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Authors

Hall, Martica

Mulukutla, Suresh

Kline, Christopher

et al.

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ORIGINAL ARTICLE

Objective Sleep Duration Is Prospectively Associated With Endothelial Health

Martica H. Hall, PhD[1]; Suresh Mulukutla, MD[2]; Christopher E. Kline, PhD[3]; Laura B. Samuelsson, MS[4]; Briana J. Taylor, MS[4]; Julian F. Thayer, PhD[5]; Robert T. Krafty, PhD[6]; Ellen Frank, PhD[1]; David J. Kupfer, MD[1]

[1]Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA; [3]Department of Health and Physical Activity, University of Pittsburgh, Pittsburgh, PA; [4]Department of Psychology, University of Pittsburgh, Pittsburgh, PA; [5]Department of Psychology, Ohio State University, Columbus, OH; [6]Department of Biostatistics, University of Pittsburgh School of Public Health, Pittsburgh, PA

Study Objectives: The mechanisms linking short sleep duration to cardiovascular disease (CVD) are poorly understood. Emerging evidence suggests that endothelial dysregulation may lie along the causal pathway linking sleep duration to cardiovascular risk, although current evidence in humans is based on cross-sectional studies. Our objective was to evaluate the prospective association between objectively assessed sleep duration and clinical indices of endothelial health.

Methods: A total of 141 medically healthy adults underwent an overnight laboratory sleep study when they were between the ages of 21 and 60 years. Total sleep time was objectively assessed by polysomnography at study entry. Endothelial health, including brachial artery diameter (BAD) and flow-mediated dilation (FMD), was measured 18.9 ± 4.6 years later. Medical health and psychiatric status were assessed at both time points. Approximately half of the sample had a lifetime history of major depressive disorder.

Results: In univariate analyses, shorter sleep duration was associated with increased BAD ($\beta = -0.24$, $p = .004$) and decreased FMD ($\beta = 0.17$, $p = .042$). BAD, but not FMD, remained significantly associated with sleep duration after adjusting for sex, age, body mass index (BMI), smoking, diabetes, hypertension, and lifetime history of major depressive disorder (MDD) at T2. The association between sleep duration and BAD was stronger than the association between BAD and an aggregate measure of CVD risk including three or more of the following risk factors: male sex, age ≥ 65 years, smoker, BMI ≥ 30, diabetes, hypertension, and MDD.

Conclusions: Objectively assessed short sleep duration was prospectively associated with increased BAD over a 12- to 30-year period.

Keywords: sleep, sleep duration, cardiovascular disease, brachial artery diameter, endothelial function.

Statement of Significance

Short sleep duration is associated with increased cardiovascular morbidity and mortality. Yet, few prospective studies have evaluated the mechanisms linking sleep duration to cardiovascular risk. We found that objectively assessed short sleep duration in healthy adults was a significant predictor of increased diameter of the brachial artery, measured 12–30 years later. Sleep duration was a stronger predictor of brachial artery diameter than was an aggregate measure of traditional CVD risk factors. These prospective data are the first to demonstrate that endothelial health may represent one pathway through which insufficient sleep increases cardiovascular risk. Future studies might use sleep extension interventions to probe causality and test reversibility, while larger community-based studies are needed to identify effect modifiers and inform precision medicine strategies.

INTRODUCTION

Short sleep duration is endemic in the industrialized world. Recent surveys indicate that anywhere between 15% and 66% of adults in first-world countries report sleeping less than 7 hours a day.[1,2] Far from benign, insufficient sleep has long-term consequences for cardiovascular health. Epidemiological studies have reported prospective associations among short sleep duration and cardiovascular morbidity and mortality including incident cases of hypertension, angina, nonfatal and fatal myocardial infarction, and sudden cardiac death.[3–7] Putative pathways through which short sleep duration adversely affects cardiovascular health include chronic inflammation, poor metabolic control, dyslipidemia, autonomic imbalance, elevated diurnal blood pressure, and obesity.[8–12] Fewer studies have evaluated sleep duration in relation to alterations of the endothelium, which may occur early in the disease process, prior to the overt manifestation of symptoms and cardiovascular events.

Brachial artery diameter (BAD) and flow-mediated dilation (FMD) are well-established noninvasive tests of endothelial health and can add important prognostic information in the evaluation of cardiovascular risk.[13,14] We are aware of only a handful of cross-sectional studies that have examined links between sleep duration and endothelial function. Reduced self-reported sleep duration in college students during a 4-week examination period and in medical residents following an overnight shift was associated with decreased brachial artery FMD.[15,16] More recently, Weil and colleagues[17] reported decreased endothelin-1 (ET-1) function in midlife adults who were self-reported short sleepers (<7 h/night) compared to those who reported sleeping 7 or more hours per night. Two other cross-sectional studies failed to show significant associations between FMD and either self-reported or objectively assessed sleep duration.[18,19] While the observational evidence linking short sleep duration and endothelial function is somewhat mixed, experimental manipulation of sleep duration via partial and total sleep deprivation in healthy adult males has been reliably linked to indices of impaired endothelial function including increased circulating E-selectin and decreased acetylcholine-induced vasodilation.[20,21] Rodent models, too, show decreased FMD in response to sleep deprivation.[22] Taken as a whole, these data are consistent with the hypothesis that short sleep duration is associated with impaired endothelial function, suggesting a novel physiological pathway through which insufficient sleep contributes to cardiovascular disease (CVD).

Significant limitations to the extant literature on sleep duration and endothelial function include reliance by most on self-reported sleep duration and the use of cross-sectional study designs. The present study prospectively examined laboratory-assessed sleep duration in medically healthy midlife adults in relation to endothelial function measured an average

of 19 years later. In addition, we expanded covariates beyond previous studies of sleep and endothelial function to include major depressive disorder (MDD), which is a known risk factor for CVD[23] and is strongly linked to disturbed sleep.[24,25] Indeed, previous studies have reported decreased endothelial health, as measured by FMD, in adults with a history of MDD.[26–28] We hypothesized that short sleep duration would be prospectively associated with increased resting BAD and decreased FMD of the brachial artery after adjusting for other factors known to influence CVD risk, including lifetime history of MDD.

METHODS

Participants

Participants for the present study were drawn from four studies originally conducted at the University of Pittsburgh between 1982 and 1999 (Time 1 [T1]). One study (MH024652 [D. J. Kupfer, PI]) assessed sleep in adults without psychiatric disorders, whereas the other three studies focused on links between sleep and clinical course of MDD and exclusively included adults with MDD (MH029618 [D. J. Kupfer, PI]; MH049115 [E. Frank, PI]; MH041884 [M. Thase, PI]). Other psychiatric disorders and substance abuse were exclusions for all four studies. Eligibility criteria common across studies stipulated that all participants be between 20 and 60 years of age and medically healthy (ie, without cardiovascular, endocrine, renal, hepatic, or neurologic disorders), as confirmed by a comprehensive medical exam and laboratory blood work at study entry (T1).

At Time 2 (T2), 339 participants from the above-mentioned studies were recontacted from 2010 through 2014 and invited to participate in a follow-up study of sleep, depression, and cardiovascular risk. Of the 177 who consented to the T2 study, two were excluded due to a current unstable major medical illness, as determined by the study cardiologist (SM), and 19 were excluded due to bipolar disorder, psychotic depression, or suicidal ideation, as determined by structured psychiatric interview. Eight participants withdrew prior to study completion, citing busy schedules ($n = 3$), protocol burden ($n = 1$), and "other" ($n = 4$) reasons for their withdrawal. An additional seven participants who lived out of state were unable to complete the cardiovascular assessments. A total of 141 participants were studied at both time points and had complete data relevant to the present report including an overnight sleep study at T1 and measures of endothelial function at T2. Sixty-three participants were drawn from the Pittsburgh Study of Normal Sleep and the remaining 78 participants were drawn from the studies of sleep and depression. This study was approved by the University of Pittsburgh Institutional Review Board (PRO10030034), and all participants provided written informed consent prior to data collection. Financial compensation was provided to study participants.

Measures

Sleep

Laboratory sleep studies at T1 were conducted in the Sleep and Chronobiology Laboratory at Western Psychiatric Institute and Clinic, with sleep times scheduled to correspond to participants' self-reported habitual sleep patterns. The polysomnographic (PSG) recording montage consisted of bilateral central electroencephalogram (EEG) leads referenced to A1+A2; right and left electrooculogram referenced to A1+A2; and bipolar submentalis electromyogram. Raw PSG signals collected at T1 were rescored at T2, pursuant to current scoring criteria.[29] Sleep duration was calculated as total sleep time, defined as the sum of non-rapid eye movement stages N1, N2, N3, and rapid eye movement sleep.

BAD and FMD

At T2, participants underwent sonographic measurement of the brachial artery in the Vascular Clinical and Translational Research Center (V-CTRC). Assessments were conducted with participants in the supine position after a 10-minute rest period by experienced V-CTRC technicians who were blind to participant characteristics, including sleep duration and lifetime history of depression. Briefly, the occlusion cuff was placed on the right forearm, and the technician obtained a clear horizontal long view of the brachial artery walls at ~2 cm or more before noting landmarks and stabilizing the probe. BAD was assessed by B-mode ultrasound (GE Vingmed Ultrasound A/S, Horten, Norway) before and after reactive hyperemia of the brachial artery induced by pneumatic cuff at a pressure of 50 mm Hg higher than systolic blood pressure for 5 minutes. The linear array transducer frequency was 3–10 MHz (9L-GE, Healthcare Japan Corporation, Hino-shi, Tokyo, Japan). Digital images of resting and hyperemic BAD were quantified by three calibrated electronic calipers, separated by 1-cm intervals. BAD was measured at baseline as the average of three resting state measures. FMD was calculated as the percent change in brachial diameter from the resting state at 60 seconds post-cuff deflation (100 × [hyperemic diameter at 60 seconds − resting diameter]/resting diameter). The timing of the V-CTRC protocol was standardized across participants to occur in the morning hours in a fasting state.

Participant Characteristics

Participant characteristics relevant to the present report were derived from the T2 assessment, which occurred 18.9 (±4.6) years (range: 12.5–30.3 years) after T1. The T2 assessment included self-report questionnaires, anthropomorphic measures and fasting blood draw, completion of a medical history and medication checklist, and the Structured Clinical Interview for DSM-IV (SCID). Age, sex, race, and current smoking were assessed by self-report. Blood pressure, measured in the supine position, was assessed by the V-CTRC technician after a 10-minute rest period using an automated sphygmomanometer (CONTEC08A, Qinhuangdao CONTEC Medical Systems Co., Ltd., China) prior to the FMD assessment. Blood pressure readings were averaged over three measurements taken at 1-minute intervals. Study staff measured participants' height and weight for calculation of body mass index (BMI; kg/m^2). Diabetes was defined as present if participants met any of the following three criteria: fasting blood glucose ≥ 126 mg/dL, HbA1c ≥ 6.5%, or use of antidiabetic medication. Hypertension was defined as present if participants met any of the following three criteria:

systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or use of antihypertensive medication. Lifetime history of depression was based on a clinician-defined diagnosis of MDD at the T1 and/or the T2 assessment, consistent with previous studies that have shown dose–response relationships between increased exposure to depression and decrements in endothelial health.[28]

Statistical Analysis

Descriptive statistics were used to characterize the study sample. Race was collapsed into a dichotomous variable of "Caucasian" and "Minority Racial Status" due to its distribution (Caucasian = 133, African American = 5, Asian = 1, Native American = 1, Other = 1). No other variable transformations were needed. Univariate linear regression was used to evaluate prospective associations among PSG-assessed sleep duration at T1 and resting BAD and FMD at T2. Sleep duration at T1 was modeled as a linear variable as only five participants slept longer than 8 hours. A second set of multivariable models were used to identify the unique prospective association between sleep duration at T1 and endothelial outcomes at T2 after adjusting for sex, age, minority racial status, smoking, BMI, diabetes, hypertension, and lifetime history of MDD. Collinearity diagnostics verified models were stable, with low variance inflation factors (all below 1.3). Exploratory analyses evaluated interactions between sleep duration at T1 and depression history to determine whether lifetime history of MDD modified the prospective association between sleep duration and endothelial function. A final set of exploratory analyses evaluated sex as an effect modifier, given sex differences in the prevalence of depression in the current sample; 69% of participants with a lifetime history of depression were female while only 40% of controls were female. Moreover, others have reported sex differences in endothelial health.[15,18]

Clinically relevant categorical definitions of sleep duration and known CVD risk factors were used in a final set of analysis of variance models to directly compare their association with endothelial function. Sleep duration was dichotomized as participants who slept less than 7 hours or 7 or more hours, comprising 33.3% and 66.7% of the sample, respectively. We summed each of the CVD risk factors included as covariates in multivariate models to create an index measure of CVD risk (range = 0–7), as shown in Figure 1A. The CVD risk index was dichotomized as 0–2 (53.9%) and three or more (46.1%) risk factors for statistical analyses.

Analyses were conducted with IBM SPSS Statistics software (version 21) and all tests were two-sided at $\alpha = 0.05$.

RESULTS

As shown in Table 1, the majority of the sample was female and reported their racial/ethnic identity as Caucasian. At T1, the age range was 21–60 years of age. At T2, an average of 18.9 ± 4.6 years later, participants were between 36 and 82 years of age. Although all participants were medically healthy at T1, diabetes and hypertension were prevalent at T2, and 46.1% had three or more traditional CVD risk factors (see Figure 1B). Nearly 60% of participants had a lifetime history of MDD; while the majority of these participants were drawn from the original depression study cohort, four participants from the Pittsburgh Study of Normal Sleep cohort developed depression during the intervening years.

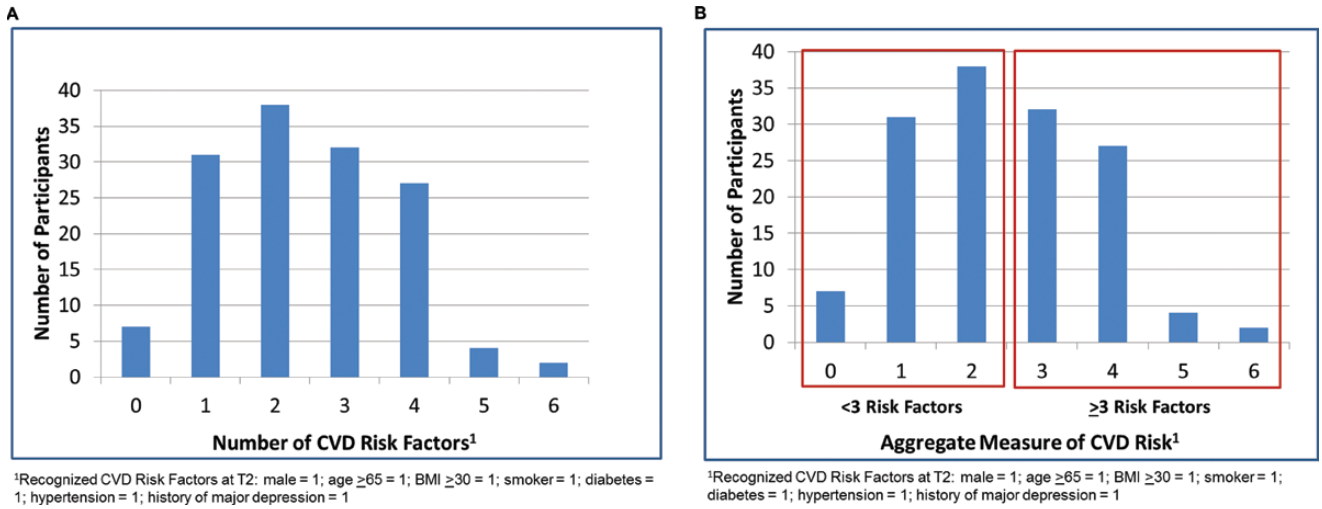
Univariate regression analyses revealed that PSG-assessed sleep duration was prospectively associated with endothelial function, predicting 5.9% of the variance in resting BAD and 2.9% of the variance in FMD (see Tables 2 and 3). Shorter sleep duration at T1 was associated with increased resting BAD ($p < .01$) and decreased FMD at T2 ($p < .05$). As shown in Table 2, sleep duration at T1 remained a significant predictor of resting BAD at T2 after adjusting for sex, age, minority racial status, BMI, smoking, diabetes, hypertension, and lifetime history of depression. The prospective association between sleep duration at T1 and FMD at T2 was no longer significant in multivariable analyses (see Table 3). The linear association between sleep duration and endothelial function, after adjusting for CVD risk factors, is depicted in Figure 2.

Categorical analyses revealed significant main effects for short sleep duration ($F = 6.15$, $p < .01$) and CVD risk index ($F = 4.60$, $p < .05$) with BAD. As shown in Figure 3A, BAD was larger in participants who slept less than 7 hours and in participants who had three or more CVD risk factors. There were no significant main effects for short sleep duration or three or more CVD risk factors with FMD (p's > .05; Figure 3B). Although the sleep duration-by-CVD risk index interaction was not significant for either outcome (p's > .05), stratified analyses revealed that BAD was significantly higher in short sleepers in both CVD risk index groups compared to participants who slept 7 or more hours and had less than three CVD risk factors (p's > .05; see Figure 3A). There was a trend for decreased FMD in participants who slept less than 7 hours and had three or more CVD risk factors compared to participants who slept 7 or more hours and had less than three CVD risk factors ($p = .091$; see Figure 3B).

Exploratory analyses indicated that lifetime history of depression significantly moderated the association between sleep duration at T1 and BAD at T2 ($\beta = 0.32$, $p < .01$); no such interaction was observed for FMD. Stratified analyses revealed that decreased sleep duration at T1 was associated with increased BAD at T2 in adults without a lifetime history of depression ($\beta = -0.38$, $p < .001$) but was unrelated to BAD in adults with a lifetime history of depression ($\beta = -0.03$, $p = .72$). A final set of exploratory analyses showed that sex did not moderate associations between sleep duration and endothelial health.

DISCUSSION

The mechanisms linking short sleep duration to CVD are poorly understood. A handful of studies suggest that endothelial dysregulation may lie along the causal pathway linking insufficient sleep to cardiovascular risk.[13–21] The present study provides the first evidence of a prospective association between sleep duration and endothelial health. We found that objectively assessed shorter sleep duration at T1 was associated with increased BAD and decreased FMD measured 12– 30 years later. The prospective association between sleep duration and BAD was independent of known CVD risk factors, including sex, age, minority racial status, BMI, smoking, diabetes, hypertension, and lifetime history of MDD. In direct comparisons, short sleep



[1]Recognized CVD Risk Factors at T2: male = 1; age ≥65 = 1; BMI ≥30 = 1; smoker = 1; diabetes = 1; hypertension = 1; history of major depression = 1

[1]Recognized CVD Risk Factors at T2: male = 1; age ≥65 = 1; BMI ≥30 = 1; smoker = 1; diabetes = 1; hypertension = 1; history of major depression = 1

Figure 1—Panel A. Prevalence of recognized cardiovascular disease (CVD) risk factors at T2. Panel B. Aggregate measure of CVD risk at T2.

Table 1—Sample Characteristics at T2[a].

	Full sample[b] (*n* = 141)	Sleep duration at T1[b]	
		<7 h (*n* = 94)	≥7 h (*n* = 47)
Age, mean (*SD*), years	60.0 (9.3)	61.5 (8.4)	56.8 (10.3)
Sex, *n* (%), female	92 (65.2)	55 (58.5)	37 (78.7)
Caucasian, *n* (%)	133 (94.3)	89 (94.7)	44 (93.6)
Body mass index, mean (*SD*), kg/m^2	30.0 (7.1)	30.2 (7.1)	29.6 (7.1)
Current smoker, *n* (%)	15 (10.6)	10 (10.6)	5 (10.6)
Hypertension, *n* (%)	67 (47.5)	47 (50.0)	20 (42.6)
Diabetes, *n* (%)	21 (14.9)	21 (14.9)	21 (14.9)
Lifetime history of major depressive disorder, *n* (%)	82 (58.2)	58 (61.7)	24 (51.1)
Sleep duration, mean (*SD*), hours	6.7 (0.8)	6.3 (0.6)	7.5 (0.4)
Resting brachial artery diameter, mean (*SD*), mm	3.4 (0.7)	3.6 (0.7)	3.2 (0.6)
Brachial artery flow-mediated dilation, mean (*SD*), %	6.5 (4.6)	6.1 (4.7)	7.3 (4.2)

[a]Means and standard deviations (*SD*) are presented for continuous variables, number (*n*) and percent (%) are presented for categorical variables.
[b]For descriptive purposes, data are presented for the sample as a whole, as well as by T1 sleep duration categories.

duration (<7 hours) and an index measure of CVD risk (≥ three risk factors) were each independently associated with increased BAD. The small but significant prospective association between sleep duration and FMD was attenuated when CVD risk factors were included in the statistical model. While our results do not imply causality, they are consistent with the hypothesis that endothelial dysfunction may play a role in the well-documented prospective association between short sleep duration and CVD.[4,30] The finding that sleep duration may be associated with future endothelial dysfunction has important clinical implications. If confirmed, our findings lay the basis for future novel studies to evaluate whether sleep extension interventions in short sleepers may improve endothelial function and attenuate cardiovascular risk.

In contrast to the strong inverse association between sleep duration and BAD observed in our study, Amir and colleagues[15] reported that BAD was unchanged following acute sleep restriction associated with call duty in a small sample of medical residents and fellows. This discrepancy may reflect methodological advantages of the present study including objective assessment of sleep, assessment of prospective associations, a larger sample size, and statistical control of possible confounds. It is also plausible that BAD may be less sensitive than FMD to acute changes in sleep duration, such as one night of acute sleep restriction. While FMD represents a dynamic response to acute challenge, which can be attenuated by oxidative stress and decreased bioavailability of endothelium-dependent nitric oxide and other vasodilators,[31] increased BAD reflects outward

Table 2—Univariate and Multivariable Models of the Prospective Associations Between Sleep Duration at T1 and Brachial Artery Diameter at T2.

Model 1 predictor	**Standardized β**	**B (95% confidence interval)**	***p***
Sleep duration at T1	−0.242	−0.003 (−0.006 to −0.001)	.004
Model 2 predictors	**Standardized β**	**B (95% confidence interval)**	***p***
Sex	−0.570	−0.824 (−1.018 to −0.630)	.000
Age	−0.116	−0.009 (−0.018 to 0.001)	.088
Minority racial status	−0.952	−0.181 (−0.556 to 0.195)	.343
Body mass index	0.298	0.029 (0.016 to 0.042)	.000
Smoker	0.077	0.172 (−0.106 to 0.450)	.223
Diabetes	−0.163	−0.314 (−0.564 to −0.065)	.014
Hypertension	−0.030	−0.042 (−0.225 to 0.142)	.654
Lifetime history of depression	−0.078	−0.109 (−0.291 to 0.073)	.236
Sleep duration at T1	−0.177	−0.003 (−0.004 to −0.001)	.007

Table 3—Univariate and Multivariable Models of the Prospective Associations Between Sleep Duration at T1 and Brachial Artery Flow-Mediated Dilation at T2.

Model 1 predictor	**Standardized β**	**B (95% confidence interval)**	***p***
Sleep duration at T1	0.171	0.016 (0.001 to 0.032)	.042
Model 2 predictors	**Standardized β**	**B (95% confidence interval)**	***p***
Sex	0.110	1.056 (−0.702 to 2.814)	.237
Age	−0.064	−0.032 (−0.121 to 0.058)	.488
Minority racial status	0.010	0.201 (−3.02 to 3.604)	.907
Body mass index	−0.126	−0.082 (−0.202 to 0.039)	.182
Smoker	−0.037	−0.551 (−3.070 to 1.968)	.666
Diabetes	0.113	1.453 (−0.805 to 3.711)	.205
Hypertension	−0.053	−0.483 (−2.144 to 1.178)	.566
Lifetime history of depression	0.106	0.985 (−0.663 to 2.634)	.239
Sleep duration at T1	0.141	0.013 (−0.003 to 0.030)	.115

remodeling of the arteries in response to prolonged exposure to physiological strain such as elevated blood pressure and the buildup of arterial plaque.[32] Together, these data suggest that a number of factors underlying sleep duration may influence their effects on endothelial health (eg, self-report versus objective assessment, sleep deprivation/restriction versus short time in bed, acute versus habitual/chronic).

Consistent with the proposal that more chronic or sustained sleep disturbances influence remodeling of the carotid and brachial arteries, several large epidemiological studies have reported higher BAD in adults with chronic sleep disturbances including sleep apnea and shiftwork.[33,34] In contrast, insomnia, which is associated with a waxing and waning symptom profile, was not associated with increased BAD in a recent study of midlife adults.[35] The strong inverse association between sleep duration and BAD in our sample was also observed in categorical analyses, where short sleep duration (<7 hours) was more strongly associated with increased BAD than an aggregate index of CVD, as measured by ≥ three risk factors, including male sex, ≥65 years of age, BMI ≥ 30, smoking, diabetes, hypertension, and history ofMDD.[36–38] These results highlight the clinical relevance of sleep duration as a novel risk factor for endothelial dysfunction.

The significant prospective association between PSG-assessed sleep duration and FMD was attenuated when covariates were included in the model. At first glance, these results seem consistent with the null findings of Cooper and colleagues who reported a correlation of 0.03 between laboratory-assessed sleep duration and FMD. However, it may be premature to conclude that sleep duration is unrelated to FMD. Careful examination of the univariate and multivariate association between sleep duration and FMD in the present study reveals that it is almost unchanged with the addition of covariates to the model (univariate $\beta = 0.17$, multivariate $\beta = 0.14$), and the confidence intervals for both models overlap. In fact, the association between sleep duration and FMD was larger than those observed for any of known CVD risk factors included in the model (absolute

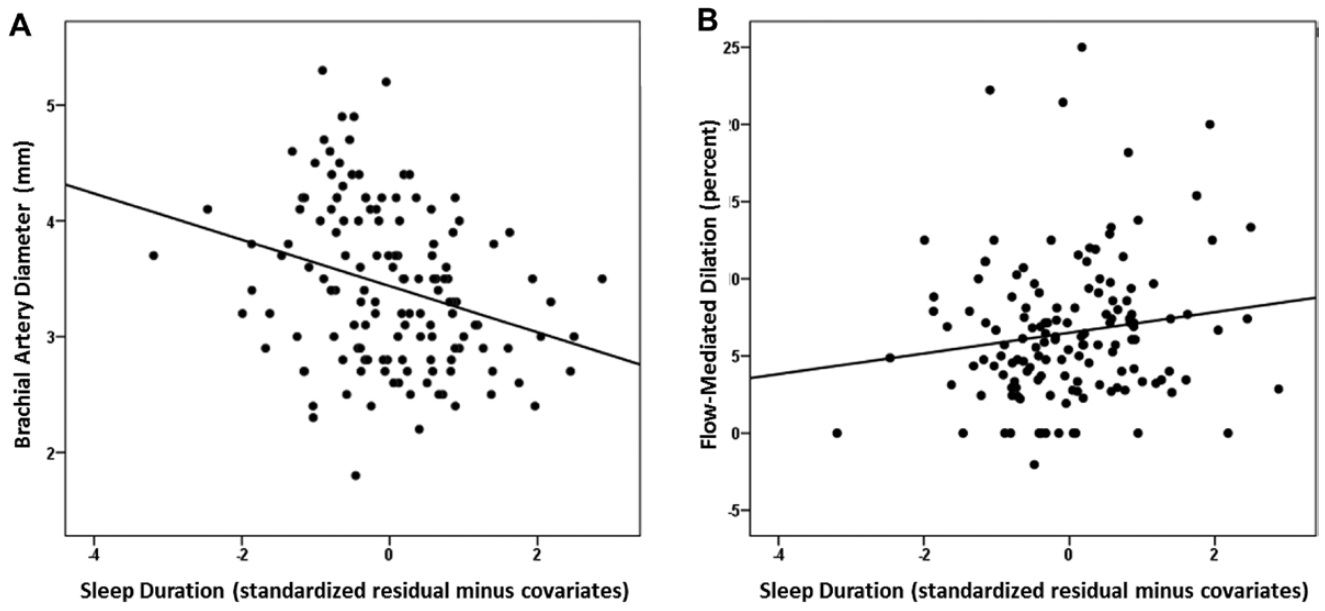


Figure 2—Panel A. Brachial artery diameter at T2 as a function of sleep duration at T1 and recognized cardiovascular disease (CVD) risk factors at T2. Panel B. Flow-mediated dilation at T2 as a function of sleep duration at T1 and recognized CVD risk factors at T2.

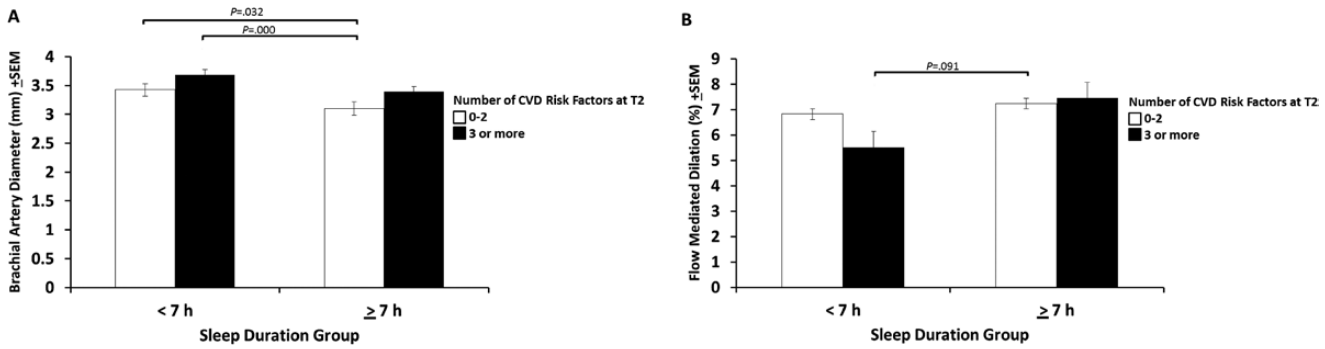


Figure 3—Panel A. Brachial artery diameter and increased cardiovascular disease (CVD) risk (≥ three risk factors) at T2 as a function of short sleep duration (<7 hours) at T1. Panel B. Flow-mediated dilation and increased CVD risk (≥ three risk factors) at T2 as a function of short sleep duration (<7 hours) at T1.

standardized β values between 0.04 and 0.13). The discrepancy between the present results and those of Cooper et al. may be related to differences in participant characteristics and study design; on average, their participants were more than 20 years younger and FMD values were more than double those observed in the present sample. In addition, participants in the study by Cooper and colleagues slept at laboratory-determined versus habitual bedtimes, which may have artificially altered participants' sleep duration. The high degree of variability in FMD observed in the present sample suggests that a larger sample size is needed to fully test associations between sleep duration and FMD; this was also true for known CVD risk factors which were, similarly, unrelated to FMD in the present sample.

There are several pathways through which short sleep duration may influence endothelial health. Experimental sleep deprivation and manipulation of intermittent hypoxia in rodent models of sleep apnea have each been shown to induce acute increases in reactive oxygen species, decreased release of nitric oxide, and alterations in transcription factors that underlie antioxidant defense mechanisms.[39–42] Decreased sleep duration may also influence oxidative stress and the endothelium via transcriptional processes specific to sleep and wakefulness in peripheral tissue.[43,44] Biological, behavioral, and psychological consequences of insufficient sleep including chronic inflammation, autonomic imbalance, decreased physical activity, and poor emotion regulation represent additional, and as-yet-untested, pathways through which short sleep duration may contribute to endothelial dysfunction and cardiovascular risk.[45–47]

The present sample afforded a unique opportunity to evaluate the influence of MDD on the prospective association between sleep duration and endothelial function. While shorter sleep duration was associated with a larger BAD in adults without a

lifetime history of MDD, no such association was observed in participants with a history of MDD. The absence of a significant association between sleep duration and BAD in participants with MDD was somewhat surprising, given that depression and short sleep duration are recognized risk factors for CVD.[5,38,47] Others have reported that FMD is significantly lower in adults with a history of MDD compared to healthy psychiatric controls.[26–28] We had anticipated that endothelial dysfunction would be most pronounced in short sleep participants with a history of depression. Yet, in the present sample, history of depression was not associated with either measure of endothelial function. The wide confidence intervals associated with history of depression suggest a high degree of variability in its association with BAD and FMD which might be a reflection of within- and between-subject differences with respect to the number and duration of depressive episodes, differences in treatment history and medication profiles over time, and differences in lifestyle and health behaviors over the 12- 30-year assessment period.[48] The wide confidence intervals also suggest that the present study may have been underpowered to detect any synergistic influence of MDD and short sleep duration on endothelial function.

Several limitations to the present study bear consideration. First, although prospective, these data do not establish causality. Short sleep duration may be prospectively associated with endothelial function because of its correlation with a third, unmeasured causal variable. Second, the sample was primarily Caucasian, limiting generalizability to other racial/ethnic groups with different sleep and cardiovascular risk profiles.[49,50] These characteristics are similar to the original cohorts from which the present sample was drawn. Nor can these data be generalized to younger or older samples in light of significant changes in objective indices of sleep duration across the life span.[51] Our response rate of 41% of participants from the original T1 studies suggests that our sample may have been biased, including the most healthy or resilient individuals. We cannot rule out the possibility that we were unable to locate or enroll the individuals who became ill, or deceased, during the years between the T1 and T2 assessments. As such, our results may underestimate associations among sleep and endothelial health. They may also underestimate the impact of depression, which was not significantly associated with endothelial health in the present sample. This finding is unusual, as previous studies have reported robust associations among FMD and MDD.[26–28] While the present study adjusted for a range of covariates, sample characteristics, including sample size, limited our ability to reliably test effect modification by key factors such as sex and age. Larger studies designed to disentangle the independent and interdependent effects of participant characteristics on sleep and endothelial health are needed. We were not able to evaluate endothelium-dependent vasodilation, as our FMD assessment did not include a nitroglycerin challenge.[52] And, although participants were medically healthy without CVD at study entry, we did not assess endothelial health at T1. Finally, a single night of laboratory-assessed sleep may not be representative of one's habitual sleep duration. Wrist actigraphy would be a useful approach to objectively quantify both cross-sectional and prospective associations among habitual sleep duration and endothelial health.

Notable strengths of the present study include its strong theoretical underpinnings, which build on and extend the extant literature, coupled with significant methodological advantages over previous studies. For instance, sleep duration was measured by laboratory polysomnography. The importance of objective sleep assessments cannot be understated in light of evidence that sociodemographic and psychosocial factors bias subjective reports of sleep duration, including the prevalence of "long" sleep duration; consistent with previous reports, very few participants had PSG-assessed sleep durations of more than 8 hours (n = 5).[53] Although BAD and FMD were not assessed at T1, participants were healthy, normal weight adults without CVD or diabetes pursuant to clinician-assessed eligibility criteria at study entry. While the prospective study design adds confidence in the hypothesis that short sleep duration influences endothelial function, these data cannot establish causality. We adjusted for covariates linked to endothelial health in large observational cohorts, even though these same covariates have not been reliably linked with FMD in laboratory-based studies similar to the present study.[15,18,26–28] Finally, previous studies of sleep duration and endothelial function have focused on convenience or community samples and have not evaluated the influence of mental health on study outcomes.

In conclusion, short objectively assessed sleep duration in healthy adults was prospectively associated with endothelial dysfunction measured 12– 30 years later. The association between short sleep duration and BAD was even stronger than that observed for recognized CVD risk factors. These data are concerning in light of the prevalence of short sleep duration in industrialized nations. On the other hand, since short sleep duration may be a modifiable risk factor, there is reason for cautious optimism; as such, interventions to ameliorate short sleep may be used in primary prevention campaigns and in conjunction with secondary efforts to alter the pathophysiology and clinical course of CVD.

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Address correspondence to: Martica Hall, PhD, Department of Psychiatry, University of Pittsburgh School of Medicine, 3811 O'Hara Street, Rm: E-1131, Pittsburgh, PA 15213, USA. Telephone: +1-412-246-6413; Fax: +1-412-246-5300; Email: hallmh@upmc.edu

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