Study Samples

The Sleep Heart Health Study (SHHS): The Sleep Heart Health Study (SHHS) is a large community-based, prospective cohort study designed to assess the cardiovascular outcomes of sleep-disordered breathing in adults[1-3]. A total of 6,441, ages $\geq$ 40 years, were recruited from multiple established "parent" cohorts to participate in a sleep exam which included an in-home polysomnography (PSG) (1995-1998), none who reported use of positive airway pressure (PAP), a mandibular advancement device or overnight oxygen therapy. As described, among the younger individuals, those reporting snoring were over-recruited[4]. Of these, 5792 studies were available on the National Sleep Research Resource (NSRR) website(www.sleepdata.org). (Strong Heart Study participants were not included because of data sharing restrictions) and 5473 had sufficient quality EEG (161 with poor EEG quality) for arousal scoring and had complete data on covariates and outcome.

The Multi-Ethnic Study of Atherosclerosis (MESA): The Multi-Ethnic Study of Atherosclerosis (MESA) is a longitudinal community-based cohort of 6,814 adults enrolled between 2000-2002 (Exam 1) from six clinical centers when participants were ages 45-85 years and free of known cardiovascular disease[5]. As described previously, 2261 participated in the sleep exam[6], performed in proximity to the Exam 5 (2010 to 2013), which included sleep questionnaires, actigraphy, and in-home PSG[7]. Participants who reported nightly use of sleep apnea treatment (including PAP, oral appliances, or oxygen therapy) were excluded. From 2035 individuals with available PSG data on the NSRR website, 1904 participants had complete data on covariates and outcomes, had sufficient quality EEG (20 with poor EEG quality) for arousal scoring, and were available for analysis.

The Osteoporotic Fractures in Men (MrOS) Study: The Osteoporotic Fractures in Men (MrOS) Sleep Study (http://mrosdata.sfcc-cpmc.net), was a community-based, prospective cohort study of 5,994 men $\geq$ 65 years enrolled between 2000-2002 from six centers across the U.S, and designed to describe the epidemiology of osteoporosis and fractures in older men(3, 8-10). A total of 3,135 men from the MrOS parent study participated in the ancillary MrOS Sleep Study (2003-2005). Sleep evaluations including full in-home PSG (similar to the MESA exam) and actigraphy were performed as described previously.(11) Participants who reported nightly use of PAP, oral appliances, or oxygen therapy) were excluded. Of the 3,135 participants who completed the sleep study, the polysomnograms of 2,907 met initial study quality criteria and were available on the NSRR website. After excluding 222 men with incomplete data and low-quality EEG (35 with poor EEG quality), 2685 were available for analysis.

In all three cohorts, Institutional Review Board approval was obtained at all study sites and all participants provided written informed consent.

Clinical Endpoints and Outcomes

Definition of Hypertension: For this report, hypertension was defined as participant reported use of anti-hypertensive medications, or an average systolic blood pressure (SBP) measurement of $\geq$ 140 mmHg or a diastolic blood pressure (DBP) measurement of $\geq$ 90 mmHg, obtained during a research clinic examination. This definition was based on the sixth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC–VI)(12). SBP and DBP were the mean value of two measurements in sitting position.

Definition of Diabetes: Diabetes was defined based on current treatment of diabetes using hypoglycemic medications or insulin in SHHS and MESA, while in MrOS, diabetes was defined based on a "yes" answer to "Is your diabetes being treated by a doctor?" Those who answered "I don't know" or were unsure were considered non-diabetic.

Definition of Sleepiness: Excessive daytime sleepiness (or sleepiness) was defined as an ESS score of 11 or more.

Definition of Incident CVD:

SHHS: In SHHS incident CVD included fatal and non-fatal myocardial infarction (MI), MI-related procedures, fatal and non-fatal stroke, congestive heart failure, coronary heart disease (CHD)-related death, and other CVD-related deaths not defined in other events as defined by adjudication procedures described before(1, 13). The follow-up time was defined as the time between the sleep study and the first CVD event or the last contact. Of 5473 participants, 727 from the New York University-Cornell site were excluded because outcome data were not available (for incident CVD analysis only). Those with preexisting CVD (defined as MI, coronary angioplasty, angina, coronary artery bypass graft, congestive heart failure, and stroke; N=802) prior to the SHHS baseline exam were excluded (3944 available for incident CVD analysis).

MESA: CVD-related events included MI, resuscitated cardiac arrest, definite angina, probable angina (if followed by revascularization), stroke, stroke death, CHD-related death, other CVD- or atherosclerotic-related death not otherwise defined in other categories, ascertained through periodic follow-up contact and adjudicated as described before(5). The

follow-up time was defined as the time between the sleep study and the first CVD event or the last contact. Those with preexisting CVD, defined as all CVD-related events at the baseline sleep study (Exam 5), were excluded from analysis of incident events (N=121). A total of 1783 individuals were available for incident CVD analysis.

MrOS: After the Sleep Visit, participants were contacted by postcard and/or phone contact every four months; 99% of contacts were completed. Incident CVD was defined as any type of fatal or non-fatal cardiovascular events, including CHD, cerebrovascular disease events, peripheral vascular disease, other CVD events, and any heart failure, adjudicated as described previously(14). For both nonfatal and fatal cardiovascular events, all documents were adjudicated by a board-certified cardiologist using a prespecified adjudication protocol developed using methods that had been successfully employed at the coordinating center for both prior randomized trials and epidemiological studies of CVD. In analysis of incident outcomes, those with a history of vascular or cardiac diseases prior to the baseline visit were excluded (1052 with a history of CVD and 46 with missing history of CVD). After excluding 30, participants with missing follow-up data, the CVD incident analysis included 1557 participants.

Polysomnography

For all three cohorts, unattended type 2 PSG was conducted using Compumedics Ltd., Abbotsville, AU equipment (the specific models varied for each cohort). All montages included central EEG, bilateral electrooculography, chin electromyography, electrocardiogram, nasal pressure (only MESA and MrOS) and thermistry (for airflow measurement), chest and thoracic inductance plethysmography, and finger pulse oximetry (Nonin, Minneapolis, MN) as previously

described (1-3, 8-10, 15, 16). Using standardized criteria, a centralized Sleep Reading Center (initially based at Case Western Reserve University, Cleveland, OH, and then at Brigham and Women's Hospital, Boston, MA; both directed by SR) scored all studies using approaches described previously(1-3, 8-10, 15, 16). To allow flexibility in defining AHI, scoring of respiratory events was based on amplitude reduction in the respiratory channels, independent of the oxygen saturation or EEG channels. Hypopneas were scored if the reduction in airflow or respiratory inductance effort was 30-90% from baseline for at least 10 seconds, while apneas were defined by a reduction in airflow exceeding 90% for at least 10 seconds. Sleep stages and arousals were scored consistently across the three cohorts consistent with published guidelines(17), with arousals identified as an abrupt increase in EEG frequency of at least 3 seconds in duration, with arousals in REM sleep also requiring an increase in chin EMG activity. Scoring of respiratory events and arousals was conducted by trained and research-certified PSG technologists who underwent regular assessment of scoring reliability, with re-training as needed. Reliability was regularly monitored, with scorer retraining as needed; the intra- and inter-scorer for respiratory event detection ranged from 0.76 to 0.99 and for arousal index varied from 0.54 (for an early set of SHHS studies) to 0.90 (for later studies).

Definitions of AHI metrics

To compare events based on desaturation versus EEG arousal, frequency of events with desaturation (≥3%) but not arousal ($AHI_{\geq 3\%Only}$) and frequency of events with arousals but no desaturation (AHI_{ArOnly}) were calculated across three cohorts. In this study, both apneas and hypopneas were required to have desaturation or arousal to be included in the experimental AHIs defined above; nonetheless, apneas with neither desaturation nor arousal were rare

(SHHS: 0.1[0, 0.6] events/hour; MESA: 0.0[0.0, 0.0] events/hour; MrOS: 0.0[0.0, 0.1] events/hour).

Linking of arousals and desaturations with events: An event was associated with arousal if a scored arousal was identified within a subject-specific search window at the end of events. The search window extended from the pre-event minimum arousal probability to the post-event minimum of the arousal probability. Probability of arousal was determined as the ensemble average of the event-aligned arousal signal ("1" indicates scored arousal at any time point while "0" indicates no arousal). Similarly, the desaturation was calculated as the difference between maximum pre-event SpO_2 and minimum SpO_2 within a subject-specific search window, described previously[18-20]. Briefly, the subject-specific search window was obtained from an ensemble-averaged desaturation curve. The average desaturation curve for each participant was determined by overlaying SpO_2 signals with respect to the end of events. This method has been previously used in hypoxic burden and heart rate response calculation[18-20].

Associations of AHI components with health outcomes

In secondary analyses, to further examine the association of events with and without arousal with different degrees of desaturation (3% versus ≥4%), five AHI variables were defined based on events (apneas or hypopneas) with: 1) events with arousal and with no or minimal desaturation (<3%); 2) events with 3% (i.e., ≥3% to <4%) desaturations and no arousal, 3) events with 3% (i.e., ≥3% to <4%) desaturation and with arousal, 4) events with ≥4% desaturation and no arousal, and 5) events with ≥4% desaturation and with arousal. Multiple logistic or Cox regression models were used to assess the association of AHIs based on events

with and without arousal and with different degrees of desaturation, after adjusting for age, sex, and race and ethnicity.

The adjusted linear associations between AHI components and log-odds of diabetes, hypertension, or sleepiness are shown in **Figure S1**. In general, associations were stronger for AHI components that required desaturation rather than arousal for event identification (**Figure S1**). In general, for analyses associating AHI with comorbidities, events with ≥4% desaturation appeared to have a higher odds ratio compared with events based on 3% (but not ≥4%) desaturation (**Figure S1**). The AHI based solely on events with associated arousals but without appreciable desaturation showed null associations, with point estimates often falling below one. In addition, the AHI based on events linked to more severe desaturations (≥4%) was associated with these comorbidities regardless of the requirement to include or exclude arousals (**Figure S1**). This difference (arousals versus no arousals) was more evident when comparing events linked with 3% desaturations (**Figure S1**). Finally, similar to the AHI based on events with arousal only, the non-respiratory arousal index showed null to negative associations with hypertension/diabetes/sleepiness in these cohorts.

Consistent with cross-sectional findings for health conditions, associations between the AHI components and incident CVD suggested stronger associations for AHI definitions requiring desaturations compared those requiring arousals, with significant associations observed for the AHI defined using ≥4% desaturation (regardless of arousal) and 3% desaturation and no arousals in both MESA and MrOS (**Figure S2**). The associations of non-respiratory arousal index with incident CVD were null.

Table S1: The associations AHI based on 3% or arousal ($AHI_{3\%OrA}$) with health outcomes are weaker and less consistent than on $AHI_{\geq3\%Only}$.

AHI	Cohort	Hypertension OR (95% CI)	Diabetes OR (95% CI)	Sleepiness OR (95% CI)	Incident CVD HR (95% CI)
	SHHS	**1.18 (1.11–1.26)***	1.07 (0.95–1.20)	**1.13 (1.06–1.21)***	1.01 (0.94–1.09)
$AHI_{3\%OrA}$	MESA	1.00 (0.89–1.13)	1.07 (0.93–1.23)	**1.20 (1.04–1.37)***	**1.32 (1.08–1.62)****
	MrOS	1.08 (0.99–1.17)°	1.04 (0.92–1.18)	**1.18 (1.05–1.31)****	1.04 (0.94–1.15)

OR, odds ratio; CI, confidence interval; HR, hazard ratio; MESA, the Multi-Ethnic Study of Atherosclerosis; MrOS, the Osteoporotic Fractures in Men study; SHHS, the Sleep Heart Health Study; $AHI_{\geq3\%Only}$, apnea-hypopnea index based ≥3% desaturation and no arousal. Covariates included age, sex, race/ethnicity, and body mass index. Bold denotes an association with *increased* risk.
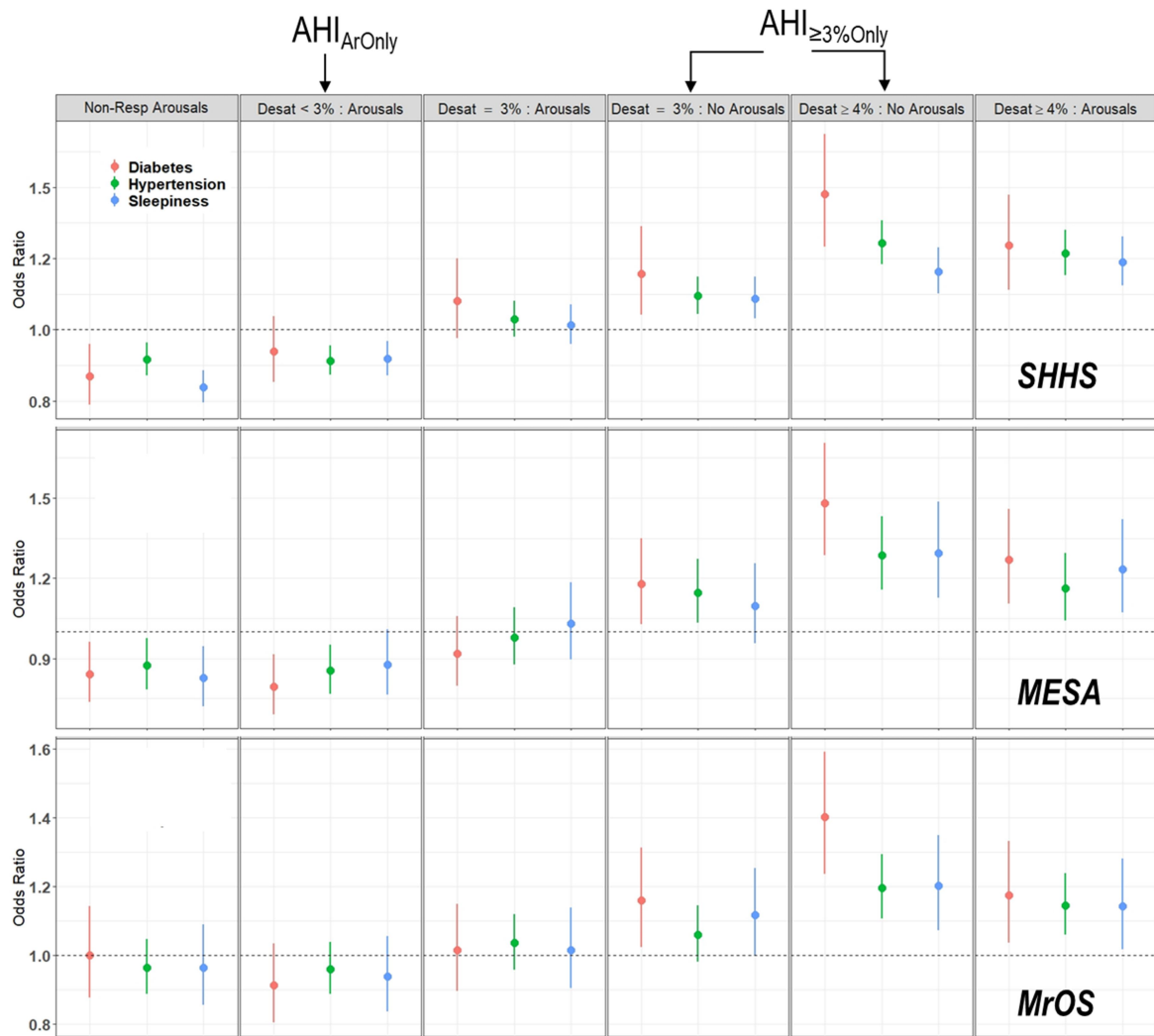°, P<0.1; *, p<0.05; **, p<0.01; ***, p<0.001.

Figure S1: The cross-sectional associations (odds ratios and 95% confidence intervals) between AHI components (per 1SD increase) and diabetes/hypertension/sleepiness. Each AHI component was modeled separately and adjusted for age, sex, and race/ethnicity in the Multi-Ethnic Study of Atherosclerosis (MESA), the Osteoporotic Fractures in Men (MrOS) study, and the Sleep Heart Health Study (SHHS).
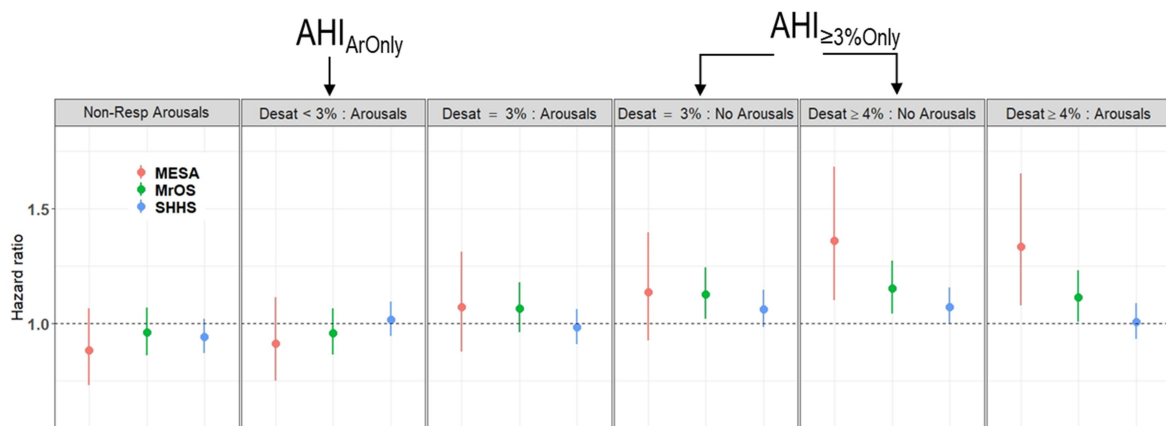
Figure S2: The associations between (hazard ratios and 95% confidence intervals) AHI components and incident CVD in the Multi-Ethnic Study of Atherosclerosis (MESA), the Osteoporotic Fractures in Men (MrOS) study, and the Sleep Heart Health Study (SHHS). Each AHI component was modeled separately and adjusted for age, sex, and race/ethnicity. In MESA, incident CVD included myocardial infarction (MI), resuscitated cardiac arrest, definite angina, probable angina (if followed by revascularization), stroke, stroke death, coronary heart disease-related death (CHD), other atherosclerotic death not defined in stroke and CHD-related death, and other CVD-related death not defined in other categories. In MrOS, incident CVD include any type of fatal or non-fatal cardiovascular events, including coronary heart disease, cerebrovascular disease events, peripheral vascular disease, cardiovascular disease events, and any heart failure. In SHHS, incident CVD included fatal and non-fatal MI, MI-related procedures, fatal and non-fatal stroke, congestive heart failure, CHD-related death, and other CVD-related deaths not defined in other events.

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