

Effects of Inadequate Sleep on Blood Pressure and Endothelial Inflammation in Women: Findings From the American Heart Association Go Red for Women Strategically Focused Research Network

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Background—Insufficient sleep increases blood pressure. However, the effects of milder, highly prevalent but frequently neglected sleep disturbances, including poor sleep quality and insomnia, on vascular health in women are unclear. We investigated whether poor sleep patterns are associated with blood pressure and endothelial inflammation in a diverse sample of women.

Methods and Results—Women who participated in the ongoing American Heart Association Go Red for Women Strategically Focused Research Network were studied ($n=323$, 57% minority, mean age= 39 ± 17 years, range= $20-79$ years). Sleep duration, sleep quality, and time to sleep onset were assessed using the Pittsburgh Sleep Quality Index (score ≥ 5 =poor sleep quality). Risk for obstructive sleep apnea was evaluated using the Berlin questionnaire, and insomnia was assessed using the Insomnia Severity Index. In a subset of women who participated in the basic study ($n=26$), sleep duration was assessed objectively using actigraphy, and endothelial inflammation was assessed directly in harvested endothelial cells by measuring nuclear translocation of nuclear factor kappa B. Vascular reactivity was measured by brachial artery flow-mediated dilation ($n=26$). Systolic and diastolic blood pressure were measured by trained personnel ($n=323$). Multivariable linear regressions were used to evaluate associations between sleep patterns and blood pressure, nuclear factor kappa B, and flow-mediated dilation. Mean sleep duration was 6.8 ± 1.3 hours/night in the population study and 7.5 ± 1.1 hour/night in the basic study. In the population study sample, 50% had poor sleep quality versus 23% in the basic study, and 37% had some level of insomnia versus 15% in the basic study. Systolic blood pressure was associated directly with poor sleep quality, and diastolic blood pressure was of borderline significance with obstructive sleep apnea risk after adjusting for confounders ($P=0.04$ and $P=0.08$, respectively). Poor sleep quality was associated with endothelial nuclear factor kappa B activation ($\beta=30.6$; $P=0.03$). Insomnia and longer sleep onset latency were also associated with endothelial nuclear factor kappa B activation ($\beta=27.6$; $P=0.002$ and $\beta=8.26$; $P=0.02$, respectively). No evidence was found for an association between sleep and flow-mediated dilation.

Conclusions—These findings provide direct evidence that common but frequently neglected sleep disturbances such as poor sleep quality and insomnia are associated with increased blood pressure and vascular inflammation even in the absence of inadequate sleep duration in women.

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Clinical Perspective

What Is New?

- We found direct evidence that highly prevalent, relatively mild sleep disturbances such as poor sleep quality, prolonged time to fall asleep, and insomnia are associated with increased blood pressure and vascular inflammation in women, even in the absence of sleep deprivation.

What Are the Clinical Implications?

- Our observations extend the relationship between sleep disorders and cardiovascular risk in women to include common but frequently neglected sleep disturbances.
- Our findings may strengthen the rationale for systematically screening women for inadequate sleep in order to prevent adverse vascular outcomes in this population.

Inadequate sleep is common, with 28% of the US adult population reporting ≤ 6 hours of sleep per night and with that, a corresponding 24% increased risk of cardiovascular disease.^{1–7} Women adopt such behavior in disproportionately greater numbers in part because of lifelong demands on them as caregivers, first to their children and later to ailing and/or elderly family members.^{8,9} Furthermore, psychosocial factors may disproportionately impact sleep among women compared with men. Women are more prone than men to depression, which is linked to insomnia.¹⁰ While the adverse effects of severe sleep disturbances, such as obstructive sleep apnea (OSA) and insufficient sleep, on metabolic and vascular health have recently been recognized in both men and women,^{11–14} the effects of highly prevalent, milder sleep disturbances, such as perceived poor sleep quality and insomnia on vascular health, are not well characterized in women, a population particularly vulnerable to these conditions.

Emerging evidence suggests sex-specific effects of sleep duration and latency on blood pressure regulation.¹⁵ Sleep deprivation among women (≤ 5 hours versus 7 hours/night) is associated with a greater risk of hypertension (odds ratio: 1.68, 95% confidence interval, 1.39–2.03) than that observed among men (odds ratio: 1.30; 95% confidence interval, 0.93–1.83); however, only 3 out of 23 reviewed studies presented findings stratified by sex.^{16–19} Prolonged sleep onset latency increases the odds of hypertension by 300% (odds ratio: 3.27; 95% confidence interval, 1.20–8.96).²⁰ In addition, OSA, an independent risk factor for hypertension that is frequently unrecognized,¹⁴ affects 17% of all American women, with even greater prevalence among postmenopausal women.²¹ Remarkably, while women are particularly vulnerable to inadequate sleep, and rate it as a leading cause of suboptimal quality of life, they are rarely asked about their sleep habits and patterns at healthcare encounters.²²

Considering the growing evidence suggesting a link between inadequate sleep and increased cardiovascular risk, we examined the associations between sleep characteristics, including sleep quality, insomnia, sleep onset latency, and OSA risk, with blood pressure in a large, diverse sample of women encompassing different life stages. Additionally, we assessed directly whether perceived sleep quality and severity of insomnia are associated with endothelial inflammation and dysfunction in the absence of sleep deprivation. We collected endothelial cells (ECs) from a subset of healthy, young women, whose sleep duration was measured objectively, to assess directly whether perceived inadequate sleep and objectively measured actual sleep duration were associated with nuclear translocation of nuclear factor kappa B (NF κ B), a marker of endothelial inflammation and a key step in the initiation and progression of cardiovascular diseases. Direct assessment of NF κ B may provide valuable, precise insight into early and subtle changes in endothelial function in conditions that affect systemic vascular endothelium.

Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request. The study has been preregistered at ClinicalTrials.gov (NCT02835261).

Study Population and Design

This study is an analysis of baseline data from an ongoing population-based prospective cohort of 323 women, aged 20 to 79 years recruited as part of the American Heart Association Go Red for Women (AHA GRFW) Strategically Focused Research Network population study at Columbia University Medical Center (CUMC AHA GRFW), a novel cohort of women representing all stages of the adult life cycle. The purpose of the CUMC AHA GRFW population study is to examine sleep patterns in relation to cardiometabolic risk factors among women throughout adulthood. Participants were visitors of patients at New York-Presbyterian Hospital/CUMC or nearby hospitals, living in the neighboring communities, or referred to the study by affiliated physicians. Bilingual staff members recruited both English- and Spanish-speaking women. All participants were required to give written informed consent and the study was approved by the CUMC Institutional Review Board.

A subset of the women participating in the prospective cohort study were also screened for eligibility to participate in the ongoing CUMC AHA GRFW basic science/clinical science studies (referred to as “basic study”), which examine the impact of prolonged mild sleep restriction on vascular endothelial function. Objective measures of habitual sleep

were obtained using actigraphy as part of the screening process for this intervention. Inclusion criteria for the basic study are as follows: (1) sleeping an average of 7 to 9 hours/night; (2) having a body mass index (BMI) between 25.0 and 33.0 kg/m² or a BMI 20 to 25 kg/m² with immediate family history of obesity, hypertension, or diabetes mellitus; (3) absence of history of chronic illness; and (4) no medication use for any chronic condition or hormones. Out of a total of 389 women who were screened, 26 women were enrolled in the basic study. As part of their participation in the CUMC AHA GRFW basic study, these women provided EC samples for NFκB activation assessments and underwent brachial artery flow-mediated dilation (FMD) measurements. Data from the baseline assessment (before any study intervention) were analyzed.

Assessment of Sleep Patterns

In the population study, sleep duration and quality were assessed using the validated Pittsburgh Sleep Quality Index.²³ The Pittsburgh Sleep Quality Index measures 7 domains including subjective sleep quality, sleep onset latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction over the last month to distinguish between poor and good sleepers. A global sum ≥ 5 indicates poor sleep.^{24–26} According to the Sleep Research Society/American Academy of Sleep Medicine consensus statement,²⁷ adequate sleep should be ≥ 7 hours/night, while sleeping < 7 hours/night is considered insufficient. The Berlin Sleep Questionnaire was used to determine the prevalence of a high-risk phenotype for OSA, using responses to questions about snoring, daytime somnolence, hypertension, and BMI.²⁸ The presence of insomnia was ascertained using the Insomnia Severity Index (ISI) Questionnaire²⁹ and categorized as (1) no clinically significant insomnia; (2) subthreshold insomnia; (3) clinical insomnia with moderate severity; or (4) severe clinical insomnia.

Sleep duration was measured using actigraph GT3X+ monitors (Actigraph LLC, Pensacola, FL) in women enrolled in the basic study. Monitors were worn on the nondominant wrist 24 hours/d for 14 days and were only removed to shower. Mean sleep duration was derived from the ActiLife software assisted with information on bedtimes and wake times derived from sleep diaries.

Assessment of Outcomes

Blood pressure

Resting systolic (SBP) and diastolic (DBP) blood pressure were assessed in the population study using a hospital-grade automated blood pressure monitor using a standard protocol

(Omron 5 Series Upper Arm [BP742]).³⁰ Participants were sitting, legs uncrossed, with arms not restricted by clothing and relaxed, for at least 5 minutes before measurements were taken. The participant's arm was supported at heart level, with palm up. Trained personnel palpated the brachial artery, positioned the cuff 1 in above the site of brachial pulsation, and centered the bladder of the cuff above the artery. The research grade automated blood pressure monitor was activated. A minimum of 2 readings were taken at intervals of at least 1 minute, and the average of those readings was used to represent the patient's blood pressure. If there was a > 5 mm Hg difference between the first and second readings, an additional reading was obtained, and the average of the 3 readings was used.

Brachial artery FMD

Vascular reactivity of the brachial artery was assessed in the basic study, in the arm contralateral to the endothelial harvesting site by FMD according to the guidelines of the International Brachial Artery Reactivity Task Force.³¹ Participants were evaluated in a quiet, temperature-controlled room after an overnight fast. After a 30-minute rest in a supine position, the brachial artery diameter was measured 6 cm proximal to the antecubital fossa using a 7 to 15 MHz linear array transducer (Philips 5500, Andover, MA). An occlusion blood pressure cuff was placed over the proximal forearm just below the antecubital fossa. FMD was measured as the dilator response to reactive hyperemia induced by a 5-minute blood pressure cuff occlusion of the upper arm. The cuff was inflated to 50 mm Hg above SBP systolic blood pressure if the SBP was > 150 mm Hg, or to 200 mm Hg if the SBP was < 150 mm Hg. SBP was < 150 mm Hg in all study participants. Resting and peak brachial artery diameter were measured (expressed in millimeters up to 1 decimal place). The brachial artery diameter was measured continuously for 2 minutes post cuff release and the maximum dilation within this period was used to calculate FMD. A blinded reader analyzed brachial artery diameters off-line using analysis software. The percent diameter change for FMD was calculated as follows:

$$\text{FMD (\%)} = \left[\frac{\text{brachial artery diameter at peak hyperemia} - \text{diameter at rest}}{\text{diameter at rest}} \right] \times 100.$$

FMD and EC harvesting were performed on the same morning within 1 hour in all participants. Nitrates were not administered during FMD to avoid possible effects on measured EC protein levels in study participants.

EC NFκB activation and inflammation

A 20-gauge angiocatheter was inserted into a superficial forearm vein in basic study participants. Under sterile conditions, 3 J-shaped vascular guidewires (Arrow, Reading, PA) were sequentially advanced into the vein up to 10 cm.

ECs were retrieved from wire tips by washing with EC dissociation buffer. Harvesting yielded \approx 2000 to 5000 ECs. ECs were recovered by centrifugation at 4°C, 150g for 6 minutes, the cell pellet was resuspended in red blood cell lysis buffer (RBC Lysis buffer, eBioscience, San Diego, CA) and incubated at 4°C for 5 minutes, then centrifuged at 150g for 6 minutes, fixed with 4% paraformaldehyde (Santa Cruz, Dallas, TX) in phosphate-buffered saline for 10 minutes, washed twice with phosphate-buffered saline, transferred to poly-L-lysine-coated slides (Electron Microscopy Sciences, Hatfield, PA), and air dried at 37°C. The slides were stored at -80°C until analyzed.^{32,33}

For assessment of NF κ B activation in harvested ECs, permeabilized harvested ECs were identified by positive staining with goat anti-human polyclonal antibodies directed against CD144 (VE Cadherin) (R&D Systems, Minneapolis, MN; 1:50) followed by fluorescein isothiocyanate-conjugated donkey anti-goat secondary antibodies (Jackson ImmunoResearch, 1:50). At least 25 consecutive ECs were analyzed from each slide. Nuclear fluorescence of NF κ B was assessed using confocal microscopy (Nikon A1, Nikon Instruments Inc, Melville, NY) and ImageJ after staining with rabbit anti-human antibodies directed against p65 subunit of NF κ B (Novus, Littleton, CO, 1:50) followed by Texas Red-conjugated donkey anti-rabbit secondary antibodies (Jackson ImmunoResearch, 1:50) and staining with 4',6-diamidino-2-phenylindole (Molecular Probes by Life Technology, Carlsbad, CA).³³ Staining for NF κ B was performed on the same day in all study participants and was analyzed within 48 hours of staining.³³

Quantitative real-time polymerase chain reaction (PCR) for mRNA expression of pro-inflammatory genes intracellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1): We used TaqMan (Life Technology) method on Applied Biosystems 7500 fast real-time PCR System. mRNA was extracted using TRIzol (Life Technology) according to the manufacturer's protocol. One microgram of mRNA was used for first-strand cDNA synthesis with SuperScript III kit (Life Technology) according to the manufacturer's protocol. The first strand cDNA was further diluted 10 times with RNase-free water and stored at -80°C . For 20 μL real-time PCR reactions, the following reagents were added: 10 μL TaqMan Gene Expression Master Mix (2X) (Life Technology), 1 μL TaqMan Gene Expression Assay (20X), 5 μL nuclease-free water, and 4 μL cDNA. This reaction mix was added to the wells as duplicates for each sample. Real-time PCR products were run on a 1% agarose gel to confirm the size of the products. All the real-time PCR primers and probes were designed to span an exon junction to eliminate the contamination of genomic DNA. TaqMan primers and probes were used for *ICAM-1* and *VCAM-1*. Results were expressed as Ct values normalized to the housekeeping gene β -actin.³³

Assessment of Other Covariates

A standardized health questionnaire was used to evaluate sociodemographic factors including age, race/ethnicity, education, employment, and income as well as medical history including menopausal status, history of chronic illness, and medication use. Anthropometric measures including height, weight, and waist circumference were obtained by trained personnel. BMI, in kg/m^2 , was calculated from weight and height.

Statistical Analysis

All data were collected on standardized forms and entered into a secure RedCap database, then exported to SAS version 9.4 (SAS Institute, Inc, Cary, NC) for statistical analyses. Participant demographic, lifestyle, and medical characteristics were described using mean \pm SD for continuous variables and frequencies for categorical variables. Sleep duration and quality were evaluated as both continuous and categorical variables (<7 versus ≥ 7 hours/night and poor quality sleep [Pittsburgh Sleep Quality Index ≥ 5] versus good quality sleep [Pittsburgh Sleep Quality Index ≤ 5], respectively). Insomnia was assessed as a continuous variable with ISI scores 0 (no clinically significant insomnia) to 28 (severe insomnia). OSA score was assessed as a continuous variable with a score of 0 (low risk) to 3 (high risk). SBP, DBP, NF κ B, and FMD were evaluated as continuous variables. Means, SDs, and ranges were used to summarize the distributions of each continuous variable. Pearson correlation coefficient was also used to calculate the correlation between BMI and NF κ B.

Statistical Analysis of the Population Study Data

Linear regressions were used to estimate the bivariate associations between each pair of outcomes and each covariate. The covariates included the sleep pattern variables, which were the main covariates of interest, and also a set of potential confounders including age, BMI, waist circumference, race/ethnicity, education, marriage, employment status, and insurance type. Wald tests were used to determine the statistical significance of the bivariate associations at significance level 0.05. All of the covariates that were significant as bivariate associations were included in a linear model to investigate their joint association with outcomes. Backward variable selection was used, in which the most insignificant covariate was removed one at a time until all the remaining covariates reached statistical significance at an alpha level of 0.05.

Statistical Analysis of the Basic Study Data

Because of the limited sample size, simple linear regressions were used to estimate the bivariate associations between

Table 1. Descriptive Characteristics of Study Population at Baseline

Variable	Population Study (n=332) Mean±SD Percentage (%)	Basic Study (n=26) Mean±SD Percentage (%)
Age, y	39±17	31±9
Age ≥39 y	138 (42%)	3 (10%)
Race		
White	199 (60%)	16 (57%)
Black	67 (20%)	5 (18%)
Asian	52 (16%)	7 (25%)
Other	14 (4%)	...
Hispanic ethnicity	85 (26%)	3 (11%)
Racial/ethnic minority	188 (57%)	14 (50%)
Married	104 (31%)	7 (25%)
Do not have health insurance	71 (21%)	5 (18%)
Employed/student	264 (82%)	24 (92%)
Education ≥ college	304 (92%)	28 (100%)
Have children	105 (32%)	4 (14%)
Premenopausal	216 (65%)	25 (89%)
Current or former smoker	75 (23%)	2 (7%)
History of chronic disease		
Diabetes mellitus	17 (5%)	...
Hypertension	66 (20%)	1 (4%)
Hypercholesterolemia	86 (26%)	4 (14%)
CVD	10 (3%)	...
BMI, kg/m ²	26.4±5.9	24.3±2.5
BMI:		
Overweight	100 (32%)	8 (31%)
Obese	61 (19%)	1 (4%)
Waist circumference, in	35.7±5.9	34.4±2.9
Waist circumference >35 in	142 (45%)	11 (42%)
SBP, mm Hg	118±15	110±14
DBP, mm Hg	73±11	69±10
Blood pressure ≥120/80 mm Hg	149 (47%)	7 (27%)
Blood pressure ≥140/90 mm Hg	38 (12%)	1 (4%)
Sleep habits		
Self-reported sleep duration (h/night)	6.8±1.3	7.5±1.1
Self-reported sleep duration <7 h	146 (44%)	5 (18%)
Sleep quality (PSQI Score)	5.7±4.0	3.5±3.0
Poor sleep quality (PSQI≥5)	167 (50%)	6 (23%)

Continued

Table 1. Continued

Variable	Population Study (n=332) Mean±SD Percentage (%)	Basic Study (n=26) Mean±SD Percentage (%)
Length of time to fall asleep, min	27±32	17±12
Insomnia severity index score	7.0±6.2	4.3±4.5
Insomnia: somewhat, moderate, or severe (ISI≥8)	119 (37%)	4 (15%)
Obstructive sleep apnea score	0.7±0.87	0.14±0.36
Obstructive sleep apnea: high risk (score=2 or 3)	55 (17%)	...
Basic science data		
Objective sleep duration (h) n=26		7.62±0.45
NFκB (mean fluorescence intensity) n=22		383.05±143.34
Brachial artery FMD (%) n=26		8.05±3.43

BMI indicates body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; FMD, flow-mediated dilation; ISI, Insomnia Severity Index; NFκB, nuclear factor kappa B; PSQI, Pittsburgh Sleep Quality Index; SBP, systolic blood pressure.

each pair of clinical outcomes and each of the baseline covariates. Wald tests were used to determine the statistical significance of the bivariate associations at a significance level of 0.05. We also assessed the associations between each clinical outcome and confounding variables including age, BMI, waist circumference, race/ethnicity, education, marriage, employment status, and insurance type. If any of those variables were significantly associated with a clinical outcome, we considered them as potential confounders and included them into the linear model to adjust for the confounding effect. If a potential confounder was no longer significant in the expanded model, it was dropped from the model.

Results

Descriptive characteristics of the study populations at baseline are shown in Table 1. The mean age was 39 years in the population study, 31 years in the basic study, and one quarter to one third of the women were married. This was a racially and ethnically diverse sample with more than half identifying as a racial and/or ethnic minority (Hispanic ethnicity and/or racial Black, Asian, American Indian, or Alaskan Native). Half of the women in the population study had a BMI >25 kg/m², compared with 35% in the basic study. The mean SBP was 118±15 mm Hg in the population study and 110±14 in the basic study, while the mean DBP was 73±11 and 69±10 mm Hg in the population and basic study, respectively.

Table 2. Multivariable Model of the Association Between Systolic Blood Pressure and Sleep Quality Adjusted for Age and BMI

Variable	Estimate	P Value
Sleep quality (PSQI score range 0–21)	0.38	0.04*
Age, y	0.29	<0.001
BMI, kg/m ²	0.96	<0.001

BMI indicates body mass index; PSQI, Pittsburgh Sleep Quality Index.

*P value significant at <0.05, n=332.

Assessment of sleep habits showed that the mean self-reported nightly sleep duration in the population study sample was 6.8 ± 1.3 hours/night compared with 7.5 ± 1.1 hours/night in the basic study. Objectively measured sleep duration was 7.62 ± 0.45 hours/night in the basic study, which was not different from self-reported sleep duration ($P=0.57$). Half of the population study sample reported having poor sleep quality, including a surprising 25% in the basic study, and 37% reported having somewhat, moderate, or severe insomnia (15% “subthreshold insomnia” in the basic study). Based on the Berlin Sleep questionnaire, the prevalence of a high-risk profile for OSA (including snoring and other factors) was 17% in the population study, and none in the basic study. Such differences in sleep quality and duration between the studies were expected because of sleep-based inclusion and exclusion criteria in the basic study.

Sleep Patterns and Blood Pressure

In the population study, sleep patterns significantly predicted SBP and DBP in multivariable-adjusted models (Tables 2 and 3). Significant univariate relations were found between SBP and sleep quality, insomnia, length of time to fall asleep, OSA score, employment status, education level, age, waist circumference, and BMI ($P<0.05$). Poor sleep quality remained as a significant predictor of greater SBP in linear regression models adjusted for age and BMI ($\beta=0.38$; $P=0.04$), after backward variable selection was used. Similarly, significant univariate relations were found between DBP and sleep

Table 3. Multivariable Model of the Association Between Diastolic Blood Pressure and Obstructive Sleep Apnea Score Adjusted for Age and BMI

Variable	Estimate	P Value
Obstructive sleep apnea score (range 0–3)	1.38	0.08
Age, y	0.13	<0.001
BMI, kg/m ²	0.57	<0.001

BMI indicates body mass index.

quality, insomnia, sleep onset latency, OSA risk score, education level, age, waist size, and BMI ($P<0.05$). After backward variable selection was used, age and BMI were significantly associated with DBP. After adjusting for age and BMI, greater OSA risk score was not associated with greater DBP in linear regression models but a nonsignificant trend was observed ($\beta=-1.38$; $P=0.08$). As expected, the association between OSA and DBP was attenuated after adjusting for BMI, because of the inclusion of BMI as 1 component of the high-risk phenotype OSA score. The P value of 0.08 suggests that the other factors included in the OSA risk score have additional independent contributions to DBP.

Sleep Patterns and Endothelial Inflammation and Function

In the subset of healthy, young women with a normal sleep duration (defined as 7–9 hours) who were participating in the basic study, greater ISI score, which indicates more severe insomnia, was associated with greater nuclear translocation of NF κ B in harvested ECs ($\beta=27.6$; $P=0.002$) (Figure). Poor sleep quality and longer sleep onset latency were also associated with greater nuclear translocation of NF κ B ($\beta=30.6$; $P=0.03$ and $\beta=8.26$; $P=0.02$, respectively), also shown in Figure. After adjusting for BMI, ISI score and sleep quality remained significantly associated with NF κ B (BMI adjusted $\beta=22.8$; $P=0.009$ and BMI adjusted $\beta=25.3$; $P=0.04$, respectively). Mean scores (\pm SD) for participants with poor versus normal sleep quality are presented using categorical variables in Table 4. No significant association between mRNA expression of pro-inflammatory genes ICAM-1 and VCAM-1 with perceived sleep quality or ISI score was observed (ICAM-1 mRNA of sufficient quality was available for 18 and VCAM mRNA for 10 participants; $P=0.14$ for the association between ICAM and sleep quality; $P=0.11$ for the association between ICAM and ISI score; $P=0.237$ for the association between VCAM and sleep quality; $P=0.79$ for the association between ICAM and ISI score). Sleep quality, sleep onset latency, and ISI score were not significantly associated with FMD. There was a significant negative correlation between BMI and NF κ B using Pearson’s correlation of -0.51 and the P value was 0.02. The sample Pearson correlation between FMD and NF κ B was -0.22 and was not significant with $P=0.37$ (using Fisher Z test).

Discussion

The major findings of this study are that poor sleep quality is associated with greater SBP in a large, racially and ethnically diverse sample of women and that perceived poor sleep quality and insomnia are associated with endothelial inflammation even when objectively measured sleep duration is

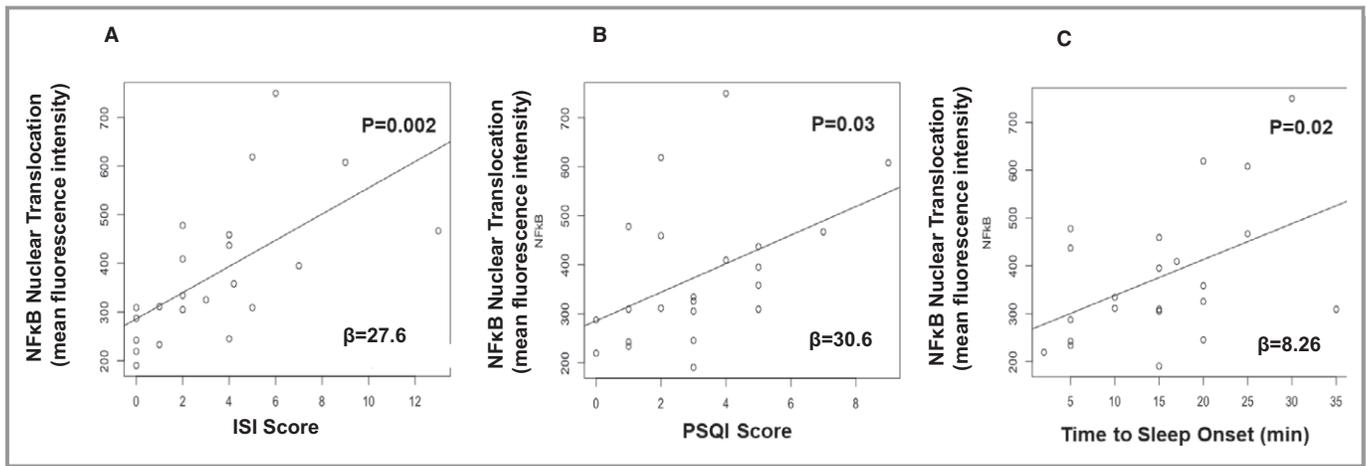


Figure. Relationship between sleep characteristics and endothelial inflammation in harvested endothelial cells in women. Nuclear factor kappa B (NFκB) activation, a marker of endothelial inflammation, is associated with (A) Greater Insomnia Severity Index, (B) poor sleep quality, and (C) longer time to sleep onset. ISI indicates Insomnia Severity Index; PSQI, Pittsburgh Sleep Quality Index.

adequate. These findings suggest that perceived inadequate sleep may increase cardiovascular risk in women.

Although it is well known that severe sleep disorders, such as OSA, are associated with impaired blood pressure, which was confirmed in the present study, and increased risk for incident hypertension and other cardiovascular diseases,^{14,34} the present study indicates that even milder, more prevalent sleep disturbances are associated with elevated blood pressure and evidence of vascular inflammation. Thus, our observations extend the relationship between sleep disorders and cardiovascular risk in women to include common but frequently neglected sleep disturbances such as perceived poor sleep quality, prolonged time to fall asleep, and insomnia.

Associations between sleep and blood pressure measures persisted even after adjustment for age and BMI (2 strong, independent predictors of vascular function). Estimates for the effect of inadequate sleep on blood pressure in our study were similar to those for depression, a well-established risk factor for CVD,³⁵ suggesting that the review of sleep patterns should be part of the routine healthcare encounter for women.

Insights into inadequate sleep-related endothelial activation have been derived from indirect measurements of nitric oxide-mediated arterial vasomotor tone in studies involving sleep deprivation.³⁶ However, the effects of perceived sleep quality and insomnia in the absence of sleep deprivation on

endothelial function are less clear. The lack of association between sleep quality and insomnia score, and brachial artery FMD in our study in a subset of women who slept more than 7 hours/night is in accordance with previous reports where FMD has been mostly unaffected by sleep quality in the absence of curtailed sleep duration.^{37–40} We tested mRNA expression of pro-inflammatory genes *ICAM-1* and *VCAM-1*, which are considered essential for transendothelial migration of monocytes into the arterial wall; however, no significant correlation with perceived sleep quality or ISI score was observed. This is likely because of the small sample size of available sufficient quality mRNA ($n=18$ and 10 for *ICAM* and *VCAM*, respectively). Additionally, in vivo studies in a large-animal model of endothelial activation (swine) showed neither histological evidence of inflammation nor significant differences in the expression of *VCAM-1* and *ICAM-1* despite a modest increase in NFκB measured by Western blot, suggesting that activation of endothelial inflammation is largely kept in check but with measurable biochemical evidence of low-level chronic NFκB activation in these models.⁴¹ This suggests that subtle, early inflammatory changes can be captured directly in a human sample consisting of several thousand ECs using sensitive methods, such as immunofluorescence of the individual ECs. Confocal microscopy is ideally suited for this task because it provides a precise view of the distribution of NFκB inside EC compartments. In our ECs, correlation between nuclear translocation of NFκB, an early marker of pro-inflammatory transcriptional changes, and parameters of sleep quality suggests an interaction.

In OSA, a condition associated with disturbed sleep, endothelial inflammation is present before onset of overt cardiovascular disease as evidenced by increased nuclear translocation of NFκB.^{32,33} Similarly, sleep deprivation activates NFκB in circulating leukocytes in healthy subjects with an

Table 4. Summary Statistics of NFκB by Sleep Quality

Variable	n	Mean	SD	P Value
Sleep quality				
Normal	16	357.2	153.3	0.95
Poor	6	429.0	104.1	

NFκB indicates nuclear factor kappa B.

effect that is more pronounced in women than men, suggesting that women may be particularly vulnerable to cardiovascular pathology after sleep loss.⁴² The catecholamine norepinephrine, whose release is enhanced by increased sympathetic tone, activates NFκB.⁴³ It is possible that increased sympathetic tone was present in women with poor sleep quality in our study as evidenced by the significant association between sleep quality and SBP. Under those circumstances, elevated blood pressure may contribute to endothelial inflammation, an early event in development of cardiovascular disease, in women having poor sleep quality, prolonged time to fall asleep, and insomnia even in the absence of restricted sleep duration or severe sleep disorders such as OSA. However, it should be noted that catecholamines and sympathetic nerve activity were not measured in this study.

The main limitation of this study is that endothelial inflammation was assessed in venous ECs harvested from healthy, young women. Local biomechanical forces that affect arterial ECs at specific sites play an essential role in determining regional susceptibility to atherosclerosis, and endothelial biopsy at specific sites of the arterial vasculature will likely be required to determine the precise mechanisms underlying atherosclerosis in inadequate sleep. However, venous and arterial ECs are exposed to the same circulating pro-inflammatory factors in inadequate sleep. Inflammatory and oxidative pathways are activated similarly in venous and arterial ECs in healthy subjects and patients with atherosclerosis.^{44,45} Furthermore, the cell culturing process rapidly erases arteriovenous gene expression differences that are present in freshly isolated ECs.⁴⁶ Thus, direct investigation of easily accessible freshly isolated human venous ECs without artifact of culture condition may be particularly useful in conditions associated with systemic endothelial activation, such as inadequate sleep.

Additionally, FMD is known to be only partially mediated by inflammation, and our smaller study sample precludes definitive conclusions regarding correlations between NFκB and FMD. However, a large study of 2701 participants from the Framingham Study reported modest, unadjusted inverse correlations between FMD and soluble markers of systemic inflammation (C-reactive protein, IL-6, and soluble intracellular adhesion molecule-1 [sICAM-1]) that were rendered non-significant after adjustment for traditional coronary risk factors.⁴⁷

An additional limitation of this study is the lack of objective assessments of OSA. However, the subset of women who underwent EC collection had no risk for OSA as measured by the Berlin questionnaire, thereby making them unlikely to have unrecognized severe sleep-disordered breathing. Also, there were no adjustments for multiple comparisons because of the small sample size. Finally, the use of 24-hour ambulatory blood pressure monitoring would have strengthened the assessment of blood pressure.

In conclusion, our findings provide direct evidence that highly prevalent, relatively mild sleep disturbances such as poor sleep quality, prolonged time to fall asleep, and insomnia are associated with increased blood pressure and vascular inflammation even in the absence of sleep deprivation. Our findings may strengthen the rationale for systematically screening women for inadequate sleep in order to prevent adverse vascular outcomes in this population.

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Disclosures

None.

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