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Original Article

ORIGINAL ARTICLE

Sleep duration regularity, but not sleep duration, is associated with microvascular function in college students

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Abstract

Study Objectives: Vascular dysfunction is a hypothesized mechanism linking poor sleep habits to an increased incidence of cardiovascular diseases (CVDs). However, the vascular profile associated with free-living sleep duration and sleep regularity has not been well elucidated, particularly in young adults. Thus, this study aimed to evaluate the associations between mean sleep duration, regularity in sleep duration, and peripheral vascular function in young adult college students.

Methods: Fifty-one healthy undergraduate students (20 ± 1 years) completed 14 days of 24-hour wrist actigraphy and subsequent vascular assessments. Macrovascular function was measured using brachial artery flow-mediated dilation (FMD) while microvascular function was measured via passive leg movement (PLM).

Results: Mean sleep duration was unrelated to FMD and PLM. Conversely, more irregular sleep duration (14-day sleep duration standard deviation [SD]) was unfavorably associated with all three measures of PLM-induced hyperemia (peak leg blood flow [LBF], $p = 0.01$; change in LBF from baseline to peak, $p < 0.01$; LBF area under the curve, $p < 0.01$), and remained significant in regression models which adjusted for sex, body mass index, blood pressure, physical activity, alcohol and caffeine consumption, and sleep duration (all $p < 0.05$). When using a median split to dichotomize "low" and "high" sleep duration SD groups, those demonstrating high variability in sleep duration exhibited ~45% lower PLM responses compared with those demonstrating low variability.

Conclusions: Irregular sleep duration is associated with poorer microvascular function as early as young adulthood. These findings support the growing body of evidence that irregular sleep patterns may be an independent and modifiable risk factor for CVD.

Statement of Significance

Obtaining adequate sleep is essential for optimal cardiovascular health, while the implications of regular sleep patterns on cardiovascular health are still emerging. There is limited information on how these sleep metrics relate to cardiovascular indices in young adulthood, especially in free-living contexts. This is the first study to objectively evaluate free-living sleep duration and sleep regularity and their associations with peripheral vascular function, a biomarker of vascular risk, in young adults. These findings support the notion that consistent sleep schedules are important for cardiovascular health at all ages. Interventions are needed to evaluate the efficacy of promoting consistent sleep duration as a strategy to optimize vascular health and reduce the risk of cardiovascular disease.

Key words: sleep duration; sleep regularity; vascular function; peripheral vasculature; passive leg movement; flow-mediated dilation; young adults

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Introduction

Habitual short sleep (i.e. <7 hour/night) has been independently linked to major cardiometabolic outcomes including hypertension [1], coronary artery disease [2], type 2 diabetes [3], and mortality [2]. Despite these known health risks, over one-third of U.S. adults routinely obtain less than the recommended 7 or more hours of sleep per night [4, 5]. Furthermore, sleep is a human behavior that often varies on a nightly basis [6, 7]. Emerging evidence has found that those who demonstrate more *variability* (i.e. less regularity) in night-to-night sleep duration also display poorer cardiometabolic health characterized by greater adiposity, inflammation, higher resting blood pressure (BP), and greater odds of metabolic syndrome when compared with those who demonstrate less variability [8–12]. Additionally, in older adults free of cardiovascular disease (CVD) at baseline, those demonstrating the highest variations in night-to-night sleep duration had more than twofold greater odds of developing CVD over ~5 years of follow-up when compared with those with the lowest variations [13]. Thus, habitual short sleep duration, and particularly irregular sleep duration, may be novel and modifiable CVD risk factors.

Vascular dysfunction is considered an independent, nontraditional risk factor for CVD [14, 15] and is one of several hypothesized physiological pathways linking short sleep duration with increased CVD risk [16–18]. Notably, vascular dysfunction is one of the earliest detectable biomarkers in the development of atherosclerosis and is a precursor to clinical manifestations [19], making it a robust preclinical assessment for evaluation, and potentially prevention, of future CVD risk in apparently healthy populations [15]. Methods for evaluating vascular function in the periphery can be broadly categorized as macrovascular assessments, which evaluate conduit artery function, or microvascular assessments, which evaluate downstream resistance vessel function [20, 21]. Both macrovascular and microvascular dysfunction are evident in young adults following repetitive bouts of restricted sleep under highly controlled experimental conditions [16–18]. This indicates that the vascular consequences of short sleep may be apparent as early as young adulthood and could contribute to an increased risk of CVD if sustained over time. However, to our knowledge, these findings have not been adequately translated to free-living settings, as the relation between habitual sleep duration and peripheral vascular function in young adulthood has only been evaluated in experimental and laboratory-based settings, and over relatively short periods (i.e. up to 1 week) [17, 22].

The underlying mechanisms linking sleep regularity with CVD incidence are currently unclear. Irregular sleep duration is a hypothesized marker of both circadian disruption and intermittent sleep deprivation [13], both of which can elicit vascular dysfunction [18, 23, 24]. Importantly, young adulthood has been characterized by more irregular sleep patterns when compared with that of older adults [25]. In particular, college students are notorious for erratic sleep schedules, with 20% of students reporting staying up all night at least once per month and 35% staying up until 3 am at least once per week [26]. However, the vascular profile associated with substantial variations in sleep duration, such as those frequently experienced by college students, is currently unknown.

Therefore, the aim of this study was to evaluate the associations between free-living sleep duration and sleep duration regularity with peripheral vascular function in young adult undergraduate college students. To comprehensively evaluate peripheral vascular function, we performed both brachial artery flow-mediated dilation (FMD) and passive leg movement (PLM) to assess conduit artery macrovascular function and resistance artery microvascular function, respectively. We hypothesized that both shorter and more irregular sleep duration would be associated with less optimal FMD and PLM responses in undergraduate students. Findings from this study could contribute to the current understanding of sleep as a potential modifiable risk factor for CVD and could assist in identifying novel intervention targets for the prevention of cardiometabolic disease risk.

Methods

Study participants and protocol

This study was approved by the Institutional Review Board at the University of Delaware and was conducted in accordance with the ethical standards of the Declaration of Helsinki. Participants were recruited from the University of Delaware and the surrounding Newark, DE region. All participants provided written informed consent prior to participation. Participants were between the ages of 18 and 25 years and nonobese (body mass index [BMI] 18.5–29.9 kg/m^2). Additionally, all participants were currently enrolled in a full-time undergraduate college student workload. Individuals were excluded from participation if they: (1) had a history of any major chronic diseases or conditions (including cardiovascular, renal, metabolic, autoimmune, chronic respiratory, or cancerous conditions, or sleep disorders), (2) were currently working night-shift-work, (3) were using sleep medication, (4) were diagnosed with depression, (5) had a resting BP >140/90 mmHg, (6) were currently pregnant, (7) were using medication that alters vascular function, or (8) were smokers (≥1 cigarette in last month).

Following consenting procedures, participants underwent a screening which included a review of medical history and measurement of resting BP, height, and weight. Eligible participants were then provided with a wrist accelerometer (MicroMotionlogger; Ambulatory Monitoring Inc., Ardsley, NY) and were instructed to wear the accelerometer for 2 weeks (14 consecutive days and nights). Participants were also provided with ActiGraph wgt3x-bt accelerometers (ActiGraph L.L.C., Pensacola, FL) for assessment of physical activity. ActiGraph accelerometers were instructed to be worn during all waking hours for 7 consecutive days which overlapped with sleep monitoring. ActiGraphs were analyzed using ActiLife software version 6.11.9. Wear time was validated using the Troiano algorithm [27] and activity variables were calculated using the Freedson Combination 1998 algorithm [28]. To be included in the final analysis, a minimum of 4 days with ≥10 hours of wear time were required [29]. Habitual alcohol consumption was estimated for each participant via self-reported questionnaire which asked "How many alcoholic beverages do you drink per week, on average?" with specific instructions to consider all beer, wine, and liquor beverages. Similarly, habitual caffeine consumption was estimated via self-reported responses to "How many caffeinated beverages do you drink per week, on average?" with specific instructions to consider all coffee, tea, and soft drinks.

Upon completion of the sleep monitoring period, participants returned to the lab for vascular function testing and an intravenous blood sample for clinical analyses of fasting glucose and

lipid profile. This testing visit was scheduled before 1:00 pm for all participants, with all participants arriving fasted and without caffeine, alcohol, or heavy exercise for a minimum of 12 hours prior to the visit. Participants were also instructed to avoid any anti-inflammatory drugs for a minimum of 24 hours prior to the visit. All testing was performed in a temperature-controlled environment (~23°C). All female participants had their vascular testing visit scheduled during the early follicular phase of the menstrual cycle. Sleep monitoring and vascular testing were performed during traditional (i.e. Fall or Spring) semesters when participants were enrolled in a full-time workload, and excluded any periods when students were on holiday breaks or during final examinations.

Sleep actigraphy protocol and sleep metric quantification

Participants were provided with MicroMotionlogger wrist accelerometers and were instructed to wear the monitor on the nondominant wrist for 24 hours a day for 14 consecutive days and nights, except for during water-based activities (i.e. showering). Relative to recommendations which suggest >7 nights of accelerometry data be used for evaluation of metrics such as sleep duration and regularity [30, 31], a conservative threshold of ≥12 nights of sleep accelerometry data was required for inclusion in final analyses to ensure reliability. Data were collected in zero-crossing mode and were stored in 60-second epochs. Actigraphy data were scored with ActionW-2 software (Ambulatory Monitoring, Inc., Ardsley, NY) using the University of California, San Diego scoring algorithm. Use of this algorithm has been validated to produce accurate and reliable sleep estimates relative to polysomnography [32]. Participants were also provided with a standardized sleep diary (the Consensus Sleep Diary [33]) to be used simultaneously with the accelerometer and were given detailed instructions on how and when to fill out the diary. Briefly, participants were instructed to record in the diary before bed and upon waking every day during the 14-day sleep monitoring period. Sleep diaries were primarily used as a supplementary reference during retrospective analysis.

Sleep duration, or the total time spent asleep from sleep onset to wake onset, was quantified for each night that the accelerometer was worn, and mean sleep duration across the 14-day monitoring period was determined for each participant. *Sleep duration regularity* was operationalized using the standard deviation (SD) of sleep duration, as this is one of the most commonly used methods for characterizing intraindividual sleep variability [6, 34].

Flow-mediated dilation

Brachial artery FMD was used to evaluate conduit artery function and was performed in accordance with current recommendations [35]. Participants rested supine in a dimly lit room for at least 20 minutes prior to FMD. An inflatable cuff was placed on participants' upper arm, 2–3 cm proximal to the elbow joint. Brachial artery duplex ultrasound imaging (Logiq *e*, General Electric Medial Systems, Milwaukee, WI) occurred with a linear array ultrasound probe (12 Hz) placed distally to the shoulder joint and proximal to the inflatable cuff. Participants remained supine throughout the duration of the protocol. Following 1 minute of resting baseline, the cuff was rapidly inflated to 250 mmHg for 5 minutes then rapidly deflated (E20 Rapid Cuff Inflation System, Hokanson, WA). Measurements of brachial artery diameter and blood flow velocity were collected continuously throughout baseline and for 2 minutes immediately following cuff deflation.

Imaging of the brachial artery and blood velocity was obtained using a transducer with a Doppler frequency of 5 MHz and with the probe appropriately positioned to maintain an insonation angle of 60° or less. The sample volume was maximized according to vessel size and centered within the vessel based on real-time ultrasound visualization. Blood velocity (V_{mean}) values (angle-corrected and intensity-weighted area under the curve [AUC]) were automatically calculated using commercially available software. End-diastolic electrocardiogram R-wave gated images were collected from the video output of the Logiq *e* for offline analysis of brachial artery vasodilation using automated edge-detection software (Medical Imaging Applications, Coralville, IA). FMD% was quantified as the maximal percentage in brachial artery diameter change following cuff release. Greater FMD% values indicate a more responsive endothelium and are associated with a lower risk of future CV events [36]. Shear rate was also calculated, as: shear rate = $8V_{mean}$/arterial diameter. Cumulative AUC values for shear rate were integrated with the trapezoidal rule and calculated as follows:

$$\sum \{y_i[x_{(i+1)} - x_i] + (1/2)[y_{(i+1)} - y_i][x_{(i+1)} - x_i]\},$$

where y is shear rate and x is time.

Passive leg movement

PLM was used to evaluate resistance vessel function and was performed as previously described [37–40] and in accordance with current recommendations [41]. Participants remained rested for at least 20 minutes prior to PLM testing. In the upright seated position, the PLM protocol consisted of 60 seconds of baseline measurements immediately followed by a 60-second bout of passive leg flexion and extension at the knee joint. Femoral artery duplex ultrasound imaging (Logic *e*, General Electric Medical Systems, Milwaukee, WI) was achieved using a linear array ultrasound probe (12 Hz) at the common femoral artery, distal to the inguinal crease but above the femoral bifurcation into the superficial and profound femoral branch. Passive movement was achieved by a member of the research team moving the participant's lower leg through a 90–180° range of motion at a rate of 1 Hz, while movement cadence was maintained by a metronome. Throughout the duration of the protocol, the unaffected leg remained extended and fully supported. Prior to the start and throughout the duration of the protocol, participants were encouraged not to assist with or resist the leg movement. Measurement of femoral artery diameter was assessed during baseline, while blood flow velocity was measured throughout the protocol.

Femoral artery diameter was determined at a perpendicular angle along the central axis of the scanned area. Blood velocity was obtained using a transducer with a Doppler frequency of 5 MHz with the probe appropriately positioned to maintain an insonation angle of 60° or less. The sample volume was maximized according to vessel size and centered within the vessel

based on real-time ultrasound visualization. Artery diameter and mean velocity (V_{mean}) values were measured. Second-by-second leg blood flow (LBF) in the femoral artery was calculated as blood flow = V_{mean} π (vessel diameter/2)^2 × 60, where blood flow is in milliliters per minute. LBF was calculated through off-line analysis of anterograde and retrograde blood flow velocities achieved during PLM using continuous ultrasound Doppler imaging. Baseline LBF was calculated via 12-second averages of anterograde and retrograde blood flow velocities, while second-by-second analysis of anterograde and retrograde blood flow velocities were used to determine LBF during the movement phase of PLM. Peak LBF was calculated as the maximal value achieved during the first 30 seconds of PLM. The change in LBF from baseline flow to peak flow (ΔPLM) was calculated as peak LBF – baseline LBF. Cumulative AUC for values of blood flow were also determined and interpreted to indicate the overall increase in blood volume achieved during movement. AUC was calculated as the sum of LBF above baseline for each second during the 60-second movement phase of PLM, according to the trapezoidal rule, as previously described. PLM-induced hyperemia is highly nitric oxide (NO)-dependent, with larger hyperemic responses indicating greater health of the vascular system [39, 41, 42].

Statistical analyses

Participant characteristics and sleep metrics are summarized using means and *SD*. A series of linear regression models were tested separately to examine the associations between sleep metrics with FMD% and PLM hyperemia. The first model was performed without adjustments (model 1). Our second model adjusted for plausible biological covariates, including sex, BMI, and resting BP (model 2). Our third model tested the associations between vascular function and sleep metrics after adjustment for vigorous-intensity physical activity level (minute/week), as this was the only physical activity variable that correlated with vascular metrics (ΔPLM Pearson's $r = 0.31$, $p = 0.04$; PLM AUC $r = 0.30$, $p = 0.04$; model 3). Lastly, we evaluated the association between sleep metrics and vascular function independent of habitual caffeine and alcohol consumption (model 4).

Secondarily, we aimed to examine the associations between sleep metrics and vascular function without the potential confounding impact of sleep duration (Supplementary Tables). Specifically, model A evaluated the associations between sleep duration SD and vascular metrics after adjusting for mean sleep duration, while model B evaluated the association between both sleep metrics and vascular function after adjusting for sleep duration on the night before vascular function testing, which was available in a subset of participants who completed the wrist actigraphy protocol on the morning of the vascular testing visit ($n = 40$).

All FMD regression models also adjusted for shear rate AUC, as it was moderately correlated with FMD% (Pearson's $r = 0.67$, $p < 0.01$). Regression results are presented as unstandardized *B* values and 95% confidence intervals (CIs).

Participants were also categorized as exhibiting low (<1.2 hours) or high (≥1.2 hours) sleep duration SD using the median as a cut point, as there is no valid cutoff point for sleep regularity. Independent *t*-tests (for continuous variables) and Fisher's Exact Test (for categorical variables) were used to compare mean participant characteristics and PLM metrics between groups, with results presented as mean and *SD*. Mean FMD% for low and high sleep duration SD groups were also compared after adjustment for shear rate AUC via analysis of covariance using a Bonferroni CI adjustment. Significance was set at $\alpha = 0.05$ for all tests. All analyses were performed using the Statistical Package for the Social Sciences (SPSS version 26.0, IBM, NY).

Results

Participants and sleep characteristics

A total of 51 participants (20 males, 31 females) completed the sleep monitoring protocol and subsequent vascular function testing. Mean ± *SD* values for participant characteristics are presented in Table 1. By design, participants were young, normotensive, and nonobese. Fasting lipid profile and glucose values were also within normal ranges for healthy young adults. Mean sleep metrics for all participants are also shown in Table 1. Participants obtained a mean of 7.1 ± 0.7 hours per night, which ranged from 5.2 to 8.4 hours of sleep per night. The mean sleep duration SD for all participants was 1.3 ± 0.4 hours and ranged from 0.4 to 2.1 hours of variability. In a 14-day timeframe, 39% of participants ($n = 20$) obtained 8 or more hours of sleep only once or twice, and 10% of

Table 1. Mean participant characteristics, sleep metrics, and peripheral vascular function metrics

	N = 51
Participant characteristics	
Age, years	20 ± 1
Sex, M/F	20/31
BMI, kg/m^2	23.8 ± 2.3
Systolic BP, mmHg	117 ± 9
Diastolic BP, mmHg	69 ± 7
Heart rate, bpm	58 ± 9
Blood chemistry	
Total cholesterol, mg/dL	158 ± 27
LDL, mg/dL	85 ± 23
HDL, mg/dL	58 ± 13
Triglycerides, mg/dL	72 ± 30
Fasting glucose, mg/dL	85 ± 7
Sleep metrics	
Mean sleep duration, hours/night	7.1 ± 0.7
Sleep duration SD, hours	1.3 ± 0.4
Health behaviors	
Vigorous intensity PA, minute/week	34 ± 33
Alcohol consumption, drinks/week	5.4 ± 5.6
Caffeine consumption, drinks/week	5.2 ± 5.4
FMD metrics	
Baseline brachial diameter, mm	3.82 ± 0.64
Peak brachial diameter, mm	4.03 ± 0.64
Shear rate AUC, s^{-1}	51,271 ± 19,417
FMD Δ, mm	0.21 ± 0.11
FMD, %	5.74 ± 3.18
PLM metrics	
Baseline LBF, mL/min	269 ± 107
Peak LBF, mL/min	764 ± 430
ΔLBF, mL/min	494 ± 365
LBF AUC, mL	153 ± 133

Values are presented as mean ± *SD*. LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; PA, physical activity.

participants (n = 5) never achieved an episode of sleep lasting ≥8 hours in duration. Additionally, 51% of participants (n = 26) experienced sleep episodes lasting <7 hours in duration on 7 or more nights.

Associations between free-living sleep metrics and FMD

Linear regression results for sleep metrics and FMD% are displayed in Table 2. In regression models which only adjusted for shear rate AUC, mean sleep duration was not associated with FMD% (model 1; Figure 1A). Similarly, adjusting for potential confounders in the series of regression models did not alter these results, as mean sleep duration remained unassociated with FMD% in all models tested (Table 2, models 2–4), including those which adjusted for sleep duration on the night before vascular testing (Supplementary Table 1, model B).

In regression models which only adjusted for shear rate AUC, although in the anticipated direction, sleep duration SD was not associated with FMD% (model 1; Figure 1B). Sleep duration SD remained unassociated with FMD% after adjustment for sex, BMI, BP, physical activity, alcohol, caffeine consumption, and habitual sleep duration. Conversely, adjusting for shear rate AUC and sleep duration on the night before vascular function testing revealed a significant inverse association between sleep duration SD and FMD% (–2.32%, 95% CI: –4.47 to –0.16, p = 0.04; Supplementary Table 1, model B).

Associations between free-living sleep metrics and PLM

PLM data were available for 49 out of 51 participants. Linear regression results for sleep metrics and PLM responses are displayed in Table 3. Mean sleep duration was not associated with PLM hyperemia in unadjusted or adjusted models. Conversely, prior to adjustment, sleep duration SD was significantly associated with all three variables of PLM hyperemia (model 1; Figure 2D–F). Specifically, every 1-hour increase in sleep duration SD was associated with a 401 mL/min lower peak LBF (95% CI: –710 to –92, p = 0.01), a 357 mL/min lower ΔLBF (95% CI: –618 to –97, p < 0.01), and a 129 mL lower LBF AUC (95% CI: –224 to –33, p < 0.01). Similarly, multivariate regression models revealed that sleep duration SD remained inversely associated with all three measures of PLM hyperemia after adjustment for sex, BMI, and resting BP (model 2),

Table 2. Associations between sleep metrics and brachial artery FMD%

	Mean sleep duration, hours			Sleep duration SD, hours		
Dependent variable	*B* (95% CI)	β	*p*	*B* (95% CI)	β	*p*
Model 1						
FMD, %	0.11 (–0.88 to 1.11)	0.03	0.82	–1.51 (–3.32 to 0.30)	–0.19	0.10
Model 2						
FMD, %	0.52 (–0.52 to 1.56)	0.11	0.32	–1.26 (–3.18 to 0.65)	–0.16	0.19
Model 3						
FMD, %	0.04 (–0.94 to 1.01)	0.01	0.94	–1.19 (–3.01 to 0.63)	–0.15	0.20
Model 4						
FMD, %	0.17 (–0.68 to 1.20)	0.04	0.74	–1.54 (–3.48 to 0.40)	–0.19	0.12

Model 1 adjusts for shear rate AUC; model 2 adjusts for shear rate AUC, sex, BMI, and resting blood pressure; model 3 adjusts for shear rate AUC and vigorous intensity physical activity; model 4 adjusts for habitual caffeine and alcohol consumption. *B*, unstandardized coefficient; β, standardized coefficient.

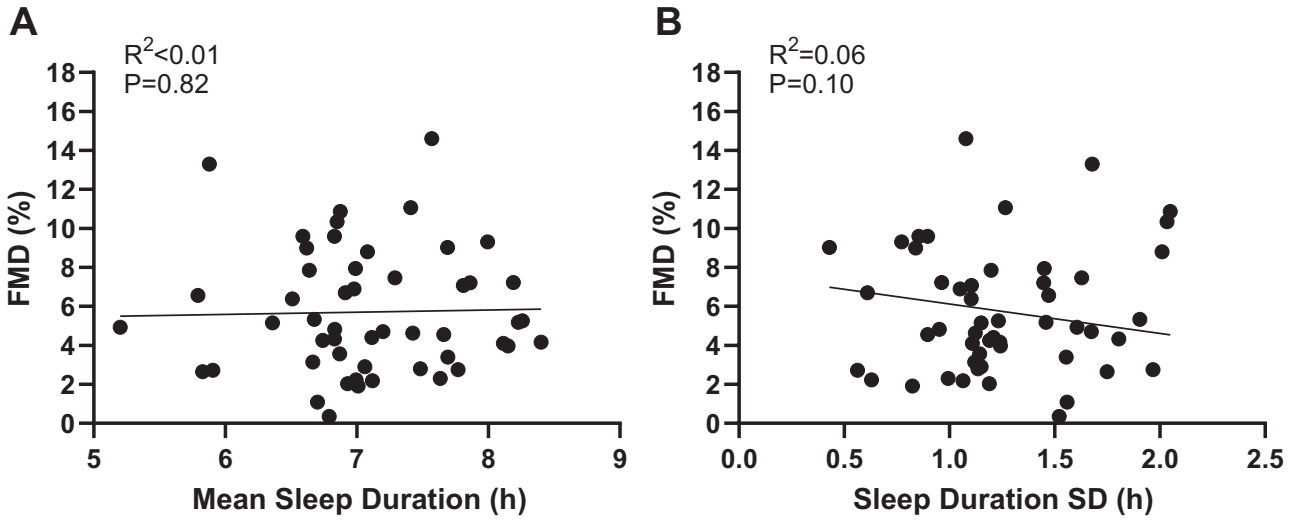

Figure 1. Scatterplots of mean sleep duration (A) and sleep duration SD (B) with brachial artery FMD%. Regression lines adjust for shear rate AUC (fixed at the mean value for illustration). R^2 values shown represent partial R^2.

Table 3. Associations between sleep metrics and PLM hyperemia

	Mean sleep duration, hours			Sleep duration SD, hours		
Dependent variable	*B* (95% CI)	β	*p*	*B* (95% CI)	β	*p*
Model 1						
Peak LBF, mL/min	−109 (−283 to 66)	−0.18	0.22	−401 (−710 to −92)	−0.36	**0.01**
ΔLBF, mL/min	−101 (−249 to 46)	−0.20	0.17	−357 (−618 to −97)	−0.37	**<0.01**
LBF AUC, mL/min	−10 (−65 to 44)	−0.06	0.70	−129 (−224 to −33)	−0.37	**<0.01**
Model 2						
Peak LBF, mL/min	−65 (249 to 120)	−0.11	0.48	−494 (−807 to −181)	−0.44	**<0.01**
ΔLBF, mL/min	−79 (−237 to 80)	−0.15	0.32	−409 (−682 to −135)	−0.43	**<0.01**
LBF AUC, mL/min	16 (−41 to 72)	0.08	0.58	−141 (−238 to −44)	−0.40	**<0.01**
Model 3						
Peak LBF, mL/min	−130 (−291 to 30)	−0.23	0.11	−333 (−631 to −35)	−0.31	**0.03**
ΔLBF, mL/min	−122 (−256 to 12)	−0.26	0.07	−300 (−548 to −52)	−0.33	**0.02**
LBF AUC, mL/min	−16 (−69 to 37)	−0.09	0.55	−109 (−204 to −14)	−0.32	**0.03**
Model 4						
Peak LBF, mL/min	−149 (−319 to 22)	−0.25	0.09	−424 (−729 to −120)	−0.38	**<0.01**
ΔLBF, mL/min	−126 (274 to 23)	−0.25	0.10	−397 (−643 to −116)	−0.40	**<0.01**
LBF AUC, mL/min	−14 (−70 to 43)	−0.07	0.63	−133 (−232 to −34)	−0.38	**0.01**

Model 1, unadjusted; model 2 adjusts for sex, BMI, and resting blood pressure; model 3 adjusts for vigorous intensity physical activity; model 4 adjusts for habitual caffeine and alcohol consumption. *p* values in bold font indicate statistical significance. *B*, unstandardized coefficient; β, standardized coefficient.

physical activity (model 3), alcohol and caffeine consumption (model 4), and mean sleep duration (Supplementary Table 2, model A). In models which adjusted for sleep duration prior to vascular function testing, associations between sleep duration SD and PLM hyperemia were attenuated; specifically, associations with peak LBF and ΔLBF were rendered nonsignificant ($p = 0.07$ and $p = 0.06$, respectively), while the inverse association with LBF AUC remained ($p = 0.03$; Supplementary Table 2, model B).

Sleep duration variability: subgroup analysis

To further explore the relation between sleep duration SD and peripheral vascular function, participants were categorized as exhibiting low (<1.2 hours; $n = 25$) or high (≥1.2 hours; $n = 26$) sleep duration SD using a median split. Group characteristics for low and high sleep duration SD groups are displayed in Table 4. Those demonstrating high sleep duration SD had a higher resting heart rate (HR) (61 ± 10 vs. 55 ± 7 bpm, $p = 0.01$) and lower high-density lipoprotein (HDL) cholesterol (54 ± 10 vs. 62 ± 14 mg/dL, $p = 0.04$) when compared with those with low sleep duration SD. Otherwise, groups were similar with regards to age, sex, BMI, resting BP, blood chemistry, physical activity, mean sleep duration, alcohol consumption, caffeine consumption, and femoral artery diameter (all $p > 0.05$).

Mean FMD% responses were not different for low and high sleep duration SD groups (5.70 ± 3.19% vs. 5.78 ± 3.22%, respectively, $p = 0.93$). Similarly, no significant differences were apparent after adjusting mean FMD% values for shear rate AUC (low sleep duration SD: 6.14 ± 3.24, high sleep duration SD: 5.31 ± 3.2%, $p = 0.24$).

Mean second-by-second blood flow responses during PLM for low and high sleep duration SD groups are illustrated in Figure 3A. PLM evoked a hyperemic response in both low and high sleep duration SD groups, however those exhibiting high sleep duration SD exhibited a significantly lower peak LBF (624 ± 336 vs. 910 ± 474 mL/min, $p = 0.02$; Figure 3B), ΔLBF (363 ± 281 vs. 631 ± 396 mL/min, $p < 0.01$; Figure 3C), and LBF AUC during PLM (112 ± 113 vs. 196 ± 140 mL, $p = 0.03$; Figure 3D) when compared with those exhibiting low sleep duration SD.

Discussion

In this cross-sectional, observational study of apparently healthy undergraduate college students, we identified that free-living sleep duration SD was consistently inversely associated with hyperemic responses to PLM, indicating less optimal peripheral microvascular function in those exhibiting more irregular night-to-night sleep durations. Additionally, when participants were categorized as exhibiting either low or high sleep duration SD using a median split, it was evident that those with more irregular sleep durations displayed a significantly attenuated response to PLM when compared with those exhibiting more regular sleep durations. Conversely, mean sleep duration was not associated with either measure of peripheral vascular function in this sample. Considering microvascular dysfunction precedes clinical manifestations of CVD [43, 44], our findings suggest that encouraging consistent night-to-night sleep durations should be considered as a novel strategy for primary prevention of CVD.

Sleep regularity is independently associated with microvascular function in healthy college students

In agreement with our hypothesis, free-living sleep duration SD was significantly inversely associated with peripheral microvascular function as assessed by PLM in a group of 51 full-time undergraduate college students. Our findings that more irregular sleep durations were associated with less robust PLM responses are notable for several reasons. First, this study was designed to include only young adults who are apparently healthy and would be assumed to have optimal or near-optimal vascular function. Thus, to be able to identify a novel, and moreover, a modifiable behavior which accounts for a significant portion of the variance in microvascular function in this sample is

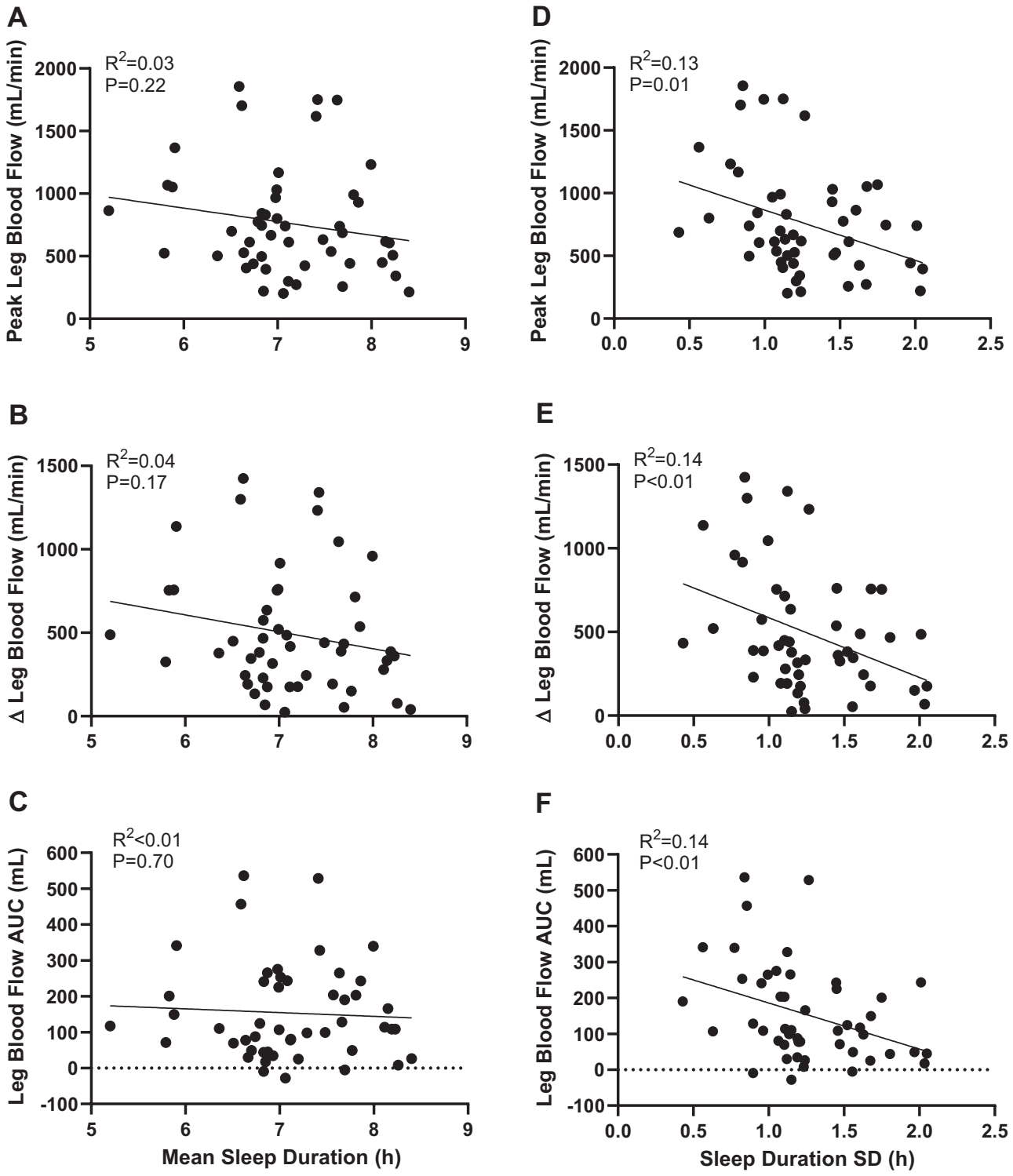

Figure 2. Scatterplots of mean sleep duration (A–C) and sleep duration SD (D–F) with PLM variables.

physiologically relevant. Second, when examining the extent of the association between sleep duration SD and microvascular function, sleep duration SD consistently performed comparably, or even outperformed several potential confounding factors that have established associations with biomarkers of vascular dysfunction (e.g. BMI [45], BP [46], physical activity [47], alcohol consumption [48], caffeine consumption [49], and habitual sleep duration [50]). We also evaluated these associations after adjustment for resting HR, which was higher, and HDL cholesterol, which was lower in those demonstrating high sleep duration SD; again, neither covariate weakened the association between sleep duration SD and PLM hyperemia (data not shown). Lastly, a healthy vascular system tightly controls vascular tone in response to various stimuli, including passive movement of a limb

Table 4. Comparisons of group means for "low" and "high" sleep duration SD groups

	Low sleep duration SD <1.2 hours (N = 25)	High sleep duration SD ≥1.2 hours (N = 26)	
	Mean ± SD	Mean ± SD	p
Participant characteristics			
Age, year	20.5 ± 1.5	20.4 ±1.3	0.10
Sex, M/F	9/16	11/14	0.78
BMI, kg/m^2	23.3 ± 2.1	24.3 ± 2.5	0.13
Systolic BP, mmHg	116 ± 9	118 ± 9	0.30
Diastolic BP, mmHg	68 ± 5	70 ± 8	0.24
Heart rate, bpm	55 ± 7	61 ± 10	**0.01**
Blood chemistry			
Total cholesterol, mg/dL	157 ± 26	160 ± 27	0.69
LDL, mg/dL	80 ± 19	89 ± 25	0.16
HDL, mg/dL	62 ± 14	54 ± 10	**0.04**
Triglycerides, mg/dL	67 ± 27	76 ± 33	0.28
Fasting glucose, mg/dL	84 ± 7	86 ± 7	0.34
Sleep metrics			
Mean sleep duration, hours/night	7.2 ± 0.6	7.0 ± 0.8	0.59
Sleep duration SD, hours	0.9 ± 0.2	1.6 ± 0.3	**<0.01**
Health behaviors			
Vigorous intensity PA, minute/week	39 ± 35	28 ± 29	0.22
Alcohol consumption, drinks/week	3.9 ± 3.8	6.9 ± 6.8	0.06
Caffeine consumption, drinks/week	6.1 ± 6.8	4.3 ± 3.6	0.21
FMD metrics			
Baseline brachial diameter, mm	3.8 ± 0.7	3.8 ± 0.6	0.79
Peak brachial diameter, mm	4.0 ± 0.7	4.1 ± 0.6	0.79
Shear rate AUC, s^{-1}	46,936 ± 16,213	55,271 ± 21,504	0.13
FMD Δ, mm	0.21 ± 0.12	0.21 ± 0.11	0.97
FMD, %	5.70 ± 3.19	5.78 ± 3.22	0.92
PLM metrics	N = 24	N = 25	
Femoral artery diameter	0.84 ± 0.12	0.82 ± 0.10	0.56
Baseline LBF, mL/min	278 ± 126	260 ± 85	0.55
Peak LBF, mL/min	910 ± 474	624 ± 336	**0.02**
ΔLBF, mL/min	631 ± 396	363 ± 281	**0.01**
LBF AUC, mL	192 ± 141	112 ± 113	**0.03**

Values are presented as mean ± SD. *p* values in bold font indicate statistical significance. LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; PA, physical activity

[51], as even one single PLM is sensitive enough to distinguish differences in NO-mediated vascular function [52–54]. Previous studies have also identified significantly lower hyperemic responses to PLM in populations that are known to demonstrate reduced NO bioavailability, such as typical aging [37, 54, 55], sedentary lifestyle [38], patients with CVDs [40, 42, 56], and patients with chronic kidney disease [57]. Moreover, in young adults, pharmacological NO blockade reduces the LBF AUC response to 1 minute of continuous PLM by up to ~80% [39, 42], suggesting that it is highly NO mediated in this population. Taken together, our observation that every 1-hour increase in sleep duration SD was independently associated with a ~100–140 mL lower LBF AUC, and that those demonstrating high sleep duration SD exhibited a ~45% lower LBF AUC than those with low sleep duration SD (Figure 3D), are both statistically and physiologically significant.

Three out of four students in this study demonstrated sleep duration SD values >1, indicating that the majority of participants experienced night-to-night sleep durations which varied by more than an hour from their mean value. Additionally, over half of participants experienced insufficient sleep (i.e. <7 hours) on ≥50% of nights during the sleep monitoring period, while more than one-third of participants only obtained "recovery" sleep (i.e. ≥8 hours) once or twice during that same timeframe. Collectively, these data support the notion that irregular sleep schedules are common in this population, and that episodes of short sleep are more frequent than "recovery" attempts. The association between sleep regularity and vascular function has not been previously examined in free-living contexts. However, our findings are in support of those by Sauvet et al., who evaluated the cutaneous microvascular responses to an experimental model of variations in sleep duration and identified microvascular impairments during repeated sleep restriction (4 hour/night) which persisted even after a night of recovery sleep (8 hours) was obtained [18]. It is possible that microvascular dysfunction which is incurred with shortened sleep episodes might require multiple nights of consistent, adequate sleep to fully recover, however this may not be achieved in those whose sleep patterns consist of frequent fluctuations in night-to-night sleep duration.

Sleep regularity is unrelated to macrovascular function in healthy college students

In the current study, there were no consistent associations apparent between sleep duration SD and brachial artery FMD in undergraduate college students. Although this is contrary to both our hypothesis and our PLM findings, there are several possible explanations for this. These assessments are evaluating peripheral vascular function in different, distinct parts of the vascular tree, as PLM-induced hyperemia is largely a result of downstream resistance vessel vasodilation, while FMD is a direct assessment of conduit artery vasodilation. Increasing evidence suggests that microvascular dysfunction precedes and predicts the development of conduit artery atherosclerosis and its associated risk factors in apparently healthy adults [44, 58, 59]. Therefore, it is plausible that microvascular dysfunction is more apparent than overt conduit artery dysfunction in the setting of poor sleep health in a young and healthy cohort. This distinction is critical as it suggests we may be identifying some of the earliest indications of the vascular risk that are associated with highly variable sleep patterns, before conduit artery or traditional CVD risk factor implications are apparent. Additionally, FMD and PLM evaluate the vascular function of anatomically different vascular beds (i.e. upper extremity vs. lower extremity, respectively). Vascular beds of different extremities are subject to differing hemodynamic forces and are known to express specific inherent characteristics [60, 61]; therefore, endothelial cells at these sites are likely to express distinct vascular phenotypes [62]. Finally, although FMD and PLM are, at least in part,

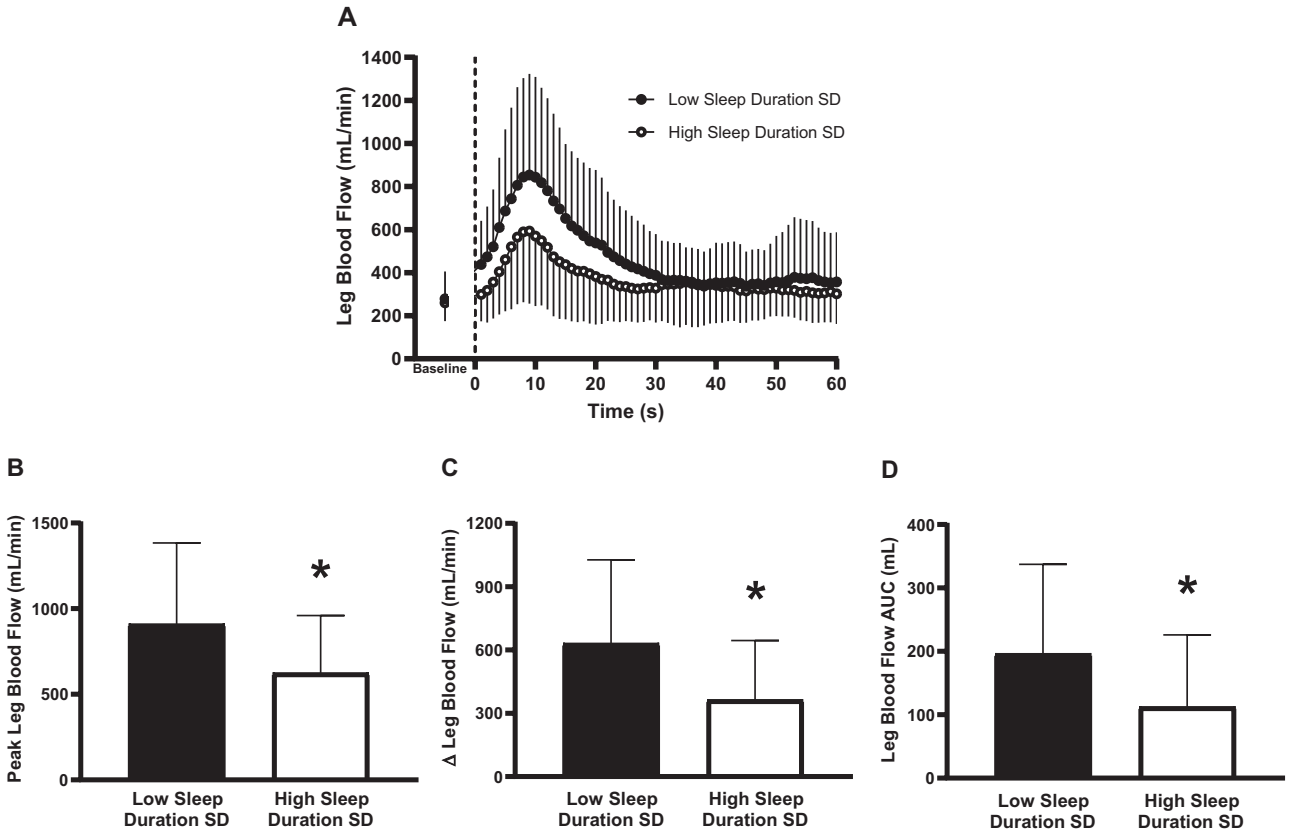

Figure 3. Blood flow responses during PLM for low (n = 24) and high (n = 25) sleep duration SD groups. Second-by-second LBF responses for each group are depicted in panel (A). Mean peak LBF (B), change in LBF from baseline to peak (C) and LBF AUC (D) are significantly attenuated in those who exhibit more irregular sleep durations. $^{*}p < 0.05$.

mediated by NO, they both rely on different combinations vasodilators and vasoconstrictors [63–65]. For example, the exact magnitude of the NO contribution varies between assessments, with recent FMD studies ranging from 24% to 33% [66, 67] and PLM up to ~80% [39, 42]. Therefore, the specific contribution of these vasoactive components may also be impacting the contradicting associations.

Mean sleep duration is not associated with vascular function in healthy college students

Contrary to our hypothesis, we did not identify any significant associations between mean sleep duration and peripheral vascular function. This contrasts findings from prior experimental studies, which have evaluated the vascular responses to repeated sleep restriction in similar populations. For example, Calvin et al. identified a significant impairment in FMD% following eight nights of sleep restriction (5.1 hour/night) in a small sample of healthy young adults when compared with controls who achieved 6.9 hours of sleep per night [17]. As previously described, Sauvet et al. also reported significant impairments in cutaneous microvascular function after six nights of sleep restriction (4 hour/night) in healthy young men [18]. Our contradictory findings could be, at least in part, due to the methodology employed in experimental studies, where participants who habitually sleep ~7–8 hour/night are typically subjected to sleep restriction protocols consisting of ~4–5 hours of sleep per night for several consecutive nights. This model of short sleep differs from free-living sleep habits in at least two distinct ways. First, this approach induces rapid sleep loss which is amplified when compared with short sleep durations reported in free-living contexts, as habitual sleep durations as short as ≤5 hours per night are only seen in ~10% of adults [68]. Comparably, in the current study, extreme sleep durations were uncommon as evidenced by a mean sleep duration of 7.1 ± 0.7 hour/night, which meets sleep duration recommendations for young adults [5]. Second, the duration of experimental sleep restriction studies may be far shorter than that of real-world sleep habits, which can persist for months or years outside of the laboratory [69, 70].

The chronicity of free-living sleep patterns may also provide insight on the significant associations identified between irregular sleep duration and microvascular function in this study. Large, persistent fluctuations in nightly sleep duration likely evokes some degree of circadian disruption due to chronic variation in night-to-night exposure to entraining sources (i.e. circadian synchronizing signals) such as light signals, food consumption, and energy expenditure [71]. In line with this, endothelial function is largely under circadian control, whereby circadian disruption evokes a dysfunctional endothelium, pathological vascular remodeling, and thrombosis [23, 24]. Collectively, our findings suggest that circadian mechanisms, rather than habitual sleep quantity, may be more pertinent for vascular homeostasis in free-living contexts, at least in apparently healthy young adults.

Comparison to prior studies

Our findings parallel several prior studies which suggest that irregular night-to-night sleep duration may contribute to excess CVD risk. For example, Ogilvie et al. previously reported that greater variability in sleep duration was independently associated with greater obesity in older adults [10] while Huang et al. found that every 1-hour increase in sleep duration SD was associated with almost double the odds of multiple metabolic abnormalities in a similar demographic [11]. More recently, Huang et al. also reported that in a group of older adults free of known CVDs at baseline, those with the most irregular sleep durations had greater than twofold increased odds of developing CVD over ~5 years of follow-up compared with those with the most consistent sleep durations [13]. The development of atherosclerosis is a gradual process which begins in childhood [72, 73], whereby vascular dysfunction, and particularly microvascular dysfunction [44], is one of the earliest manifestations of this process and is apparent prior to any morphological changes to the vasculature [19]. Vascular dysfunction has an established role in the development of CVDs [19, 73], is predictive of future cardiovascular events [44, 74], and is implicated in the pathophysiology of metabolic conditions including insulin resistance, obesity, and diabetes [75]. Taken together, our findings emphasize the possibility that the microvascular impairments identified in young adults with irregular sleep schedules may represent not only a biomarker, but also a potential contributor to the development of cardiometabolic diseases which could have cumulative implications if irregular sleep patterns are sustained into older adulthood.

Experimental considerations

There are several aspects of our study design that should be considered when interpreting these findings. Although many experimental studies have demonstrated that sleep disruption evokes vascular dysfunction [16–18], the current study design is cross-sectional and observational, therefore we cannot assume any directionality of the association identified between sleep regularity and microvascular function. Additionally, identifying the precise mechanisms responsible for vascular dysfunction in the context of irregular sleep duration is outside the scope of this study design, therefore future studies are needed to interrogate the underlying mechanisms for these observations. Although measures of peripheral vascular function are heavily relied upon in cardiovascular research [21, 76], they have not yet been adopted in clinical settings, and no distinct cut points or normative values currently exist for FMD or PLM. However, FMD and PLM values in the present study are comparable to those previously reported in similar populations [37, 55, 77, 78]. By design, our study only included young, healthy adults within a very narrow age range. Although this may limit the generalizability of our findings, this was intentional as it also effectively limited the impact of possible confounders. Nevertheless, future studies, which evaluate these associations in diverse populations, are also needed. Although screening for sleep disorders was specifically incorporated in our review of medical history, we did not use additional validated methods to confirm that sleep disorders were not present, potentially confounding our findings. We were not powered to evaluate sex-specific relations between sleep metrics and peripheral vascular function in this study. Similarly, due to sample size limitations, we were unable to include all potential covariates in one regression model. However, the associations across regression models in this study were highly consistent and are in agreement with previous findings on sleep duration SD and cardiovascular health in other populations [10–13, 79], supporting the validity of our results. While patterns of sleep duration were the focus of this study, several other metrics which represent irregular sleep patterns have also emerged [11, 13, 79–81] and should be evaluated for their role in preclinical CVDs in future investigations. Finally, our methodology for evaluating sleep was very strong, as we objectively estimated sleep metrics over 14 consecutive days and nights using wrist actigraphy, which has been validated for its ability to estimate sleep parameters over consecutive nights [82] and has high agreement with polysomnography measures [32, 83]. Participants also used standardized nightly sleep diaries in conjunction with wrist actigraphy as an additional confirmatory measure of sleep parameters, to further ensure precise sleep evaluation per recommendations [33, 84].

Conclusion

Irregularity in night-to-night sleep duration, rather than mean sleep duration, was associated with decreased peripheral microvascular function, and this could not be explained by traditional CVD risk factors. Thus, this study provides novel insight on the cardiovascular implications of nightly fluctuations in sleep duration that are commonly experienced in young adulthood, and particularly in college students. Additionally, our findings could provide a possible explanation for previous studies which may have been unsuccessful in identifying associations between sleep quantity and cardiovascular indices when relying on habitual sleep duration assessments alone, as this overlooks any fluctuations in night-to-night sleep duration which may occur. Sleep regularity is a modifiable behavior, therefore promoting consistent, adequate sleep may provide a novel strategy for maintaining ideal cardiovascular health and mitigating the risk of CVD that increases with age.

Supplementary material

Supplementary material is available at *SLEEP* online.

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