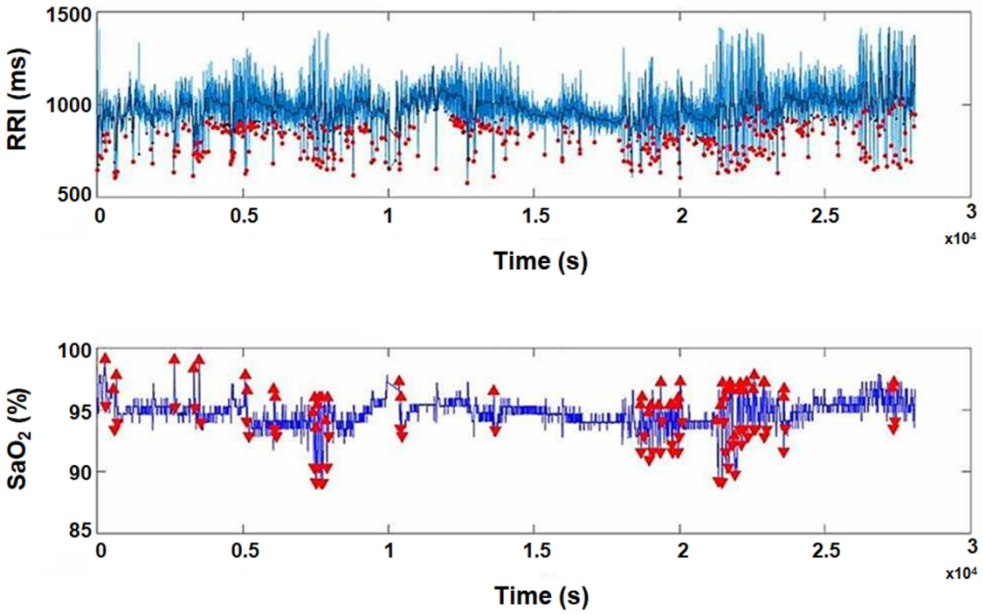


Figure 2

140x90mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

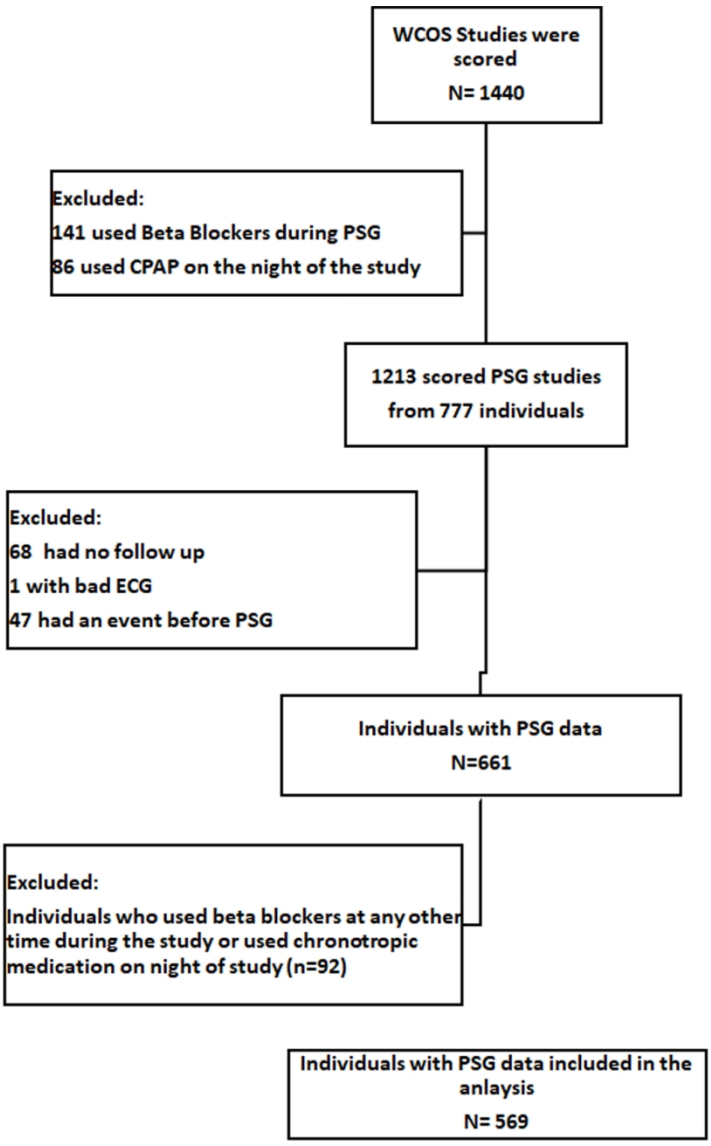


Figure 3

90x150mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

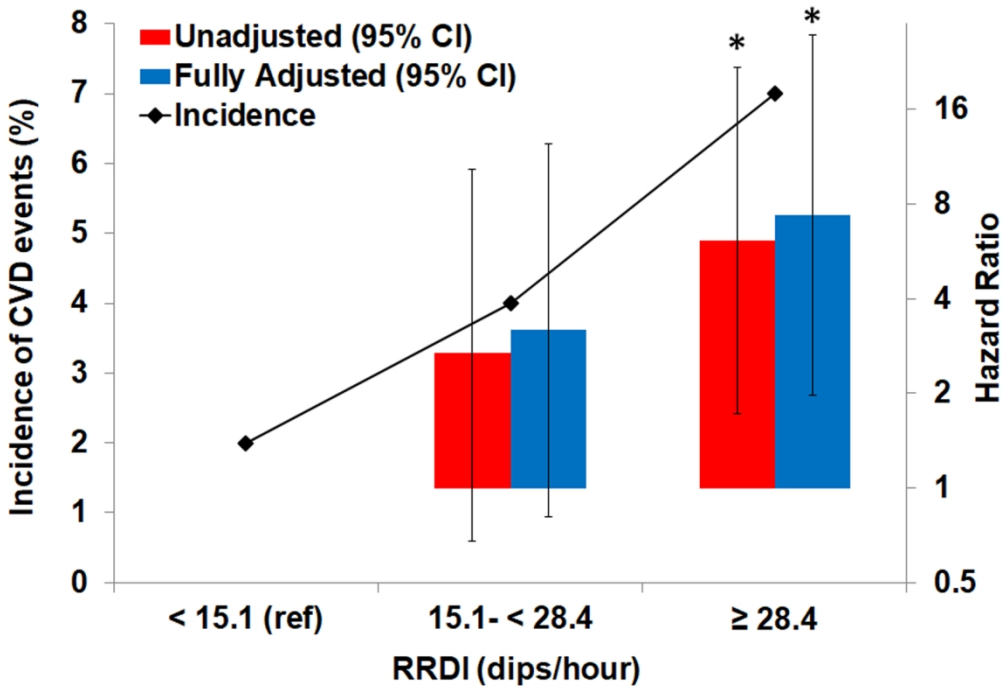
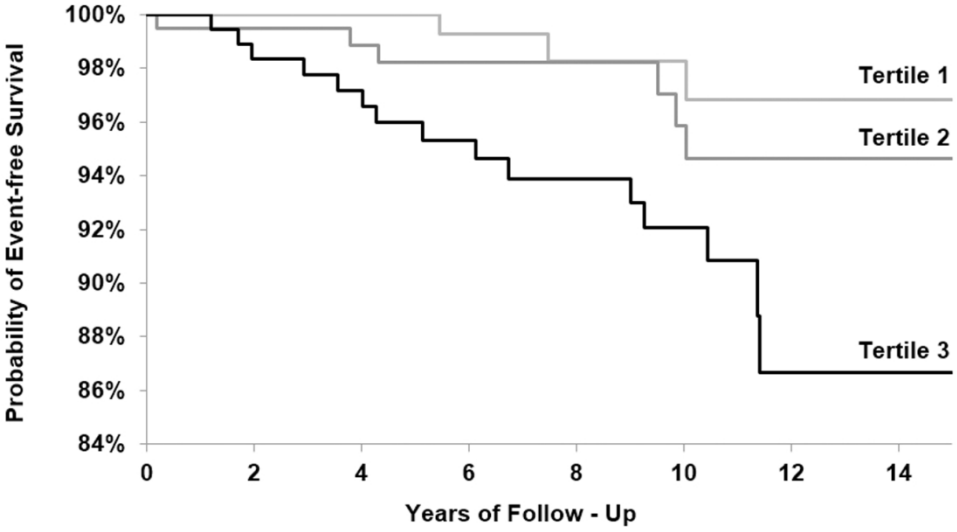


Figure 4

150x109mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.



Log-Rank p=0.0139 for total RRDI

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

Figure 5

119x90mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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 (Hydrocholorothiazide / Bisoprolol combo)
Carvediol (Coreg, Cartrol)
Pindolol (Visken)
Diltiazem HCL (Cardizem, Dilacor, Taztia XT, Tiazac)
Amiodarone HCl (Cordarone, tikosyn)

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

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

	Item No.	Recommendation	Page No.	Relevant text from manuscript
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 crietria[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 (WSCS), 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*

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

		design early in the paper		
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.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

		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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Quantitative variables	*11*	*Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why*	*9*	The RRDI values were examined as a continuous variable and as a categorical variable divided into tertiles [lower 25% (low), middle 50% (medium), and upper 25% (high)].
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*	*31*	Figure 3: The Wisconsin Sleep Cohort Study (WSCS) sample

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

		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		

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

		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 (≥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

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

For peer review only

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

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

accelerations that can be translated into an executable program or a plug-in for sleep scoring software and can be used in any sleep study across the world. The study has some limitations. First, we used the self-reported diagnosis of CVD (including dates of diagnosis). However, there is evidence that self-reported CVD is very reliable and accurate as noted in the AusDiab cohort. Barr et al. reported more than 99% of self-reported CVD events were correctly verified in the patients' medical records; only 0.2% of those denying any CVD event being recorded as having had an event on the medical record [33]. 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.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.

Interpretation	*20*	*Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence*	*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.
Generalisability	*21*	*Discuss the generalisability (external validity) of the study results*	*19*	this study may not be applicable to individuals with arrhythmia, frequent ectopic beats and in case of cardiac pacemakers.
Other information				
Funding	*22*	*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*	*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 [CVRI].

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2019-030559 on 17 July 2019. Downloaded from http://bmjopen.bmj.com/ on April 23, 2024 by guest. Protected by copyright.