ORIGINAL ARTICLE

Objectively measured sleep characteristics and prevalence of coronary artery calcification: the Multi-Ethnic Study of Atherosclerosis Sleep study

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ABSTRACT

Background We tested whether objectively measured indices of obstructive sleep apnoea (OSA) and sleep quality are associated with coronary artery calcification (CAC) prevalence independent of obesity, a classic confounder.

Methods 1465 Multi-Ethnic Study of Atherosclerosis participants (mean age 68 years), who were free of clinical cardiovascular disease, had both coronary CT and in-home polysomnography and actigraphy performed. OSA categories were defined by the Apnea-Hypopnea Index (AHI). Prevalence ratios (PRs) for CAC >0 and >400 (high burden) were calculated.

Results Participants with severe OSA (AHI ≥30; 14.6%) were more likely to have prevalent CAC, relative to those with no evidence of OSA, after adjustment for demographics and smoking status (PR 1.16; 95% CI 1.06 to 1.26), body mass index (1.11; 1.02 to 1.21) and traditional cardiovascular risk factors (1.10; 1.01 to 1.19). Other markers of hypoxaemia tended to be associated with a higher prevalence of CAC >0. For CAC >400, a higher prevalence was observed with both a higher arousal index and less slow-wave sleep. Overall, associations were somewhat stronger among younger participants, but did not vary by sex or race/ethnicity.

Conclusions In this population-based multi-ethnic sample, severe OSA was associated with subclinical coronary artery disease (CAC >0), independent of obesity and traditional cardiovascular risk factors. Furthermore, the associations of the arousal index and slow-wave sleep with high CAC burden suggest that higher nightly sympathetic nervous system activation is also a risk factor. These findings highlight the potential importance of measuring disturbances in OSA as well as sleep fragmentation as possible risk factors for coronary artery disease.

Key messages

What is the key question?

- Are indices of obstructive sleep apnoea (OSA) and sleep quality associated with coronary artery calcification (CAC) prevalence independent of obesity?

What is the bottom line?

- In this large population-based sample, there was evidence that indices of sleep apnoea, arousal and sleep quality were all associated with prevalent CAC and/or a high CAC burden.

Why read on?

- The current findings enhance the existing literature suggesting an association between OSA and CAC, and provide novel evidence that more frequent arousals and less slow-wave sleep are associated with a high burden of subclinical atherosclerosis.

INTRODUCTION

Obstructive sleep apnoea (OSA) has been associated with an elevated risk of incident cardiovascular disease (CVD) in several observational studies,[1 2] and among patients with OSA, treatment has been associated with lower CVD morbidity and mortality.[3] Despite this suggestive evidence, important key questions remain. As noted in an American Heart Association/American College of Cardiology Foundation Scientific Statement, it remains unclear 'whether sleep apnoea is important in initiating the development of cardiac and vascular disease'.[1]

One way to address this question is through examination of the association between OSA and markers of subclinical CVD, such as coronary artery calcification (CAC). The Multi-Ethnic Study of Atherosclerosis (MESA) has previously shown CAC to be predictive of incident clinical CVD risk.[4]

Information examining associations between objective measurements of sleep and CAC is sparse. Of the five existing studies which objectively measured sleep apnoea, all found sleep apnoea to be associated with CAC prevalence in basic models,[5–9] however in some instances the association was non-significant after adjustment for body mass index (BMI).[5 6] It is important to note that all but one[9] of these studies were relatively small (N<260), and two were conducted among individuals with suspected sleep disorders.[7 8] In addition to sleep apnoea, several other sleep phenotypes, such as abnormal (short or long) sleep duration and insomnia have also been evaluated in relation to CAC[8 10] and CVD risk.[11 12] Results have been mixed, and interpretation is challenging as many of the existing studies had small sample sizes and several used sleep data based on self-report. Self-reported measures of typical sleep are only modestly correlated with objectively measured sleep characteristics,[13]

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and no study has yet evaluated the influence of sleep stages and arousal frequency in relation to CAC. Sleep disturbances may influence atherogenesis through several pathways, such as hypoxaemia or sympathetic nervous activation,[1] but no previous study has comprehensively assessed the independent contributions of alternative measures of sleep disturbances.

A related question is whether racial/ethnic variation in sleep disturbances may contribute to the well-established racial/ethnic differences in CVD risk.[14] Few studies have objectively measured sleep characteristics in racially/ethnically diverse populations. However, the existing literature suggests that sleep disorders vary by race/ethnicity.[15] African American individuals appear to be disproportionately affected by OSA and short sleep duration, relative to Caucasian participants.[16] Much less is known about other racial/ethnic groups, although recent work from the MESA Sleep study suggests that after accounting for BMI, Chinese individuals have a higher prevalence of OSA than Caucasian participants.[17] Similarly, the prevalence of OSA varies by age and sex,[4] as do CVD incidence rates,[18] but it is not clear whether OSA may underlie this variation.

Data from the MESA were used to test the hypotheses that objectively measured OSA, and adverse levels of other metrics of sleep quality and quantity, are associated with greater CAC prevalence and CAC severity, independently of obesity and possible mediating traditional cardiovascular risk factors. We also explored whether associations between sleep characteristics and CAC prevalence varied by age, sex or race/ethnicity.

MATERIALS AND METHODS

Design overview

The MESA Study[19] (http://www.mesa-nhlbi.org/) began when 6814 men and women who were free of clinical CVD and aged 45–85 years were recruited from six US communities between 2000 and 2002: Chicago, Illinois; Los Angeles County, California; New York, New York; Forsyth County, North Carolina; St Paul, Minnesota; and Baltimore, Maryland. At baseline, participants self-identified as non-Hispanic African American (28%), Chinese (12%), Caucasian (38%) or Hispanic (22%). Five clinical exams have taken place, the most recent of which (exam 5) was conducted between April 2010 and December 2011, and attended by 4651 individuals (78% of the original MESA participants who were alive). CAC was measured, among those with no contraindications, in a subset of 3305 exam 5 participants. MESA participants who took part in exam 5 and were not on current treatment with positive airway pressure, oral devices or oxygen (n=95) were also invited to take part in the MESA Sleep ancillary study, during which overnight in-home polysomnography and 7-day actigraphy were conducted, and sleep questionnaires were completed. A total of 2237 participants underwent polysomnography, of whom 1581 also had CAC measured at MESA exam 5. From this sample, we further excluded participants with prevalent myocardial infarction, stroke, congestive heart failure, or who had undergone interventional cardiology procedures (n=116). The final analytical sample for most analyses was 1465. All participants gave informed consent, and local Institutional Review Boards approved the study protocols for the main MESA exams, and for the MESA Sleep ancillary study.

Sleep ascertainment

In-home polysomnography was conducted using the Compumedics Somte system (Compumedics, Abbottsville, Australia) employing techniques similar to those previously described.[20] The sensors and recording montage consisted of central, occipital and frontal electroencephalograms, bilateral electrooculograms, chin EMG, and thoracic and abdominal respiratory inductance plethysmography, airflow (by nasal-oral thermocouple and pressure recording from a nasal cannula), ECG, leg movements (piezoelectric sensors) and finger pulse oximetry. Nocturnal recordings were transmitted to the centralised reading centre at Brigham and Women's Hospital and data were scored by trained technicians using current guidelines. For our primary analyses we defined OSA according to the Apnea-Hypopnea Index (AHI) which includes all apnoeas (regardless of desaturation or arousal) and all hypopnoeas with ≥4% oxygen desaturation. Arousals, transient awakenings from sleep lasting <10 s, were characterised by the American Academy of Sleep Medicine criteria.[21] Sleep stages were identified for each 30 s epoch using American Academy of Sleep Medicine scoring criteria.[22] Other polysomnography measures included measures of sleep disordered breathing (average oxygen saturation during sleep; percentage time during sleep with an oxygen saturation <90%; percentage sleep time occupied by apnoeas or hypopnoeas), percentage time in stage N3 (slow-wave sleep) and the arousal index in stages REM and NREM. Inter- and intra-scorer intraclass correlation coefficients for the AHI ranged from 0.95 to 0.99, for the arousal index from 0.84 to 0.99, and for percentage time in stage N3 from 0.79 to 0.99.

Actigraphy was performed using the Actiwatch Spectrum wrist actigraph (Philips Respironics, Murrysville, Pennsylvania, USA) worn on the participant's non-dominant wrist. Output was sent to the Sleep Reading Center at Brigham and Women's Hospital where records were scored with use of a sleep diary. Summary variables examined included average sleep duration, sleep efficiency (proportion of the sleep period asleep), and time wake after sleep onset (WASO). A minimum of 3 days of data with >50% reliable data were required to meet minimal standards for analysis. Inter-scorer reliability for average sleep duration, sleep efficiency and WASO were 0.91, 0.97, and 0.91, respectively.

CAC measurement

Details of the procedures for CAC measurement, scanner quality assurance, and scan reading have been described previously.[4] Briefly, CAC was assessed by chest CT with a multidetector CT system at all six sites, using standardised protocols. A cardiologist or radiologist interpreted all scans at the MESA CT reading centre (Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center), blinded to participant data. Agatston scores were quantified. In previous MESA exams, the kappa statistics for intra- and inter-reader reproducibility of CAC prevalence were both 0.92. Intraclass correlation coefficients for intra- and inter-reader reproducibility of CAC scores exceeded 0.99. For the present analysis, CAC was considered prevalent (CAC >0) if the Agatston score was greater than 0. High CAC burden (CAC >400) was defined by Agatston scores >400.

Other variables

Sex, age, cigarette smoking status (current, former, never) and use of anti-hypercholesterolemics, anti-hypertensives and diabetes medications were self-reported. BMI was calculated as weight over height squared (kg)/(m^2). Resting blood pressure was measured three times in the seated position using a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon, Tampa, Florida, USA). The average of the last two measurements was used in analyses. Participants were asked to

fast for at least 8 h before their visit. Serum glucose was measured by rate reflectance spectrophotometry using thin film adaptation of the glucose oxidase method on the Vitros analyser (Johnson & Johnson Clinical Diagnostics, Rochester, New York, USA). HDL cholesterol was assessed in EDTA plasma using the cholesterol oxidase method (Roche Diagnostics) after precipitation of non-HDL cholesterol with magnesium/dextran, and LDL cholesterol was calculated in plasma specimens having a triglyceride value <400 mg/dL using the Friedewald formula. Serum assays were performed at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, Minnesota, USA).

Data analysis

The descriptive characteristics of participants are presented as means and proportions, stratified by clinical categories of OSA presence/severity (no OSA, AHI 5–14, AHI 15–30, AHI >30). For the primary analysis, relative risk regression (binomial regression) was used to explore the association between sleep phenotypes and CAC prevalence (CAC >0). OSA prevalence/severity was modelled according to the aforementioned clinical categories. Sleep duration was categorised as: <399 min, 399–444 min (reference; corresponds to the 50th to 75th percentiles of the MESA distribution) and >444 min. All other sleep phenotypes were modelled per 1 SD. Non-linearity was tested using generalised additive models with any CAC as the endpoint and adjustments for age, gender, race, site, education, income and smoking status. Additional models examined high CAC burden (CAC >400) as the outcome.

A series of models were conducted: model 1 adjusted for age, race/ethnicity, sex, centre, education (<high school, high school or some college, college degree or higher), income (<US$20 K, US$20 K to <US$50 K, ≥US$50 K) and smoking status (current, former, never). Model 2, our primary model, further adjusted for BMI. Model 3, an 'overadjusted model', additionally adjusted for traditional CVD risk factors, some of which are believed to be on the pathway through which OSA influences CAC prevalence. Specifically, we added to model 3 prevalent diabetes, systolic blood pressure, hypertension medication use, HDL-C, LDL-C, use of lipid-lowering medications, and high-sensitivity C-reactive protein (hs-CRP). Regarding interpretation, if magnitudes of association are attenuated with adjustment for model 3 covariates, it implies that the association between OSA and CAC is mediated through these factors. Model 4 was also adjusted for sleep duration categories in order to evaluate whether sleep duration was a confounder of the association between OSA and CAC. Effect modification of the OSA and CAC association by age, sex and race/ethnicity was explored by including cross-product terms with model 1 adjustments.

RESULTS

The 1465 participants in our analytical sample were on average 68 years old, and 54% were female, 35.9% Caucasian, 12.5% Chinese, 27.4% African American and 24.2% Hispanic. Of the total sample, 14.6% had severe OSA (AHI >30), 18.0% had moderate OSA (AHI 15–30), 32.6% had mild OSA (AHI 5–14) and 34.8% had no OSA (AHI <5). As shown in table 1, participants with severe OSA were slightly older, more likely to be male, to have greater adiposity (as assessed by BMI or waist circumference), and to have worse CVD risk factor profiles (ie, more diabetes, elevated systolic blood pressure, higher total cholesterol, low HDL-C, and greater usage of blood pressure and lipid-lowering medications). CAC (>0) was found in 64.0% of the cohort, while CAC >400 was found in 14.9%. Associations of demographic, behavioural, cardiovascular and sleep characteristics stratified by CAC categories are presented in online supplementary e-table 1. In general, relative to participants with no CAC, those with a high CAC burden tended to be older, male, and have a more adverse cardiovascular risk factor profile.

Obstructive sleep apnoea and prevalent CAC

Participants with severe OSA were 1.16 (95% CI 1.06 to 1.26) times more likely to have prevalent CAC relative to participants with no evidence of OSA, after adjustment for demographics and smoking status (table 2). The association remained significant after additional adjustment for BMI (1.11; 1.02 to 1.21) and traditional CVD risk factors which may be mediators of the association between OSA and CAC (1.10; 1.01 to 1.19). Results were essentially unchanged with additional adjustment for estimated glomerular filtration rate (eGFR) or sleep duration category. There were no interactions by either sex or race/ethnicity. The association between severe OSA and CAC >0 was slightly stronger among younger than older participants (median population age=67 years): p-interaction <0.01; prevalence ratio (95% CI) ≤67 years=1.22 (1.03 to 1.45), >67 years=1.16 (1.04 to 1.28). No associations were seen between mild or moderate OSA and CAC prevalence, regardless of degree of adjustment.

When CAC >400 was considered as the outcome, although severe OSA was associated with a qualitatively greater prevalence of CAC >400, the association was not statistically significant in any of the models considered. There was no interaction by age.

Other sleep phenotypes and prevalent CAC

Associations between other sleep phenotypes and prevalent CAC (>0) are presented in table 3. With the exception of sleep duration, sleep phenotypes were modelled linearly (per 1 SD). There was no evidence of non-linearity. In models adjusted for demographics, prevalence of CAC >0 was significantly associated with average oxygen saturation during sleep, percentage of time at oxygen saturation <90%, percentage of sleep time spent in apnoea or hypopnoea, number of apnoea/hypopnoea events per night, and AHI (modelled continuously). Associations were slightly attenuated after adjustment for BMI. With additional adjustment for traditional cardiovascular risk factors, only percentage time in apnoea and hypopnoea and the AHI remained significant. There was little evidence that measures of sleep architecture, sleep fragmentation or sleep duration were associated with CAC >0. There were no interactions between sleep phenotypes and race or sex on CAC >0. However, several age interactions were noted (all p<0.01) when age was modelled continuously, and with adjustment for model 1 covariates. For the following phenotypes, associations were stronger among younger individuals (see online supplementary e-table 2): average oxygen saturation, percentage sleep time in oxygen saturation <90%, number of apnoea/hypopnoea events per night, AHI, the overall arousal index, and the non-REM arousal index.

A greater prevalence of CAC >400 was observed with higher scores on the arousal indices (total, REM and non-REM) (table 4). In fully adjusted models, each SD higher score on the total arousal index was associated with a prevalence ratio of 1.14 (1.02 to 1.29) for CAC >400. An inverse association between percentage time in stage N3 (slow-wave sleep) and prevalence of CAC >400 was also observed in the fully adjusted

Table 1 Descriptive characteristics by OSA severity: Multi-Ethnic Study of Atherosclerosis 2010–2013

	OSA severity category				
	Normal (AHI <5)	Mild (AHI 5–14)	Moderate (AHI 15–29)	Severe (AHI ≥30)	p Trend
N (%)	510 (35)	478 (33)	263 (18)	214 (15)	
Demographics and behaviours					
Age (years)	66.9±9.0	68.9±9.2	69.1±9.2	67.9±9.0	0.03
Male gender, n (%)	165 (32.3)	214 (44.8)	155 (58.9)	136 (63.6)	<0.001
Race/ethnicity, n (%)					
Caucasian	192 (37.6)	181 (37.9)	89 (33.8)	65 (30.2)	0.04
Chinese	68 (13.3)	49 (10.3)	36 (13.7)	30 (14.0)	
African American	150 (29.4)	133 (27.8)	64 (24.3)	54 (25.2)	
Hispanic	100 (19.6)	115 (24.1)	74 (28.1)	65 (30.4)	
Education, n (%)					
Less than HS	67 (13.2)	68 (14.3)	40 (15.2)	37 (17.3)	0.26
HS or some college	156 (30.6)	168 (35.4)	84 (31.9)	78 (36.5)	
College degree	286 (56.2)	239 (50.3)	139 (52.9)	99 (46.3)	
Income, n (%)*					
<US$20 K	94 (19.1)	92 (19.4)	54 (21.0)	54 (25.7)	0.41
US$20 K to <US$50 K	164 (33.3)	171 (36.1)	91 (35.4)	64 (30.5)	
≥US$50 K	235 (47.7)	211 (44.5)	112 (43.6)	92 (43.8)	
Smoking status, n (%)					
Never	250 (49.3)	216 (45.3)	127 (48.9)	94 (43.9)	0.16
Former	217 (42.8)	224 (47.0)	125 (47.9)	106 (49.5)	
Current	40 (7.9)	37 (7.8)	9 (3.5)	14 (6.5)	
Pack-years	8.6±16.7	11.1±21.5	9.7±17.8	9.2±17.9	0.64
Anthropometry					
BMI (kg/m^2)	26.6±5.0	28.9±5.0	29.6±5.3	31.7±6.0	<0.001
Waist size (cm)	93.6±14.0	100.1±13.5	102.1±12.8	106.5±14.7	<0.001
Cardiovascular risk factors					
Diabetes, n (%)	71 (14.0)	90 (19.0)	63 (24.1)	51 (23.8)	0.001
Systolic BP (mm Hg)	121.1±21.4	123.0±19.4	122.4±19.1	126.0±19.8	0.007
BP medication, n (%)	232 (45.5)	255 (53.4)	137 (52.1)	124 (57.9)	0.009
Total cholesterol (mg/dL)	190.0±34.9	184.7±34.4	182.7±36.4	180.9±35.8	<0.001
HDL-C (mg/dL)	60.7±17.7	54.9±15.2	52.3±14.4	50.8±13.5	<0.001
LDL-C (mg/dL)	109.4±31.4	107.8±30.5	107.9±31.3	105.2±33.3	0.13
Lipid medication, n (%)	155 (30.4)	177 (37.0)	97 (36.9)	84 (39.3)	0.05
eGFR categories					0.43
≥90	195 (38.6)	159 (33.5)	87 (33.2)	78 (36.5)	
60–89	259 (51.3)	259 (54.5)	148 (56.5)	119 (55.6)	
<60	51 (10.1)	57 (12.0)	27 (10.3)	17 (7.9)	
Agatston score, median					
Overall	6.2	32.6	31.8	62.9	
Among those with CAC >0	84.3	125.5	123.7	117.4	

*Income refers to total gross family income.
Numbers are mean±SD, unless otherwise noted as n (%) or median. p Values are based on χ^2 tests for categorical variables, and trend tests for continuous variables.
AHI, Apnea-Hypopnea Index; BMI, body mass index; BP, blood pressure; CAC, coronary artery calcification; eGFR, estimated glomerular filtration rate; HS, high school; MESA, Multi-Ethnic Study of Atherosclerosis; OSA, obstructive sleep apnoea.

model with a prevalence ratio per 1 SD higher percentage time in stage N3 of 0.77 (0.64 to 0.92). No meaningful interactions by age, sex or race were observed.

DISCUSSION

In this large, cross-sectional study of racially/ethnically diverse middle-aged and older individuals, objectively measured indices of sleep disturbances were found to be associated with CAC after a number of potential confounders were considered. Measures of OSA, in particular both the AHI and measures of overnight hypoxaemia, were positively associated with the prevalence of subclinical atherosclerosis, defined by CAC >0. When OSA as a categorical measure was considered, individuals with severe OSA, defined by an AHI >30, had an approximately 10% increased adjusted prevalence of CAC, compared to individuals without OSA. In contrast, a high CAC burden (CAC >400) was significantly associated with high sleep fragmentation (arousal index) and low sleep quality (time in stage N3, slow-wave sleep). In particular, each SD increase in the arousal index was associated with a 14% higher prevalence of CAC >400, while each SD decrease in stage N3 (slow-wave sleep) was associated with a 30% higher prevalence of CAC >400. Both of these sleep exposures are associated with increased levels of sympathetic nervous system activation and hypertension.[23] The current data provide the first evidence we are aware of linking these sleep quality metrics to CAC

Table 2 Prevalence ratios (95% CIs) of OSA and coronary artery calcification (CAC): the Multi-Ethnic Study of Atherosclerosis 2010–2013

	OSA category				
	Normal (AHI <5)	Mild (AHI 5–14)	Moderate (AHI 15–29)	Severe (AHI ≥30)	p Trend
N total (%)	510 (34.8)	478 (32.6)	263 (18.0)	214 (14.6)	
CAC >0 (n=937)					
N	280	318	177	162	
Model 1	1.00	1.05 (0.97 to 1.14)	1.03 (0.94 to 1.13)	1.16 (1.06 to 1.26)	0.001
Model 2	1.00	1.04 (0.96 to 1.12)	1.00 (0.91 to 1.10)	1.11 (1.02 to 1.21)	0.03
Model 3	1.00	1.04 (0.97 to 1.12)	1.00 (0.91 to 1.09)	1.10 (1.01 to 1.19)	0.07
Model 4	1.00	1.04 (0.96 to 1.12)	1.00 (0.91 to 1.10)	1.10 (1.01 to 1.20)	0.05
CAC >400 (n=218)					
N	62	72	44	40	
Model 1	1.00	0.95 (0.70 to 1.28)	1.09 (0.81 to 1.48)	1.16 (0.85 to 1.58)	0.44
Model 2	1.00	0.93 (0.68 to 1.26)	1.05 (0.76 to 1.45)	1.10 (0.79 to 1.53)	0.42
Model 3	1.00	1.00 (0.73 to 1.38)	1.07 (0.75 to 1.51)	1.20 (0.85 to 1.69)	0.33
Model 4	1.00	1.00 (0.71 to 1.39)	1.07 (0.75 to 1.53)	1.20 (0.86 to 1.68)	0.33

Model 1: adjusted for age, race/ethnicity, sex, centre, education (<high school, high school or some college, college degree or higher), income (<US$20 K, US$20 K to <US$50 K, ≥US $50 K) and smoking status (current, former, never).
Model 2: adjusted for model 1 + BMI.
Model 3: adjusted for model 2 + traditional CVD risk factors (prevalent diabetes, systolic blood pressure, hypertension medication use, HDL-C, LDL-C, use of lipid-lowering medications).
Model 4: adjusted for model 3 + sleep duration category.
AHI, Apnea-Hypopnea Index; BMI, body mass index; CVD, cardiovascular disease; OSA, obstructive sleep apnoea.

burden, and suggest that disturbances in sleep architecture associated with higher nightly levels of sympathetic nervous system activation associate with a high burden of subclinical atherosclerosis.

OSA and CAC prevalence

Our results extend and support findings from previous studies evaluating the association between OSA and CAC prevalence. These MESA findings are unique as OSA was objectively

Table 3 Prevalence ratios (95% CIs) of measures of sleep disordered breathing, sleep architecture, sleep fragmentation and sleep duration and coronary artery calcification prevalence (CAC >0): the Multi-Ethnic Study of Atherosclerosis 2010–2013*

		CAC >0					
		Model 1		Model 2		Model 3	
	1 SD	PR (95% CI)	p Value	PR (95% CI)	p Value	PR (95% CI)	p Value
Hypoxaemia and disordered breathing							
Average oxygen saturation in sleep	1.7	0.96 (0.94 to 0.99)	0.005	0.98 (0.95 to 1.01)	0.12	0.98 (0.95 to 1.01)	0.25
Percentage sleep time SAO_2 <90%	8.6	1.03 (1.01 to 1.05)	0.004	1.02 (1.00 to 1.04)	0.05	1.02 (1.00 to 1.04)	0.13
Percentage time in apnoea + hypopnoea	12.4	1.05 (1.02 to 1.08)	0.001	1.03 (1.00 to 1.06)	0.03	1.03 (1.00 to 1.06)	0.04
Apnoea/hypopnoea events per night	109	1.05 (1.02 to 1.07)	0.001	1.03 (1.00 to 1.06)	0.02	1.03 (1.00 to 1.05)	0.07
AHI (continuously measured)	16.5	1.05 (1.02 to 1.08)	<0.001	1.04 (1.01 to 1.07)	0.01	1.03 (1.00 to 1.06)	0.03
Sleep architecture							
Percentage time in stage N3	9.0	1.00 (0.96 to 1.04)	0.94	1.00 (0.97 to 1.04)	0.93	1.00 (0.97 to 1.04)	0.83
Sleep fragmentation							
Arousal index	12.0	1.02 (0.99 to 1.06)	0.10	1.02 (0.99 to 1.05)	0.26	1.02 (0.99 to 1.05)	0.25
Arousal index—REM	11.8	1.03 (1.00 to 1.06)	0.05	1.02 (0.99 to 1.06)	0.13	1.02 (1.00 to 1.06)	0.10
Arousal index—NREM	12.8	1.02 (0.99 to 1.05)	0.11	1.02 (0.99 to 1.05)	0.25	1.02 (0.99 to 1.04)	0.26
Average sleep efficiency, %	3.5	0.98 (0.95 to 1.01)	0.18	0.98 (0.96 to 1.01)	0.24	0.98 (0.95 to 1.01)	0.20
Average sleep WASO	16.7	1.01 (0.98 to 1.04)	0.44	1.01 (0.98 to 1.04)	0.38	1.01 (0.98 to 1.04)	0.47
Sleep duration							
399–444 min		Ref		Ref		Ref	
<399 min		1.04 (0.96 to 1.12)	0.37	1.02 (0.94 to 1.11)	0.59	1.03 (0.95 to 1.11)	0.46
>444 min		0.99 (0.91 to 1.09)	0.88	0.99 (0.91 to 1.08)	0.83	0.98 (0.90 to 1.07)	0.71
3 degrees of freedom test			0.47		0.71		0.46

*All continuous variables have PR expressed per SD increment. Only 1390 observations were present for certain measures based on actigraphy (ie, sleep efficiency, WASO and sleep duration).
Model 1: adjusted for age, race/ethnicity, sex, centre, education (<high school, high school or some college, college degree or higher), income (<US$20 K, US$20 K to <US$50 K, ≥US $50 K) and smoking status (current, former, never).
Model 2: adjusted for model 1 + BMI.
Model 3: adjusted for model 2 + traditional CVD risk factors (prevalent diabetes, systolic blood pressure, hypertension medication use, HDL-C, LDL-C, use of lipid-lowering medications).
AHI, Apnea-Hypopnea Index; BMI, body mass index; CVD, cardiovascular disease; PR, prevalence ratio; SAO_2, oxygen saturation; WASO, time wake after sleep onset.

Table 4 Prevalence ratios (95% CIs) of measures of sleep disordered breathing, sleep architecture, sleep fragmentation, sleep duration and high coronary artery calcification burden (CAC >400): the Multi-Ethnic Study of Atherosclerosis 2010–2013*

		CAC >400					
		Model 1		Model 2		Model 3	
	1 SD	PR (95% CI)	p Value	PR (95% CI)	p Value	PR (95% CI)	p Value
Hypoxaemia and disordered breathing							
Average oxygen saturation in sleep	1.7	1.03 (0.83 to 1.29)	0.77	1.04 (0.81 to 1.34)	0.77	1.18 (0.89 to 1.56)	0.25
Percentage sleep time SAO_2 <90%	8.6	0.93 (0.68 to 1.29)	0.68	0.93 (0.66 to 1.31)	0.66	0.78 (0.52 to 1.19)	0.25
Percentage time in apnoea + hypopnoea	12.4	1.02 (0.92 to 1.13)	0.73	1.02 (0.92 to 1.13)	0.72	1.04 (0.93 to 1.16)	0.50
Apnoea/hypopnea events per night	109	1.06 (0.95 to 1.19)	0.30	1.06 (0.95 to 1.19)	0.31	1.02 (0.90 to 1.16)	0.76
AHI (continuously measured)	16.5	1.02 (0.91 to 1.14)	0.80	1.02 (0.90 to 1.14)	0.78	1.02 (0.91 to 1.15)	0.71
Sleep architecture							
Percentage time in stage N3	9.0	0.86 (0.70 to 1.06)	0.15	0.86 (0.70 to 1.06)	0.15	0.77 (0.64 to 0.92)	0.005
Sleep fragmentation							
Arousal index	12.0	1.09 (0.97 to 1.21)	0.15	1.09 (0.97 to 1.21)	0.15	1.14 (1.02 to 1.27)	0.02
Arousal index—REM	11.8	1.14 (1.01 to 1.30)	0.04	1.15 (1.01 to 1.30)	0.04	1.15 (1.02 to 1.29)	0.02
Arousal index—NREM	12.8	1.08 (0.96 to 1.22)	0.18	1.08 (0.96 to 1.21)	0.18	1.14 (1.02 to 1.28)	0.03
Average sleep efficiency, %	3.5	1.02 (0.92 to 1.14)	0.68	1.02 (0.92 to 1.14)	0.68	1.00 (0.89 to 1.13)	0.97
Average sleep WASO	16.7	1.07 (0.97 to 1.19)	0.19	1.07 (0.97 to 1.19)	0.19	1.03 (0.91 to 1.17)	0.62
Sleep duration							
399–444 min		Ref		Ref		Ref	
<399 min		0.68 (0.49 to 0.95)	0.02	0.66 (0.47 to 0.94)	0.02	0.75 (0.55 to 1.03)	0.08
>444 min		0.95 (0.68 to 1.32)	0.74	0.94 (0.68 to 1.31)	0.72	0.84 (0.56 to 1.26)	0.40
3 degrees of freedom test			0.06		0.05		0.19

*All continuous variables have prevalence ratio expressed per SD increment.
Model 1: adjusted for age, race/ethnicity, sex, centre, education (<high school, high school or some college, college degree or higher), income (<US$20 K, US$20 K to <US$50 K, ≥US $50 K) and smoking status (current, former, never).
Model 2: adjusted for model 1 + BMI.
Model 3: adjusted for model 2 + traditional CVD risk factors (prevalent diabetes, systolic blood pressure, hypertension medication use, HDL-C, LDL-C, use of lipid-lowering medications).
AHI, Apnea-Hypopnea Index; BMI, body mass index; CVD, cardiovascular disease; PR, prevalence ratio; WASO, time wake after sleep onset.

measured using state-of-the-art in-home polysomnography equipment, and the study sample was diverse, community-based, and quite large relative to other studies which have objectively measured sleep. OSA is known to be extremely underdiagnosed in the community,[24] and study samples identified in clinical settings (most often sleep clinics) are likely not representative of the general population. Of the existing studies on OSA and CAC prevalence, our design is most similar to that of the population-based Heinz Nixdorf Recall study, which recently evaluated this association in 1604 German participants.[9] This study used limited channel sleep monitoring, which precluded quantitative assessments of sleep architecture, sleep fragmentation and hypoxaemia, and which may underestimate sleep apnoea severity and obstructive/central subtypes. The study also did not collect objective measurements of sleep duration, as was done in MESA with the use of actigraphy. They found objectively measured OSA to be associated with CAC prevalence among men aged ≤65 years and women of any age. Although in MESA there was no interaction by sex, associations between OSA and CAC tended to be stronger among younger (≤67 years) MESA participants. The idea that OSA may exert a stronger influence on atherosclerotic risk among younger individuals is also supported by findings from the Sleep Heart Health Study which revealed that OSA was an independent predictor of coronary heart disease in men ≤70 years of age, but not in older men or women of any age.[2] Our MESA findings furthermore show that the relationship with OSA persisted after adjusting for sleep duration.

The two other population-based studies of OSA and prevalent CAC were much smaller than the MESA and Heinz Nixdorf Recall study samples, and in both instances the associations were not independent of BMI.[5 6] In MESA the association between OSA and CAC was modestly attenuated after adjustment for BMI. Obesity is a strong risk factor for the development of both OSA[24] and CAC,[25] and as such is an important potential confounder of the association. However, in both MESA and the Heinz Nixdorf Recall study associations between the AHI and CAC prevalence persisted even after accounting for BMI and other established CVD risk factors which have been hypothesised to mediate the association. These results support converging evidence which suggests that severe OSA is a risk factor for subclinical atherosclerosis. MESA participants with moderate or mild OSA did not have a higher CAC prevalence.

In our sample, there was no statistically significant association between OSA and CAC >400. However, as fewer people had CAC >400, power was more limited to detect an association. The fact that the magnitudes of the prevalence ratios were similar to those for CAC >0, and in some instances stronger than those for CAC >0, suggests that OSA may also be associated with CAC >400.

OSA causes repetitive acute hypoxemic episodes and sleep disruption, which are believed to initiate a range of pathophysiological mechanisms, including sympathetic nervous system activation, that may act to promote CVD. As has been reviewed elsewhere, OSA has been implicated in the pathogenesis of systemic inflammation, oxidative stress, a prothrombotic state, hypertension and diabetes.[1] Hypoxaemia occurring in association with OSA has been associated with both metabolic disturbances[26] and atherosclerosis.[27] Furthermore, converging evidence also indicates that sleep interventions may improve

vascular risk factors.[1] Multiple guidelines recognise OSA as a secondary cause of hypertension and advise assessment and treatment of OSA in patients with refractory hypertension.[28] Some evidence also suggests that OSA treatment may improve glucose metabolism[29 30] and inflammatory marker[31] profile. Therefore, although speculative, it is possible that *if* OSA does influence CAC development through these existing cardiovascular risk factors, that interventions for OSA may slow CAC progression.

It is of interest that despite the strong potential pathophysiological links between OSA and hypoxaemia-related stresses and atherosclerosis, the association between OSA and CAC was relatively modest, and only present with severe OSA. This is consistent with the epidemiological literature that has shown relatively weaker associations of OSA with coronary heart disease compared to OSA and stroke,[2 32] and suggests the possibility that the influence of OSA on CVD risk may vary across CVD outcomes.

Other sleep phenotypes and CAC prevalence

In addition to evaluating the association between OSA and CAC prevalence, we also assessed whether other objective measurements of sleep architecture were associated with CAC. Notably, for CAC >400, a level of CAC that indicates a severe CAC burden, sleep disruption as measured by an elevated arousal index and less stage N3 were both associated with a higher prevalence. Frequent arousals are accompanied by chemoreflex-mediated increases in sympathetic activity to the peripheral blood vessels and consequent vasoconstriction, endothelial dysfunction and higher blood pressure.[1] Stage N3 sleep is the stage when parasympathetic tone is highest and overnight blood pressure is lowest. Reductions in N3 sleep have been associated with an increased incidence of hypertension[23] as well with decreased insulin sensitivity,[33] and both elevated blood pressure and diabetes have been shown to be associated with CAC development.[34] These data indicate that polysomnography measures that quantify the degree of disruption in sleep may provide unique information regarding sleep-related CVD risk.

The CARDIA study previously reported an association between short sleep duration and CAC.[16] Short sleep duration may reflect curtailed time in bed, or reduced sleep time occurring as a consequence of frequent arousals or increased wake time after sleep onset. The differences in findings between MESA and CARDIA with regards to sleep duration and CAC prevalence may reflect differences in the ages of the two populations. The MESA findings suggest that in older populations, measures of sleep fragmentation are more strongly associated with CAC than are measures of sleep duration.

Strengths and limitations

The strengths of this study are the relatively large population-based multi-ethnic sample, the use of both polysomnography and multiple-day actigraphy to objectively measure sleep characteristics, and the standardised assessment of CAC. Importantly, sleep studies conducted with in-home polysomnography, such as those performed here, have been shown to be extremely consistent with hospital polysomnography (log-transformed, r=0.96 for AHI) and highly reproducible.[31] Furthermore, the analytical sample was free of established CVD, allowing inferences to be made for subclinical disease. Although relatively large for a study which used polysomnography and 7-day actigraphy to objectively measure sleep characteristics, power was limited to detect effect sizes of small magnitude and for subgroup analyses. This was particularly evident when CAC >400 was used as the outcome. Furthermore, the design was cross-sectional. As such, it is not possible to determine the temporality of the association between sleep characteristics and CAC prevalence, and selection bias may have occurred. To date, no studies have been published exploring the association between OSA and CAC incidence or progression.

Conclusions

Objective measurements showed that severe OSA and other indices of nocturnal hypoxaemia were associated with greater CAC prevalence, independent of BMI and traditional cardiovascular risk factors, in this cross-sectional analysis of data from the community-based MESA. There also was evidence that an elevated arousal index and a low percentage of time spent in stage N3 sleep were associated with a high CAC burden (CAC >400). These specific measures of sleep disruption have been linked to elevations in sympathetic nervous system activation. Overall, associations were somewhat stronger in younger relative to older individuals, but there was no evidence of differences by sex or race/ethnicity. Our findings support existing evidence suggesting that OSA is associated with risk of incident coronary artery disease. However, prospective data are needed to evaluate the temporal relationship between OSA and CAC development.

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