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ORIGINAL RESEARCH

Sleep Variability, Eating Timing Variability, and Carotid Intima-Media Thickness in Early Adulthood

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BACKGROUND: Day-to-day variability in sleep patterns and eating timing may disrupt circadian rhythms and has been linked with various adverse cardiometabolic outcomes. However, the extent to which variability in sleep patterns and eating timing relate to atherosclerotic development in subclinical stages remains unclear.

METHODS AND RESULTS: Generally healthy adults (N=62, 29.3±7.3 years, 66% female) completed 14 days of sleep and dietary assessments via wrist accelerometry and photo-assisted diet records, respectively. Variability in sleep duration, sleep onset, eating onset (time of first caloric consumption), eating offset (time of last caloric consumption), and caloric midpoint (time at which 50% of total daily calories are consumed) were operationalized as the SD across 14 days for each variable. Separate regression models evaluated the cross-sectional associations between sleep and eating variability metrics with end-diastolic carotid intima-media thickness (CIMT) measured via ultrasonography. Models adjusted for age, sex, systolic blood pressure, sleep duration, and total energy intake. Each 60-minute increase in sleep duration SD and sleep onset SD were associated with a 0.049±0.016 mm (P=0.003) and 0.048±0.017 mm (P=0.007) greater CIMT, respectively. Variability in eating onset and offset were not associated with CIMT; however, each 60-minute increase in caloric midpoint SD was associated with a 0.033±0.015 mm greater CIMT (P=0.029). Exploratory post hoc analyses suggested that sleep duration SD and sleep onset SD were stronger correlates of CIMT than caloric midpoint SD.

CONCLUSIONS: Variability in sleep patterns and eating timing are positively associated with clinically relevant increases in CIMT, a biomarker of subclinical atherosclerosis, in early adulthood.

Key Words: actigraphy ■ circadian misalignment ■ diet record ■ sleep health ■ subclinical atherosclerosis

Atherosclerotic cardiovascular disease (CVD) remains the leading cause of death in the United States and worldwide.[1,2] Although clinical manifestations of overt CVD do not typically appear until late adulthood,[1] vascular aging begins as early as youth and progresses asymptomatically for several decades, secondary to the accumulation of clinical and behavioral risk factors.[3–5] Importantly, lifestyle habits are often established early in adulthood,[6] and even modest improvements in cardiovascular health during early-to-middle adulthood have been shown to yield long-term protection against overt CVD.[7,8] Thus, identifying novel behavioral strategies to optimize vascular health in these life stages has clear implications for mitigating the global CVD burden.[2]

The circadian timing system plays a marked role in regulating cardiovascular physiology via its established influence on ~24-hour rhythms in autonomic function,

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CLINICAL PERSPECTIVE

What Is New?

- Day-to-day variability in sleep patterns (ie, timing, duration) and eating timing has been increasingly linked with traditional cardiovascular disease risk factors and overt disease presence; however, there are limited data evaluating these associations with physiological biomarkers of atherosclerosis in subclinical stages.
- We found that metrics indicative of night-to-night variability in sleep patterns and day-to-day variability in eating timing were positively associated with carotid intima-media thickness, a surrogate marker of subclinical atherosclerosis, in nonshift working adults between the ages of 18 to 45 years.
- Exploratory analyses suggest that sleep variability metrics, most notably sleep duration SD, were stronger correlates of carotid intima-media thickness than caloric midpoint SD in this sample.

What Are the Clinical Implications?

- Intraindividual variability in sleeping and eating behaviors are pervasive in modern society and may be a contributing factor to the high rates of atherosclerotic cardiovascular disease.
- Encouraging consistency in nightly sleep patterns and daily eating timing may represent novel behavioral strategies to mitigate the development of atherosclerosis in subclinical stages, which has important implications for delaying or preventing the onset of overt cardiovascular disease later in life.
- Additional studies using larger and more diverse samples, as well as longitudinal designs, are needed to determine the extent to which variability in sleeping and eating behaviors predict progression of subclinical atherosclerosis over time.

inflammation, endocrine function, and metabolism.[9,10] Importantly, circadian rhythm disruption can occur when behavioral schedules (eg, sleeping, eating) and biological rhythms are temporally misaligned.[11] Intraindividual variability in sleep and eating patterns are considered common causes of circadian rhythm disruption in the general public and may have an adverse impact on cardiometabolic health across the lifespan.[12] In older adults, epidemiological studies have shown that each 60-minute increase in the SD of sleep onset timing and sleep duration independently predicts a ~20% to 40% higher risk of incident CVD[13] and a ~20% to 30% higher odds of metabolic syndrome.[14] In adolescents and younger adults, greater variability in sleep patterns (eg, timing,[15–17] duration[15,17–19]) and eating timing (eg, timing of caloric intake[20,21]) have been increasingly associated with a range of unfavorable cardiometabolic parameters, decades before clinical signs of CVD typically emerge. For example, in a cross-sectional assessment of 188 children, higher night-to-night variability in weeknight bedtime and sleep duration were positively associated with various anthropometric measures of adiposity, including body mass index (BMI) and waist circumference.[16] Similarly, in a sample of 115 women (mean age 33±12 years), higher day-to-day variability in evening caloric intake independently predicted significant increases in systolic and diastolic blood pressure over only 1 year of follow-up.[20] These data converge to suggest that greater consistency in sleep patterns and eating timing may represent behavioral strategies to optimize primary CVD prevention paradigms. Considering the pervasiveness of unstructured sleeping and eating schedules in today's 24-hour society,[22,23] encouraging consistency in these modifiable behaviors may enable marked reductions in CVD morbidity and mortality.

Primary outcomes of former studies examining the link between sleep variability and eating timing variability with CVD risk have largely focused on traditional risk factors (eg, adiposity,[16,17,20,21] blood pressure[14,20,24]) or overt disease presence.[13,14,24] Meanwhile, there remains a paucity of evidence on the possible link between sleep variability and eating timing variability with subclinical biomarkers of vascular aging (eg, structural vascular wall changes[25]), when preventing the progression to overt disease is still attainable. Alterations in vascular wall structure capture some of the earliest morphological evidence of subclinical atherosclerosis,[26,27] independently predict incident CVD in younger adults,[28] and improve the accuracy of CVD prediction compared with traditional risk factors alone.[29] Accordingly, elucidating the link between sleep variability and eating timing variability with biomarkers of subclinical atherosclerosis such as arterial wall thickness could enhance the clinical significance of prior research and further inform CVD prevention paradigms targeting early adulthood (eg, 18–45 years[30]).

On this basis, the aim of this study was to identify the associations between sleep variability and eating timing variability with carotid intima-media thickness (CIMT), a widely used surrogate marker for subclinical atherosclerosis,[28,29] in free-living adults ages 18 to 45 years. We hypothesized that greater variability in night-to-night sleep patterns and day-to-day eating timing would be associated with a greater CIMT.

METHODS

Study Participants

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. An overview of the study design can be found in Figure S1. Participants were recruited using various strategies including printed flyers and mass-media advertisements targeting Newark, Delaware, and surrounding regions. All participants provided written informed consent before participation. The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Participants included generally healthy men and women between the ages of 18 and 45 years. Individuals were excluded for the following: a history of any major chronic disease or condition, including cardiovascular, renal, metabolic, autoimmune, or cancerous conditions; a recent history of COVID-19 infection (<60 days) or vaccination (<14 days); diagnosis of a sleep disorder (eg, insomnia, restless leg syndrome, sleep apnea) or at high risk for a sleep disorder (Insomnia Severity Index score >14[31]; STOP-Bang [snoring, tiredness, observed apnea, blood pressure, BMI, age, neck size, gender] Questionnaire score ≥3[32]); resting blood pressure >140/90 mm Hg; BMI <18.5 or >35 kg/m^2; use of any supplements or medications for sleep; diagnosis of depression or at risk for depression (Center for Epidemiologic Studies Depression Scale score ≥16[33]); current night-shift work; use of any medications that significantly alter cardiovascular physiology (including antihypertensive and antihyperlipidemic drugs); women who reported being currently pregnant or breastfeeding; women who reported being peri- or postmenopausal; suspected alcohol use disorder (Alcohol Use Disorders Identification Test score ≥15[34]); current tobacco use (≥1 cigarette in the past month); current engagement in weight loss (eg, calorie-restricted diet) or extended fasting regimen(s); unstable body weight (>4.5 kg change[35]) over the past 3 months; or lack of regular access to a smartphone.

Wrist Accelerometry and Nightly Sleep Variability Metrics

Sleep metrics were estimated using a wrist accelerometer (Actiwatch Spectrum Plus; Philips Respironics, Inc.) worn continuously on the nondominant wrist, in conjunction with a standardized sleep diary.[36] Participants were instructed to wear accelerometers for 14 consecutive days immediately before the CIMT assessment and to remove the device only when engaging in water-based activity (eg, swimming, showering). Data were collected in 30-second epochs and processed using Philips Actiware software (version 6.1.0). Nights with >1 hour of missing data were considered invalid.[37] Rest intervals were identified following a standardized protocol that incorporates sleep diaries, activity level, and light exposure.[38] Sleep–wake scoring was based on the medium threshold setting for sleep/wake detection using the algorithm provided by the manufacturer. Actiwatch accelerometers have been validated against polysomnography, the gold standard method for sleep assessment (sensitivity >0.90, accuracy >0.80).[39,40]

Metrics of sleep health that have implications for circadian rhythmicity and have been previously linked to cardiovascular outcomes were estimated for each night of wear. Specifically, sleep timing,[41,42] operationalized as the timing of sleep onset (clock time at start of each nocturnal sleep period), and sleep duration,[43,44] defined as the time elapsed between sleep onset and wake onset (in minutes), were generated. Considering that the premise of this study was to examine the association between intraindividual (ie, night-to-night) variability in sleep patterns with CIMT, our independent variables were operationalized as the SD of each metric across the 14-day monitoring period for each participant (ie, sleep onset SD, sleep duration SD).[13,15,18]

Diet Records and Eating Timing Variability Metrics

Time-stamped, photo-assisted diet records were collected over 14 consecutive days that overlapped with sleep monitoring, immediately before the CIMT assessment. Diet records were obtained via a smartphone application (MealLogger, Wellness Foundry) and were immediately available to research staff via internet. Photo-assisted methods of dietary assessment, which use images of food intake to assist traditional dietary assessment methods (eg, written diet records), are increasingly popular for estimating eating behavior in free-living conditions.[45,46] These approaches have been shown to improve the accuracy of conventional dietary assessment methods by adding detail from images to participants' self-reports[46] and can produce accurate estimates of energy and nutrient intake when paired with sound methods that facilitate data quality and completeness.[45,47] Accordingly, research staff used various strategies to enhance completeness and accuracy of records. This included messaging with participants within the logging application or via text messages, thorough review with participants via scheduled phone calls at the end of days 1 and 8, as well as an in-person review on day 15 (ie, immediately upon completion of the monitoring period). Diet records were analyzed using Nutrition Data System for Research software (University of Minnesota, Minneapolis, MN). Diet records were 100% quality checked after entry into Nutrition Data System for Research by a registered dietitian or by research staff under the direction of a registered dietitian.

Temporal metrics of caloric intake that have implications for the regulation of circadian rhythms and have been previously linked with cardiometabolic health

outcomes were characterized from photo-assisted diet records for each day of diet monitoring. Specifically, eating onset and eating offset[48–50] were defined as the time of first and last caloric intake after awakening and before sleep, respectively. Additionally, the calorically weighted midpoint (ie, caloric midpoint)[51] was defined as the time at which 50% of each individual's total daily calories were consumed. Caloric midpoint provides an estimate of caloric distribution across the eating window, whereas eating onset and offset define the eating window.[52] Considering the premise of this study was to examine the association between intraindividual (ie, day-to-day) variability in eating timing with CIMT, our independent variables were operationalized as the SD of each metric across the monitoring period for each participant (ie, eating onset SD, eating offset SD, and caloric midpoint SD).[20,52]

CIMT Assessment

CIMT was measured noninvasively using high-resolution B-mode ultrasonography (Logiq *e*, General Electric Medial Systems, Milwaukee, WI) at the level of the common carotid artery, in accordance with current recommendations proposed by the European Society of Cardiology.[53] Briefly, longitudinal unilateral images of the right common carotid artery were acquired using a linear array ultrasound transducer (10 MHz) centered below the common carotid artery bulb, with the edge of the bulb to the left of the image. Participants rested supine in a dimly lit room for a minimum of 20 minutes before and throughout the duration of this assessment. CIMT was measured as the distance between the lumen-intima and media-adventitia interfaces on the far wall of the artery, excluding focal plaques. Measures were collected by 2 highly trained operators within 1 laboratory and according to rigorous standard operating procedures.[53] Measurements of CIMT were made at end-diastole, on R-wave gated still frames, using automated edge-detection software (Carotid Studio, Quipu, Pisa, Italy). High-quality images of arterial segments ≥10 mm in length were obtained from a minimum of 10 cardiac cycles, and measurements were averaged. All data collection and processing procedures were conducted under the supervision of study investigators with extensive experience conducting ultrasound assessments of human vasculature (E.K.H., M.A.W.) to ensure that only high-quality, reliable images were used in the current analysis.

Covariates

Age,[54] sex,[55] resting systolic blood pressure,[56,57] and sleep duration[57,58] were selected as a priori covariates. Age and sex were self-reported at screening. Brachial blood pressure was obtained immediately before CIMT assessments, in the fasted state (≥12 hours), with participants resting supine in a dimly lit room for a minimum of 20 minutes before and during assessment. Measures were obtained in triplicate and averaged. Mean sleep duration was derived from 14-day wrist accelerometry, as previously described.

Additional sociodemographic, biological, and lifestyle factors, including race and ethnicity, education, BMI, sleep onset, sleep efficiency, wake after sleep onset, eating onset, eating offset, caloric midpoint, total energy intake, diet quality, and physical activity level, were also considered as candidate covariates. These factors were selected for inclusion in final regression models on an empirical basis, that is, if they were correlated with the outcome variable at $P<0.10$. Participants were classified as a racial or ethnic minority group member (binary variable) if they reported Hispanic ethnicity or reported their race as Black, Asian, Native American, Pacific Islander, Asian Pacific American, or Alaskan Native. Highest education level, categorized as high school diploma, college degree (undergraduate/4-year), or graduate degree, was self-reported at screening. For BMI, height was assessed using a wall-mounted stadiometer, and weight using a calibrated scale, then BMI was calculated (weight in kilograms divided by height in m^2). Sleep onset, eating onset, eating offset, and caloric midpoint were obtained via 14-day wrist accelerometry or diet records, as previously described. Metrics of sleep continuity, including sleep efficiency (time between sleep onset and wake onset scored as "sleep," expressed as a percentage) and wake after sleep onset (number of minutes scored as "wake" between sleep onset and wake onset), were also obtained from 14-day wrist accelerometry. Physical activity level was operationalized as moderate-vigorous physical activity, in minutes/day, obtained via 14 days of waist-worn accelerometry (ActiGraph wGT3X+, ActiGraph, LLC) that overlapped with sleep and diet monitoring. Activity intensity was estimated using activity counts according to the Freedson Combination 1998 algorithm (ActiLife software v. 6.13.4).[59] A minimum of 10 hours of wear-time was required to be considered a valid day.[60] Total energy intake (kcal/day) and diet quality (Healthy Eating Index-2015 score[61]; scores range from 0 to 100, with higher scores reflective of higher diet quality based upon greater alignment with the *Dietary Guidelines for Americans*) were obtained from 14-day diet records.

Statistical Analysis

To characterize the sample, summary statistics were generated for all variables—mean and SD for continuous variables, and frequency and percentage for categorical variables. Pearson's correlations were used to evaluate bivariate associations between independent variables (sleep variability metrics: sleep onset SD,

sleep duration SD; eating timing variability metrics: eating onset SD, eating offset SD, caloric midpoint SD) and CIMT. Separate multivariable linear regression models were used to elucidate associations between each independent variable and CIMT after adjustment for age, sex, systolic blood pressure, and sleep duration (all selected a priori), as well as total energy intake (selected empirically; see Table S1). Exploratory post hoc analyses were conducted on metrics of sleep variability and eating timing variability found to be associated with CIMT in separate multivariable regression models. Specifically, metrics of sleep variability and eating timing variability were included together as predictors in adjusted regression models, to explore the extent to which these associations were independent of one another (ie, each variable's relative contribution to CIMT). All multivariable models were checked for normality via the Shapiro–Wilk test, homoscedasticity via the Breusch–Pagan test, multicollinearity via inspection of the variance inflation factors, and linearity by visual inspection of the residuals versus predicted values plot, to ensure that all assumptions were met. Significance was set at $P<0.05$ for all tests. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS; version 28.0, IBM, NY).

RESULTS

Participant Characteristics

A flow diagram that summarizes participant enrollment is provided in Figure S2. The analytic sample consisted of 62 adults who were aged 29.3±7.3 years on average, 66.1% female, with mean resting systolic blood pressure of 113±9 mm Hg, diastolic blood pressure of 70±7 mm Hg, and BMI of 24.4±3.1 kg/m^2. In terms of race and ethnicity, the sample was predominantly non-Hispanic White (67.7%) but also consisted of participants identifying as Asian (11.3%), Black (8.1%), Hispanic (8.1%), and multiracial (4.8%). In terms of highest educational attainment, 19.4% had a high school diploma, 38.7% had an undergraduate degree, and 41.9% had a graduate degree. All participant characteristics are summarized in Table 1 and Table S2.

An average of 13.4±1.3 nights of sleep data (range: 9–14) and 13.8±0.7 days of dietary data (range: 9–14) were obtained per participant. Participants had an average sleep onset of 23:31±1.1 hours and an average sleep duration of 450.5±40.6 minutes per night. In terms of sleep variability, average sleep onset SD was 56.2±27.4 minutes and average sleep duration SD was 66.2±28.0 minutes. The average timing of eating onset and eating offset were 08:44±1.4 and 20:24±1.1 hours, respectively, and average caloric midpoint was 15:14±1.3 hours. In terms of eating timing variability, participants exhibited an average eating onset SD of 76.7±38.3 minutes, eating offset SD of 83.2±34.4 minutes, and caloric midpoint SD of 152.7±37.5 minutes.

Table 1. Participant Characteristics (n=62)

	Mean±SD or n (%)
General characteristics	
Age, y	29.3±7.3
Sex, female	41 (66.1)
Racial or ethnic minority group, yes	20 (32.3)
Highest education level	
High school diploma	12 (19.4)
College degree (undergraduate/4-year)	24 (38.7)
Graduate degree	26 (41.9)
Insomnia Severity Index, score	3.0±2.4
STOP-Bang	
Low probability of OSA (0–2)	62 (100)
Moderate probability of OSA (3, 4)	0 (0)
High probability of OSA (5–8)	0 (0)
Body mass index, kg/m^2	24.3±3.0
Systolic blood pressure, mm Hg	113±9
Diastolic blood pressure, mm Hg	70±7
Carotid intima-media thickness, mm	0.55±0.07
Moderate-vigorous physical activity, min/day	57.0±30.7
Actigraphy-derived sleep metrics	
Sleep onset, clock hour	23:31±1.1
Sleep duration, min/night	450.5±40.6
Sleep efficiency, %	92.2±2.5%
Wake after sleep onset, min	35.2±12.3
Sleep onset SD, min	56.2±27.4
Sleep duration SD, min	66.2±28.0
Eating metrics	
Total energy intake, kcal/day	2240±755
Diet quality, HEI score*	66.9±11.7
Eating onset, clock hour	08:44±1.4
Eating offset, clock hour	20:24±1.1
Caloric midpoint, clock hour	15:14±1.3
Eating onset SD, min	76.7±38.3
Eating offset SD, min	83.2±34.4
Caloric midpoint SD, min	152.7±37.5

HEI indicates Healthy Eating Index; and OSA, obstructive sleep apnea.
*HEI scores range from 0 to 100.

Associations Between Sleep and Eating Variability Metrics With CIMT

The association between sleep onset SD and CIMT was not significant at the bivariate level ($r=0.20$, $P=0.13$; Figure 1A); however, after adjustment for covariates, a significant positive association between sleep onset SD and CIMT emerged. Specifically, each 60-minute increase in sleep onset SD was associated

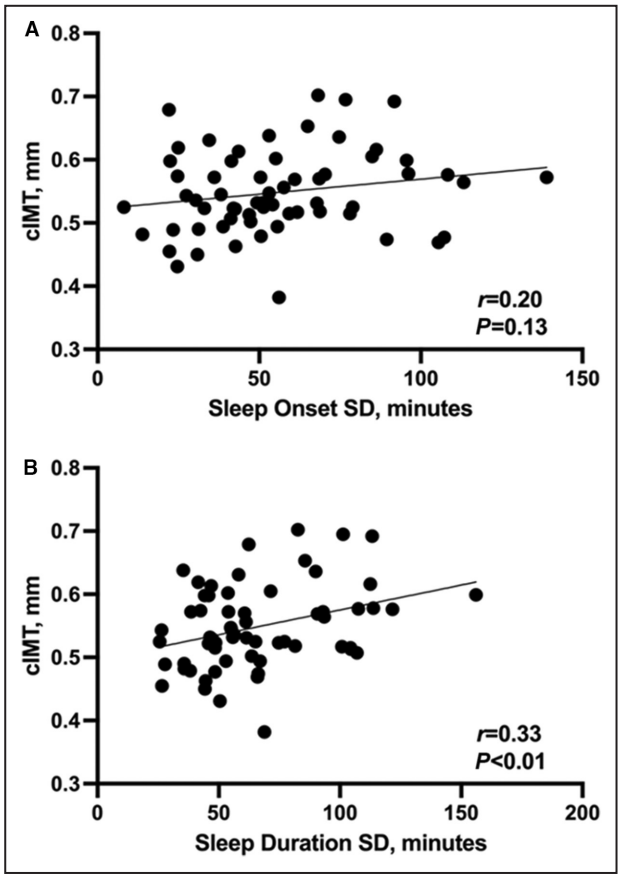

Figure 1. Scatterplots illustrating the bivariate associations between sleep variability metrics of sleep onset SD (A) and sleep duration SD (B) with CIMT.
CIMT indicates carotid intima-media thickness.

with a 0.048±0.017 mm greater CIMT value (P<0.01; Table 2). Additionally, there was a significant positive correlation between sleep duration SD and CIMT (r=0.33, P<0.01; Figure 1B). This association remained after adjustment for covariates, such that each 60-minute increase in sleep duration SD was associated with a 0.049±0.006 mm greater CIMT value (P<0.01; Table 3).

There were no significant correlations between eating onset SD (r=0.08, P=0.52; Figure 2A) or eating offset SD (r=0.08, P=0.54; Figure 2B) with CIMT at the bivariate level. Similarly, eating onset SD and eating offset SD were not associated with CIMT after adjustment for covariates (eating onset SD: B=0.015±0.013; P=0.22; eating offset SD: B=0.012±0.015; P=0.41). Alternatively, there was a significant positive correlation between caloric midpoint SD with CIMT (r=0.31, P=0.01; Figure 2C), which remained significant after adjusting for covariates, such that each 60-minute increase in caloric midpoint SD was associated with a 0.033±0.015 mm greater CIMT value (P=0.03; Table 4).

Exploratory Post Hoc Multivariable Regression Models of CIMT

Given the significant associations identified between sleep onset SD, sleep duration SD, and caloric midpoint SD with CIMT in separate regression models, these metrics of sleep variability and eating timing variability were included together in exploratory models of CIMT. In an adjusted model that included both sleep onset SD and caloric midpoint SD, associations between both metrics with CIMT were attenuated, with sleep onset SD trending toward significance (B=0.044±0.016; P=0.05), whereas caloric midpoint SD was not significant (B=0.018±0.016; P=0.26) (Table 5). In an adjusted model that included both sleep duration SD and caloric midpoint SD, the association between sleep duration SD and CIMT remained significant (B=0.044±0.016; P<0.01), whereas the association between caloric midpoint SD did not (B=0.025±0.014; P=0.08; Table 6).

DISCUSSION

The goal of this study was to characterize the associations between sleep variability and eating timing variability with CIMT in early adulthood. These associations are important given that intraindividual variability in sleeping and eating patterns are exceedingly common in today's 24-hour society.[22,23,62] Herein, we show that higher night-to-night variability in sleep onset timing and sleep duration are positively associated with higher CIMT values. We also demonstrate a novel association between day-to-day variability in eating timing and vascular health, such that those who concentrate their caloric intake at varying clock times each day tend to exhibit higher CIMT values than those who concentrate their caloric intake around a more consistent time each day. Collectively, these data extend a growing body of evidence that variability in sleeping patterns and eating timing are linked with clinically relevant indices of CVD risk and that this link is apparent decades before typical manifestation of overt disease.

Prior studies examining the association between irregular sleep patterns and CVD risk have identified a positive signal at both early and advanced stages of CVD development; however, direct physiological evidence at subclinical stages remains scarce. At the advanced stage, Huang et al used data from the Multi-Ethnic Study of Atherosclerosis to demonstrate the positive association between sleep variability and incident CVD in older adults.[13] Analyses indicated that each 60-minute increase in sleep duration SD was independently associated with a 39% increased risk of incident CVD, whereas each 60-minute increase in sleep onset SD was independently associated with an 18% increased risk of incident CVD, over a median

Table 2. Multivariable Regression Model Testing the Association Between Sleep Onset SD and CIMT (n=62)

	Independent variable: CIMT, mm				
	Unstandardized B±SE	Standardized beta	*t*	95% CI	*P* value
Intercept	0.261±0.147		1.781	−0.033 to 0.555	0.080
Age, per y	0.004±0.001*	0.388*	3.224*	0.001 to 0.006*	0.002*
Sex (ref male)	0.019±0.020	0.138	0.979	−0.020 to 0.059	0.332
Systolic blood pressure, per mm Hg	0.001±0.001	0.191	1.563	−0.041 to 0.335†	0.124
Sleep duration, per 60-min	−0.001±0.020	−0.009	−0.076	−0.041 to 0.038†	0.940
Energy intake, per 100-kcal	−0.002±0.001	−0.173	−1.277	−0.004 to 0.001†	0.207
Sleep onset SD, per 60-min	0.048±0.017*	0.331*	2.798*	0.014 to 0.083*	0.007*

CIMT indicates carotid intima-media thickness.
*Values are statistically significant.
†Values are scaled upwards by a factor of 10^2 to aid in data visualization.

follow-up of 4.9 years. Several studies now show that irregular sleep patterns are also linked with a range of CVD risk factors in life stages that coincide with early atherosclerotic development. For example, irregular sleep patterns have been observationally associated with increased adiposity,[16,17] inflammation,[15,63,64] elevated blood pressure,[65] and autonomic dysfunction,[19] in samples consisting of mostly adolescents and young adults. Our findings extend this evidence by illustrating that irregular sleep patterns are in fact associated with an in vivo surrogate for subclinical atherosclerosis in early adulthood—a life stage when prevention of progression to overt CVD may still be readily attainable.

The current study also complements the limited evidence that sleep variability is associated with biomarkers of vascular health, which are often recognized as precursors to overt CVD.[66] We previously identified an inverse association between actigraphy-estimated sleep duration SD, obtained over 14 consecutive nights, and passive leg movement-induced hyperemia, an assessment of microvascular function,[67] in 50 apparently healthy college students.[18] As with the current study, our prior analysis revealed that the association between higher sleep variability and poorer vascular health was not confounded by traditional CVD risk factors or other health behaviors, such as resting blood pressure, physical activity level, average sleep duration, or BMI. Another small study in 31 college students recently demonstrated that higher sleep timing variability, operationalized as the SD in sleep midpoint, was inversely associated with macrovascular function as measured by brachial artery flow-mediated dilation.[68] A notable drawback of these previous studies is the vascular assessments (ie, passive leg movement and flow-mediated dilation), which are used predominantly in cardiovascular research rather than clinical practice, despite having implications for future vascular risk.[69,70] The current study bolsters prior findings by demonstrating that the relationship between sleep variability and vascular health is also apparent when using CIMT, a clinical diagnostic tool that is commonly used to evaluate vascular aging.[26] In terms of magnitude, we found that each 60-minute increase in sleep duration SD and sleep onset SD was associated with ~0.05 mm higher CIMT, approaching the SD for CIMT values within our sample (0.07 mm). The magnitude of this association is clinically relevant, given that each 1-SD increase in CIMT has been shown to independently predict a 40% higher risk of first-time CVD events in a large-scale study of adults under the age of 45 years.[28]

Table 3. Multivariable Regression Model Testing the Association Between Sleep Duration SD and CIMT (n=62)

	Independent variable: CIMT, mm				
	Unstandardized B±SE	Standardized beta	*t*	95% CI	*P* value
Intercept	0.284±0.143		1.990	−0.002 to 0.569	0.052
Age, per y	0.003±0.001	0.362	3.095*	0.001 to 0.005*	0.003*
Sex (ref male)	0.018±0.019	0.128	0.923	−0.021 to 0.057	0.360
Systolic blood pressure, per mm Hg	0.001±0.001	0.177	1.467	−0.050 to 0.322†	0.148
Sleep duration, per 60-min	−0.005±0.011	−0.046	−0.393	−0.046 to 0.031†	0.696
Energy intake, per 100-kcal	−0.001±0.001	−0.096	−0.718	−0.003 to 0.002†	0.476
Sleep duration SD, per 60-min	0.049±0.016*	0.345*	3.076*	0.017 to 0.081*	0.003*

CIMT indicates carotid intima-media thickness.
*Values are statistically significant.
†Values are scaled upwards by a factor of 10^2 to aid in data visualization.

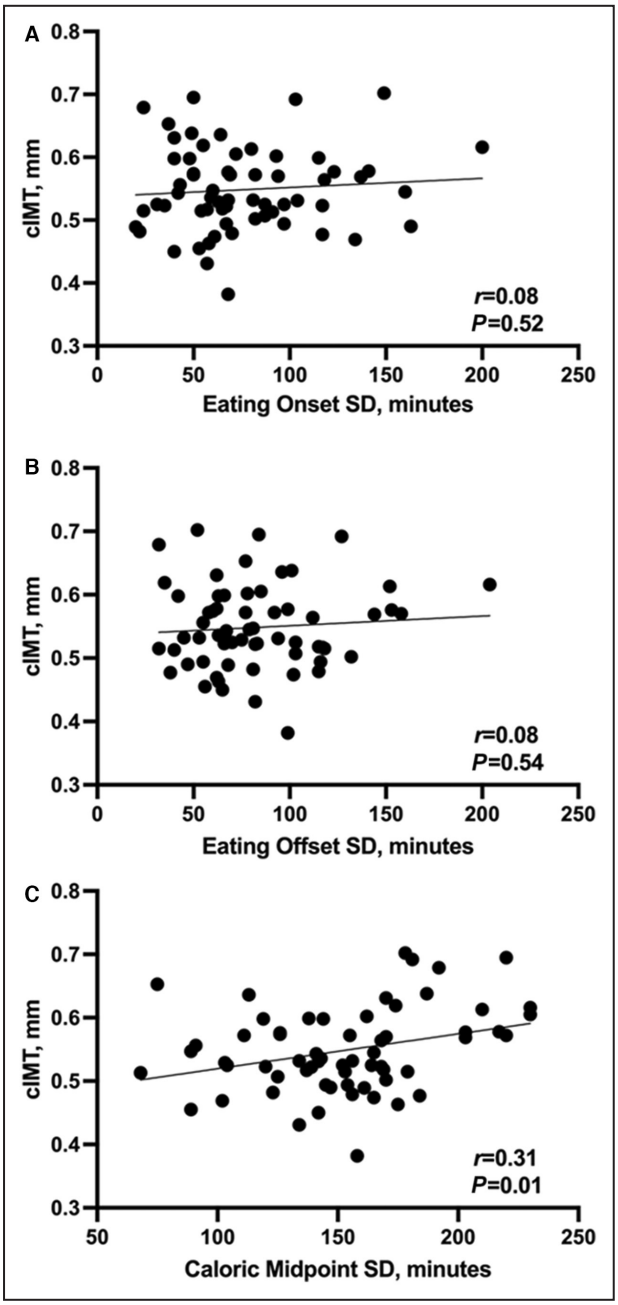

Figure 2. Scatterplots illustrating the bivariate associations between eating timing variability metrics of eating onset SD (A), eating offset SD (B), and caloric midpoint SD (C) with CIMT.
CIMT indicates carotid intima-media thickness.

The significant positive association between caloric midpoint SD and CIMT identified here is particularly novel and notable, as epidemiologic data on day-to-day variability in eating timing and its implications for cardiometabolic health are scarce. Makarem et al previously evaluated the association between eating timing variability with traditional CVD risk factors in 115 racially and ethnically diverse women (33±12 years).[20] Metrics of daily eating timing variability (ie, SD of eating timing metrics) and eating jetlag (ie, weekday-weekend differences in eating timing metrics) were variably associated with worsening cardiometabolic health parameters, including increased BMI, waist circumference, and hemoglobin A1c, over 1 year of follow-up. Additionally, each 10% increase in variability in the percentage of total daily calories consumed after 5:00 PM was associated with a ~3 mm Hg increase in systolic blood pressure and a ~2 mm Hg increase in diastolic blood pressure, across the same time frame. In another study by Zerón-Rugerio et al, cross-sectional analyses in 1106 emerging adults (21.0±2.5 years, 78% women) revealed that greater weekday-weekend differences in eating midpoint (ie, halfway point between eating onset and offset) were associated with a higher BMI[21]; however, day-to-day variability in eating timing was not examined. Nevertheless, it is reasonable to hypothesize that the poorer cardiometabolic health profile identified in those with irregular eating schedules could contribute to pathophysiological vascular changes over time. Although underlying mechanisms for these observations remain uncertain, the timing of energy intake is a known regulator of endogenous circadian rhythms in peripheral metabolic tissues.[50] Thus, greater day-to-day variability in eating timing may increase the likelihood that food intake will occur at metabolically inappropriate times, while also leading to a state of chronic misalignment among central and peripheral circadian rhythms, plausibly contributing to poorer cardiometabolic health.[71]

The association between eating timing variability and CIMT was not significant when operationalized as eating onset SD or eating offset SD in our sample. These results suggest that consistency in the timing where energy intake is concentrated may be more important for vascular health in early adulthood, rather than consistency in the time-of-day at which caloric intake is initiated or terminated. It is possible that this finding may be partially due to how these metrics of eating timing variability are quantified. Caloric midpoint, or "calorically weighted midpoint," incorporates the magnitude of energy intake and illustrates how this intake is distributed within the 24-hour day; meanwhile, eating onset and eating offset are determined by the first and last clock time when calories are consumed, respectively, regardless of the size of the meal. Metabolic responses to meals are positively associated with number of calories consumed[72]; thus, eating onset and eating offset—and consequently, their SD values—may have limited precision due to their lacking consideration of caloric content. However, this divergence remains speculative, as there is an absence of empirical research that has sought to determine which metric is most appropriate for measuring the timing of caloric consumption as a circadian time cue. Nevertheless, our finding will require further

Table 4. Multivariable Regression Model Testing the Association Between Caloric Midpoint SD and CIMT (n=62)

	Independent variable: CIMT, mm				
	Unstandardized B±SE	Standardized beta	*t*	95% CI	*P* value
Intercept	0.306±0.147		2.075	0.011 to 0.601	0.043
Age, per y	0.003±0.001	0.346	2.862*	0.001 to 0.005*	0.006*
Sex (ref male)	0.030±0.021	0.218	1.475	−0.011 to 0.072	0.146
Systolic blood pressure, per mm Hg	0.001±0.001	0.092	0.699	−0.001 to 0.003	0.488
Sleep duration, per 60-min	−0.005±0.012	−0.046	−0.381	−0.047 to 0.032†	0.705
Energy intake, per 100-kcal	0.000±0.001	0.006	0.006	−0.003 to 0.003†	0.995
Caloric midpoint SD, per 60-min	0.033±0.015*	0.305*	2.240*	0.003 to 0.062*	0.029*

CIMT indicates carotid intima-media thickness.
*Values are statistically significant.
†Values are scaled upwards by a factor of 10^2 to aid in data visualization.

examination, ideally using larger and more generalizable samples, as well as prospective study designs with sufficient long-term follow-up to examine atherosclerotic development and progression over time.

Although sleeping and eating are distinct time cues for the endogenous circadian clock and have independent implications for CVD risk, they are also interrelated behaviors that likely fluctuate in tandem.[73,74] For example, we recently quantified the day-to-day associations between nighttime sleeping and next-day eating behaviors over 14 days in healthy, nonshift working adults, which revealed that a 60-minute delay in sleep timing predicts a ~40- to 60-minute delay in eating onset and a ~20-minute delay in eating offset the following day.[75] Additionally, each 60-minute reduction in sleep quantity predicts a ~20-minute advance in eating onset and a ~10-minute delay in eating offset the following day. Metrics of eating timing were found to similarly predict the timing and duration of subsequent nighttime sleep, albeit to a smaller degree. Given these pronounced associations between nighttime sleep patterns and daytime eating timing, the possible overlap in their influence on cardiovascular health, and the known challenges with behavior change, determining which behavior may be a more potent target for primary CVD prevention is of interest. Our exploratory post hoc regression models revealed that sleep variability metrics, most notably sleep duration SD, were stronger correlates of CIMT than eating timing variability in this sample. These lines of research collectively point to sleep variability as a particularly compelling intervention target that could result in numerous downstream health benefits, ranging from improved consistency in eating timing to reduced risk of atherosclerosis. However, to our knowledge, this is the first study to concomitantly examine sleep variability, eating timing variability, and their association with cardiovascular health, underscoring the need for additional research that expands on this theory.

Sleep duration SD illustrates variability in 2 important dimensions of sleep health—quantity and timing—as fluctuations in sleep duration will inherently yield changes in the timing of sleep (ie, onset, offset, or both). Therefore, those with a higher sleep duration SD may be more likely to experience periods of insufficient sleep, with or without recovery, concomitant with circadian rhythm disruption. Recent evidence in adolescents has confirmed that irregular sleep patterns are associated

Table 5. Exploratory Multivariable Regression Model Testing the Associations Between Sleep Onset SD and Caloric Midpoint SD with CIMT (n=62)

	Independent variable: CIMT, mm				
	Unstandardized B±SE	Standardized beta	*t*	95% CI	*P* value
Intercept	0.251±0.146		1.175	−0.042 to 0.545	0.092
Age, per y	0.004±0.001	0.390	3.250*	0.001 to 0.006*	0.002*
Sex (ref male)	0.025±0.020	0.180	1.240	−0.015 to 0.066	0.220
Systolic blood pressure, per mm Hg	0.001±0.001	0.136	1.041	−0.001 to 0.003	0.303
Sleep duration, per 60-min	−0.001±0.012	−0.009	−0.072	−0.041 to 0.038†	0.943
Energy intake, per 100-kcal	−0.001±0.001	−0.091	−0.598	−0.004 to 0.002†	0.552
Sleep onset SD, per 60-min	0.038±0.019	0.262	1.973	0.000 to 0.077	0.054
Caloric midpoint SD, per 60-min	0.018±0.016	0.170	1.140	−0.014 to 0.050	0.259

CIMT indicates carotid intima-media thickness.
*Values are statistically significant.
†Values are scaled upwards by a factor of 10^2 to aid in data visualization.

Table 6. Exploratory Multivariable Regression Model Testing the Associations Between Sleep Duration SD and Caloric Midpoint SD with CIMT (n=62)

	Independent variable: CIMT, mm				
	Unstandardized B±SE	Standardized beta	*t*	95% CI	*P* value
Intercept	0.251±0.141		1.776	−0.032 to 0.533	0.081
Age, per y	0.003±0.001	0.380	3.297*	0.001 to 0.006*	0.002*
Sex (ref male)	0.026±0.020	0.186	1.325	−0.013 to 0.065	0.191
Systolic blood pressure, per mm Hg	0.001±0.001	0.104	0.830	−0.001 to 0.003	0.410
Sleep duration, per 60-min	−0.003±0.011	−0.031	−0.268	−0.043 to 0.033†	0.790
Energy intake, per 100-kcal	−0.000±0.001	−0.001	−0.007	−0.003 to 0.002†	0.994
Sleep duration SD, per 60-min	0.044±0.016*	0.305*	2.709*	0.011 to 0.076*	0.009*
Caloric midpoint SD, per 60-min	0.025±0.014	0.232	1.763	−0.003 to 0.053	0.084

CIMT indicates carotid intima-media thickness.
*Values are statistically significant.
†Values are scaled upwards by a factor of 10^2 to aid in data visualization.

with disrupted patterns of light exposure, the strongest circadian time cue,[76] which has been causally linked with adverse vascular changes in animal models.[77–80] For example, environmentally induced circadian disruption via inversion of the light–dark cycle was found to promote atherosclerotic development in hyperlipidemic female APOE*3-Leiden.CETP mice via increased lesion macrophage content, gene expression of inflammatory markers and oxidative stress, and protein expression of CCL2 (chemokine [C-C motif] ligand 2), a chemokine that actively recruits monocytes to sites of endothelial injury.[79] Studies that have further controlled for the confounding impact of a high-fat diet indicate that increased circulation of very-low-density/low-density lipoprotein particles are key mechanisms that contribute to exacerbated atherosclerosis following abnormal light exposure patterns.[77,80] Experimental and observational studies in humans provide additional insight, as many mechanisms known to promote atherosclerosis have been observed in those with irregular or rapidly inverted sleep/wake schedules (eg, increased inflammation,[11,15,63,64] autonomic dysfunction,[11,19] elevated blood pressure[11,65]). In terms of sleep quantity, an extensive experimental literature suggests that sleep deprivation results in increased sympathetic activity, increased expression of proinflammatory cytokines, a prothrombotic state, reduced glucose tolerance, and increased insulin resistance, collectively promoting a physiological environment that favors atherosclerosis development.[81] Consistent with this experimental research is rigorous observational evidence demonstrating that habitual insufficient sleep is associated with adverse vascular changes including endothelial dysfunction,[82] arterial stiffness,[83] and coronary artery disease.[44] Taken together, the relatively strong relationship between sleep duration SD and CIMT identified here is substantiated by a considerable body of evidence.

Our results should be interpreted in the context of several design limitations. Our sample consisted of mostly young, generally healthy adults, with limited sociodemographic diversity, so findings may not be generalizable to older adults, those with chronic health conditions, or more diverse populations. Although the results of this investigation align with prior experimental and longitudinal findings, our cross-sectional design prohibits us from drawing conclusions regarding the causality or directionality of the associations identified here. Thus, there remains a need for interventions that test the impact of circadian alignment paradigms (ie, regularity in sleep patterns and eating timing) on biomarkers of vascular aging. Additionally, there are numerous ways to characterize variability in sleep patterns and eating timing, many of which were not addressed in this study. We focused on SD-based metrics as our research question centered on behavioral variability at a more granular scale (ie, day to day), rather than broader weekly fluctuations (eg, social jetlag).[84] It could be argued that day-to-day variability may be more relevant to the general population as it considers those who do not always adhere to conventional weekday versus weekend schedules. Additionally, the use of SD as a metric of variability in daily behaviors has become increasingly common (especially in sleep literature), can be easily operationalized, and has been previously linked with other vascular biomarkers as well as overt CVD.[13,18,68] Nevertheless, we recognize the exigency for further research identifying the most optimal metrics for estimating behavioral variability as it relates to cardiovascular health. Finally, other methods for examining eating behavior exist, such as 24-hour diet recalls and questionnaires; however, there are important limitations to these approaches, such as an inability to assess day-to-day variability and recall bias, which may hinder their ability to capture nuances in daily eating behavior over longer assessment periods.[85] On the other hand, objective methods such as continuous glucose monitoring could be useful in verifying the timing of caloric intake and should be

considered in future research. Strengths of this study include the rigorous design that consists of 14 consecutive days of sleep and dietary assessments, obtained via mostly objective methods and with minimal missing data, the comprehensive consideration of plausible confounding factors, and the use of a clinically relevant surrogate of subclinical atherosclerosis as the study outcome variable.

CONCLUSIONS

In summary, this study provides new evidence of a positive association between sleep variability and eating timing variability with CIMT—an established surrogate marker of subclinical atherosclerosis—in nonshift working adults between the ages of 18 and 45 years. Moreover, these associations are independent of other established risk factors for CVD, with a magnitude that is both statistically and clinically meaningful. These results align with several former experimental and observational studies that have linked circadian rhythm disruption and sleep loss with an elevated risk of atherosclerosis. From a clinical perspective, this growing literature collectively points to irregular sleep patterns and eating timing as primary CVD prevention targets that should be considered in early adulthood. Future studies with larger samples and prospective, longitudinal designs are needed to expand on these findings and to determine if similar associations are present when evaluating subclinical CVD progression. Moreover, elucidating the multilevel (ie, individual, social, environmental) determinants of sleep variability and eating timing variability will be critical to informing intervention strategies that promote consistency in these metrics.

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Disclosures

None.

Supplemental Material

Tables S1–S2
Figures S1–S2

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