SLEEP DURATION AND CAROTID INTIMA MEDIA THICKNESS

SUPPLEMENTAL MATERIAL

Supplemental Methods

Study participants

Participants were recruited from the Chicago study center, which is one of four sites within the ongoing multicenter cohort of the Coronary Artery Risk Development in Young Adults (CARDIA) Study. All participants in the CARDIA cohort study that were not pregnant at the year 15 exam were invited to participate in the ancillary sleep study, (which took place between years 15 and 20). As noted in the manuscript, among the 814 Chicago participants invited to participate, 82% (669) consented. Supplementary Table A highlights the distribution of study characteristics of the entire CARDIA cohort compared to the ancillary sleep study. In addition, a prior analysis demonstrated no significant differences in the self-reported sleep time between ancillary sleep study participants and non-participants from the CARDIA cohort as measured in the year 15 general sleep duration questionnaire.[1]

Subjective sleep characteristics

The secondary aim of this study was to evaluate whether subjective measures of sleep quality are associated with CIMT using self-reported measures of sleep. Validated measures were used to assess daytime sleepiness, global sleep quality and apnea risk.

As part of the ancillary study, participants completed validated sleep questionnaires: the Pittsburgh Sleep Quality Index (PSQI), the Epworth Sleepiness Scale (ESS), and the Berlin Questionnaire (BQ).[2-4] These subjective measures were assessed twice at the same time as the actigraphy data were collected.

The PSQI is a 19-item questionnaire that assesses the individual's sleep quality over the previous month. Scores range from 0-21, and a higher score indicates poorer sleep quality. When identifying cases of sleep disorders, this measure has a sensitivity of 89.6% and a specificity of 86.5% with a cutpoint of 5.[2, 5, 6] The ESS is a measure of one's likeliness of dozing off or falling asleep in certain situations. It is an 8-item questionnaire with scores ranging from 0-24, where values greater than 10 are considered to be indicative of significant sleepiness.[3, 7] The BQ measure classifies individuals as being at high risk for having sleep apnea if they score positive for two of the following

three categories 1) frequent or loud snoring 2) daytime fatigue or 3) BMI > 30 kg/m^2 or presence of hypertension.[4] It is a validated measure of sleep apnea risk with a sensitivity of 86%, a specificity of 77%, a positive predictive value of 89%, and Cronbach's correlation between 0.86-0.92.[4] The self-reported sleep duration measure was assessed at year 20 at the CARDIA follow-up visit. Participants were asked "During the past month, how many hours of actual sleep did you get at night?"

Additional indicators of sleep quality in the secondary analysis included frequent snoring from the BQ. The participants were asked a set of snoring-related questions on the BQ including 1) Do you snore? 2) How loud is your snoring? 3) How often do you snore? 4) Has your snoring ever bothered other people? 5) Has anyone noticed that you quit breathing during your sleep? The scoring method for this questionnaire is outlined elsewhere,[4] however if the individual answered at least 2 of the 4 questions related to high frequency of snoring, they were categorized as positive for the snoring measure, which was operationalized as a dichotomous variable.

Sleep fragmentation, which is a common feature of sleep disorders and may be related to a number of pathological stimuli, was determined by actigraphy. It was calculated by taking the sum of the proportion of time spent sleeping when the subject is moving, as well as the proportion of immobile periods lasting 60 seconds or less. An average over the 6 days of actigraphy monitoring was calculated. This value was then divided by its standard deviation.

Covariates

All covariates were based on the year 20 CARDIA study visit. Sociodemographic variables included in this analysis were age, gender, race/ethnicity, and level of education, which were assessed based on self report from questionnaires. Level of education was categorized into four levels: < high school, high school degree or GED, some college, and college graduate. BMI was calculated as weight (to the nearest 0.2 kg) divided by the square of height in meters (to the nearest 0.5 cm). Waist circumference was measured to the nearest 0.5 cm at the minimal abdominal girth or "natural waist", which is defined as half way between the iliac crest and the lowest lateral portion of the rib cage. Blood pressure was measured three times in the seated position after a five minute resting period, and

the measure was calculated based on the average of the last two readings using an Omron HEM-90XL sphygmomanometer (Bannockburn, IL.) Participants were asked to fast 12 hours prior to clinical visit, and to avoid smoking and physical activity at least 2 hours before the exam. Standard protocols were used and blood plasma samples and analysis for triglycerides, total cholesterol, and HDL cholesterol, C-reactive protein (CRP), and interleukin-6 (IL-6) were measured using assays and processed at a Central Lab as outlined according to standard procedures.[8] Lipid concentrations were measured by the University of Washington Northwest Lipid Research Clinic Laboratory. Triglycerides and HDL-cholesterol were measured by enzymatic procedures, HDL-cholesterol was measured after dextran sulfate-magnesium precipitation, and LDL cholesterol was derived using the Friedewald Formula.[9] CRP and IL-6 assays were measured by University of Vermont, Department of Pathology (Colchester, VT). A high-sensitivity ELISA measured serum CRP using a BN-II analyzer.[10] Diabetes was assessed based on a fasting plasma glucose ≥126 mg/dL, or hemoglobin A1c ≥ 6.5%, or if the participant reported taking diabetes control medications. Smoking was also based on self report, and categorized as never, former and current smoker. Alcohol consumption was categorized as non-drinker, 0-7 drinks per week, and >7 drinks per week. Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression (CES-D) scale, an instrument that has been previously validated and well studied.[11] We used a cut point of 16 to identify individuals with depressive symptoms (higher score indicates more severe symptoms). The physical activity index combines frequency and intensity reported in the physical activity measures.[12, 13] Total daily caffeine intake (milligrams per day) was calculated based on caffeine-containing beverages and foods reported in the validated, interviewer-administered dietary history questionnaire.[14]

Supplemental results

Characteristics of study participants differed by men and women. Among men, sleep duration was significantly associated with race, education, systolic and diastolic blood pressure, smoking and depressive symptoms; and CIMT was significantly associated with age, education, BMI, waist circumference, systolic and diastolic blood pressure, alcohol and depression. Among women, sleep

duration was significantly associated with race, education, BMI, waist circumference, systolic and diastolic blood pressure, HDL cholesterol, alcohol, smoking status and depression; and CIMT was significantly associated with all demographics and covariates except depression.

Regarding the additional subjective measures of sleep, findings indicated that measures of self-reported sleep quality, including the PSQI, ESS and BQ were not significantly associated with CIMT in the fully adjusted models. In analyses adjusting for age and race, the only significant subjective measure was the BQ among women (β=0.043, 95% CI 0.014, 0.073, p=0.01), which became weaker and no longer significant after further adjustment of CVD risk factors (β=0.010, 95% CI -0.037, 0.056, p=0.69). In fully adjusted models, the ESS and PSQI were not significant among women (β=0.015, 95% CI -0.031, 0.061, p=0.51; β=0.001, 95% CI-0.038, 0.040, p=0.98); and the BQ, ESS and PSQI were not significant among men (β=-0.036, 95% CI -0.105, 0.034, p=0.31; β=-0.0163, 95% CI -0.074, 0.041, p=0.58; β=0.030, 95% CI -0.020, 0.080, p=-0.23). This analysis also evaluated snoring behaviors (the snoring component of the BQ) and sleep fragmentation, and their association with CIMT. Neither of these variables was a significant predictor of CIMT and did not improve the fit of the models; and these were assessed both with the sleep duration variable included and excluded from the model.

Site-specific CIMT associations also differed by gender and the bulb was a key driver in the association between sleep duration and CIMT among men. Sleep duration was a strong and significant predictor of thickness of the bulb among men in models adjusted for age and race (β=-0.06, 95% CI -0.094, -0.025, p<0.01) as well the models adjusted for demographics and CVD risk factors (β=-0.07, 95% CI -0.125,-0.023, p<0.01). Adjusted models were not significant for the ICA (β=-0.01, 95% CI -0.037, 0.009, p=0.24) or the CCA (β=-0.01, 95% CI -0.026, 0.003, p=0.11) among men; or the bulb, ICA or CCA among women (β=-0.01, 95% CI -0.035, 0.021, p=0.66; β=-0.01, 95% CI -0.035, 0.012, p=0.34; β=-0.01, 95% CI -0.008, 0.017, p=0.438), respectively.

In addition to the results highlighted in the manuscript, we also investigated the concurrent association between sleep and CIMT using the self-reported measure from the 20-year CARDIA follow-up assessment. The association between self-reported sleep duration and CIMT was not significant for either men or women in the gender-stratified models that were adjusted for age, race, smoking education, depression, BMI, systolic blood pressure, LDL cholesterol, HDL cholesterol and diabetes (β=-0.011 95% CI -0.024, 0.001, p=0.07 among women; β=-0.010 95% CI -0.024, 0.003, p=0.14 among men). The observed estimates of effect for the association between self-reported sleep duration and CIMT were also very similar for both men and women.

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Supplemental Tables

Table A. Distribution of Study Population compared to CARDIA cohort*

Demographics	CARDIA Sleep Study Participants	CARDIA cohort*	P-value†
Participants	N=617	N=4448	
Age			
37 - 41 years	18.31	19.92	
42 - 46 years	30.15	28.49	0.653
47 - 52 years	51.54	51.59	
Sex			
Male	42.14	45.97	0.073
Female	57.86	54.03	
Race			
Black	43.44	52.70	<0.001
White	56.56	47.30	
Education			
Less than high school degree	5.36	4.02	
High school degree	16.56	20.63	0.022
Some college	25.49	27.58	
College graduate	52.60	47.76	
IMT measures			
Carotid IMT (mean)	0.70 (0.1)	0.72 (0.1)	0.060
Internal carotid artery (mean)	0.64 (0.2)	0.62 (0.2)	0.016
Carotid bulb (mean)	0.79 (0.2)	0.81 (0.2)	0.066
Common carotid artery (mean)	0.66 (0.1)	0.68 (0.1)	<0.001
Carotid IMT (mean of max)	0.88 (0.2)	0.90 (0.2)	0.028
Covariates			
Body mass index			
≥ 30 kg/m^2 (obese)	35.05	38.79	
25 - 30 kg/m^2 (overweight)	33.44	33.18	0.136
< 25 kg/m^2 (normal)	31.5	28.03	
Waist circumference			
> 90 cm	47.08	52.08	0.024
≤ 90 cm	52.92	47.92	
Systolic blood pressure			
> 120 mmHg	32.41	34.72	0.272
≤ 120 mmHg	67.59	65.28	
Diastolic blood pressure			
> 80 mmHg	20.62	22.06	0.431
≤ 80 mmHg	79.38	77.94	

High-density lipoprotein cholesterol			
< 52 mg/dL (below median)	49.35	50.10	0.734
≥ 52 mg/dL (above median)	50.65	49.90	
Low-density lipoprotein cholesterol			
> 109 mg/dL (above median)	49.17	47.31	0.404
≤ 109 mg/dL (below median)	50.83	52.69	
Diabetes			
Yes	7.62	5.91	0.098
No	92.38	94.09	
Alcohol			
> 7 drinks/week	28.77	32.71	
1-7 drinks/week	44.05	40.14	0.170
Non-drinker	27.18	27.14	
Smoking			
Current smoker	29.80	35.00	
Former smoker	39.54	34.34	0.105
Never smoker	30.66	30.66	

Abbreviations: IMT, intima-media thickness

*Those participants not included in the CARDIA ancillary sleep study

†p-values reflect t-tests, chi-square tests or ANOVA test for those with 3 or more categories

Table B. Linear regression of sleep duration (hrs) with CIMT, stratified by sex, adjusting for individual variation of sleep time by the 6 days of data collection

	Male (N=260)		**Female (N=357)**	
	β	**P-value**	**β**	**P-value**
Sleep duration (h)*	-0.28	<0.001	-0.005	0.485
Sleep duration (h)**	-0.28	<0.001	-0.003	0.655
Sleep duration (h)***	-0.27	<0.001	-0.004	0.604
Sleep duration (h)****	-0.28	0.003	-0.001	0.944

*Adjusted for age, race

**Adjusted for age, race, standard deviation of 6 days of sleep time

***Adjusted for age, race, the range of 6 days of sleep time

****Adjusted for age, race, coefficient of variation of 6 days of sleep time

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