ORIGINAL RESEARCH

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Experimental Sleep Restriction Causes Endothelial Dysfunction in Healthy Humans

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***Background*—**Epidemiologic evidence suggests a link between short sleep duration and cardiovascular risk, although the nature of any relationship and mechanisms remain unclear. Short sleep duration has also been linked to an increase in cardiovascular events. Endothelial dysfunction has itself been implicated as a mediator of heightened cardiovascular risk. We sought to determine the effect of 8 days/8 nights of partial sleep restriction on endothelial function in healthy humans.

***Methods and Results*—**Sixteen healthy volunteers underwent a randomized study of usual sleep versus sleep restriction of two-thirds normal sleep time for 8 days/8 nights in a hospital-based clinical research unit. The main outcome was endothelial function measured by flow-mediated brachial artery vasodilatation (FMD). Those randomized to sleep restriction slept 5.1 hours/night during the experimental period compared with 6.9 hours/night in the control group. Sleep restriction was associated with significant impairment in FMD (8.6±4.6% during the initial pre-randomization acclimation phase versus 5.2±3.4% during the randomized experimental phase, P=0.01) whereas no change was seen in the control group (5.0±3.0 during the acclimation phase versus 6.73±2.9% during the experimental phase, P=0.10) for a between-groups difference of −4.40% (95% CI −7.00 to −1.81%, P=0.003). No change was seen in non-flow mediated vasodilatation (NFMD) in either group.

***Conclusion*—**In healthy individuals, moderate sleep restriction causes endothelial dysfunction.

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Growing evidence suggests a link between short sleep duration[1–4] and mortality, with those who sleep less than 7 hours per night experiencing a 12% to 35% increased risk of death compared to those who sleep 7 hours per night.[2,3,5] Retrospective data suggest a trend towards increased cardiovascular events[6] and prospective data show an increase in cardiovascular mortality.[7–10] Meanwhile, voluntary sleep restriction is common, with 28% of the US adult population reporting 6 or fewer hours of sleep per night and those who do are 24% more likely to have cardiovascular disease.[11] Thus, sleep deprivation may be a common and preventable cardiovascular risk factor.

Some studies have suggested that vascular dysfunction may be an important link between sleep deprivation and cardiovascular disease including atherosclerosis.[12,13] Flow-mediated dilatation (FMD) is reduced after a single 24-hour work shift[14] and a study of chronic stress with sleep restriction was also shown to be associated with reduced FMD.[15] Total sleep deprivation may also reduce microvascular reactivity.[16] It was recently reported that a single night of sleep restriction of <4 hours per night compared to >7 hours of sleep was associated with reduced coronary flow reserve, consistent with the idea that that sleep restriction may be causally related to cardiovascular events through dysregulation of vascular function.[17]

While these prior studies are provocative, no study has yet examined the effects of partial, intermediate-duration sleep restriction that mimics that seen in everyday life, on arterial

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vascular function.[18,19] Given that sleep restriction is largely voluntary and potentially correctable, understanding the mechanisms by which insufficient sleep promotes the development of cardiovascular disease is clinically important, with clear public health implications.[20] We therefore tested the hypothesis that chronic partial sleep deprivation would be associated with impaired endothelial function.

Methods

This was a 1:1 randomized, parallel-group study of sleep deprivation versus control sleep, stratified by sex, conducted at the Clinical Research Unit at St. Marys Hospital, part of the CTSA of Mayo Clinic (NCT01334788). The study design, outlined below, has been previously described[21] and was chosen because we believed this length of moderate sleep deprivation would likely produce physiologic effects. Individuals gave written informed consent. This study was approved by the Mayo Clinic Institutional Review Board (IRB # 08-006780).

Subjects

Eligible individuals were between the ages of 18 to 40 years, normal weight (body mass index 18.5 to 24.9 kg/m^2), sedentary (defined as less than four 20-minute episodes of moderate or vigorous intensity physical activity in the prior 4 weeks), had no medical conditions requiring ongoing treatment, and taking no medications other than oral contraceptive pills for birth control. Exclusion criteria were pregnancy or plans to become pregnant in the next year, tobacco use, anemia, any sleep disorder, and inability to follow the study protocol.

Screening Evaluation

Subjects underwent a screening evaluation consisting of a physical examination, dietary surveys, assessment of hemoglobin concentration, a urine pregnancy test, and an overnight polysomnogram (PSG). Subjects left our facility the morning after the PSG wearing a digital actigraph (Actiwatch 2; Philips Respironics, Amsterdam, the Netherlands) and wore it continuously for at least 1 week while engaging in their usual activities.

Inpatient Phase

One week to 1 month after the screening examination, subjects were admitted to the Clinical Research Unit and began the 15-day and 14-night inpatient phase of the study. Temperature and lighting were controllable and left to the discretion of the subjects who were allowed access to clocks and were aware that awakening would consistently occur at 06:00 during the study. The first 3 days and 3 nights consisted of an acclimation phase during which subjects were allowed to sleep ad lib. The experimental phase consisted of the subsequent 8 days and 8 nights. A computer-generated list of random numbers was used to create simple randomization to the sleep deprivation or to the control group 1:1 that was stratified by sex. Allocation concealment and blinding of participants and study staff except the lead physician and lead sleep technologist until the experimental phase was achieved through the use of a single protocol with identical procedures except for the provision that bedtime would be according to randomization status during the experimental phase. On the morning of the fourth day, participants and staff were informed of the randomization status. During the experimental phase, those randomized to sleep deprivation were asked to stay awake between 06:00 and their bedtime which was calculated to give an in-bed time equal to two-thirds of their usual sleep time using data from the actigraph. Those randomized to the control group were allowed to sleep ad lib. During the experimental phase of the study protocol, nurses checked on each subject every 30 minutes and recorded their activities between 07:00 and bedtime. After the experimental phase, subjects entered the recovery phase for 4 days/3 nights during which all subjects continued to be woken at 06:00 and all were allowed to sleep ad lib.

Sleep Monitoring

PSGs were performed at the screening examination and each night during the inpatient phase of the study. PSGs were digitally recorded (Siesta, Compumedics, Abbotsford, Victoria, Australia) and scored using Profusion3 PSG software. Recorded variables included 7 channel electroencephalography, 2 channel electro-oculography, oronasal airflow by pressure transducer and thermocouple sensors, submental and limb electromyograms, 3-lead electrocardiography, transcutaneous pulse oximetry, thoracic and abdominal respiratory effort by inductance plethysmography, snoring by tracheal microphone or piezo crystal sensor and body position by body sensor and video monitoring.

During the daytime, wakefulness was assessed by continuous 7-channel electroencephalography, 2-channel electro-oculography, submental and electromyograms, and 3-lead electrocardiography using the Siesta device.

Scoring of sleep stages, disordered breathing events, oxygen desaturation, and periodic limb movement was performed by an experienced polysomnographer and results reviewed by a qualified physician[22] as previously described.[21]

During the study, subjects were allowed ad lib food and drink without restrictions.

Endothelial Function

Endothelial function was assessed by brachial artery flow mediated dilatation as previously described[23] with the exception of free access to food during the entire study. Briefly, endothelial function was assessed by ultrasound measures of flow-mediated (endothelium-dependent) vasodilation (FMD) using reactive hyperemia. Non-flow mediated (endothelium-independent) vasodilation (NFMD) was assessed in the brachial artery after nitroglycerine 0.4 mg sublingual administration.[24–27] Testing was done in the morning prior to arising from bed at the end of the acclimation period (day 3), the end of the experimental period (day 11), and the end of the recovery period (day 15) and included measurement of supine blood pressure prior to testing. Changes in FMD and NFMD are expressed as percentage from baseline values.

Biochemical Measurements

Venous blood was collected on the last day of each study period by standard venipuncture between 06:10 and 06:30 prior to arising from recumbency. Adiponectin, leptin, and total ghrelin were measured by radioimmunoassay (Millipore, Billerica, MA). Glucose, and high sensitivity C-reactive protein (hsCRP) were measured in a chemistry analyzer (Roche Cobas C311; F. Hoffmann-La Roche LTD, Basel, Switzerland). Insulin and cortisol was measured by automatic immunoassay (Beckman DXi; Beckman Coulter, Inc, Brea, CA). Interleukin-6 (IL-6), tumor necrosis factor- α (TNF-α), intercellular adhesion molecule-1 (I-CAM), vascular adhesion molecule-1 (V-CAM), and endothelin-1 (ET-1) were measured by enzyme-linked immunosorbent assay. CCL2 and CCL5 were also measured by ELISA using kits from RnD system (Minneapolis, MN).

Statistical Analysis

The primary outcome measure was change in FMD between the acclimation (day 3) and experimental (day 11) time points. The deprived and control groups were compared using the *t* test. As a confirmatory test the deprived and control groups were compared using analysis of covariance (ANCOVA) including the acclimation measure as a covariate.

Secondary analyses included examination of NFMD and associations between changes in FMD with changes in other physiologic parameters. Covariates of interest as potential predictors of FMD were assessed using correlations and included change in caloric intake per day and changes in serum concentrations of glucose, insulin, hsCRP, IL-6, TNF-α, I-CAM, V-CAM, CCL-2, CCL-5, ET-1, cortisol, adiponectin, leptin, and total ghrelin. Bonferroni correction was used to adjust for multiple comparisons, and a family wide *P* value of 0.05 was used to judge significance.

Data are summarized as frequencies for categorical variables and means with standard deviation (mean±SD) for continuous variables. Log-transformation of the data was used for the variables that had a skewed distribution, and scatter plots were examined to assess the reasonableness of using correlations as a descriptive. Changes over time (from phase to phase) were assessed by first taking within patient differences and groups were compared using 2-sample techniques, for example 2-sample *t* test or analysis of covariance. Differences in proportions were tested using the χ^2 test or Fisher exact test. Analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC). For all comparisons $P<0.05$ was considered significant.

Results

The study sample consisted of 17 subjects who were randomized to sleep deprivation or to control sleep. High-quality data were available for analyses from 16 subjects, which included 5 men and 3 women in each group (Table 1). FMD data were unavailable on 1 male subject randomized to control due to technical limitations allowing us to analyze FMD data from 15 subjects, and NFMD data were not available on 1 male subject randomized to sleep deprivation, allowing us to analyze NFMD data from 15 subjects.

Baseline sleep parameters were normal in all subjects by study design as previously reported[21] without abnormalities in oxygen saturation or apnea-hypopnea index. Total sleep time in the sleep-deprived group fell from an average of 6.5±1.1 hours/night during acclimation to 5.1±0.37 hours/night during sleep restriction while the control group sleep time was 7.4±1.2 hours/night during acclimation and 6.9±0.8 hours/night during the experimental phase. As previously reported,[21] sleep restriction was associated with an increase in caloric intake with those randomized to sleep restriction consuming an additional 542 kcal/day during the experimental phase ($P=0.008$), compared to a

Table 1. Baseline Subject Characteristics

	Sleep Deprived	Control	*P* Value
Number	8	8	
Age, y	24.1±4.5	25.1±5.0	0.70
Gender, n	5 men, 3 women	5 men, 3 women	1.0
BMI, kg/m^2	22.4±2.5	22.4±1.2	0.98

Values are presented as mean±SD. BMI indicates body mass index.

non-significant change of −118 kcal/day (*P*=0.52) in the control group. No differences were seen in activity-related energy expenditure or change in body weight between groups (+1.1±1.1 kg versus +0.8±1.3 kg, *P*=0.66).

Subjects randomized to sleep restriction showed significant impairment in FMD (8.6±4.6% during the acclimation phase versus 5.2±3.4% during the experimental phase, *P*=0.01) whereas no change was seen in the control group (5.0±3.0% during the acclimation phase versus 6.73±2.9% during the experimental phase, *P*=0.10) for a between groups difference of −4.40% (95% CI −7.00 to −1.81%), *P*=0.003, Figur). Due to the imbalance in acclimation phase measures we repeated the analysis using analysis of covariance using our acclimation phase values as covariates. This analysis continued to show a significant difference between the deprived and controls groups (diff=−3.47, 95% CI −5.96 to −0.97, *P*=0.011).

In contrast, subjects randomized to sleep restriction showed no change in NFMD (18.9±3.9% during acclimation versus 18.5±6.9% during the experimental phase, *P*=0.97) and no change was seen in the control group (15.0±5.3% during acclimation versus 16.8±4.8% during the experimental phase, *P*=0.42) for a non-significant between group difference of −0.66% (95% CI −4.29 to 2.96%, *P*=0.70, Figure).

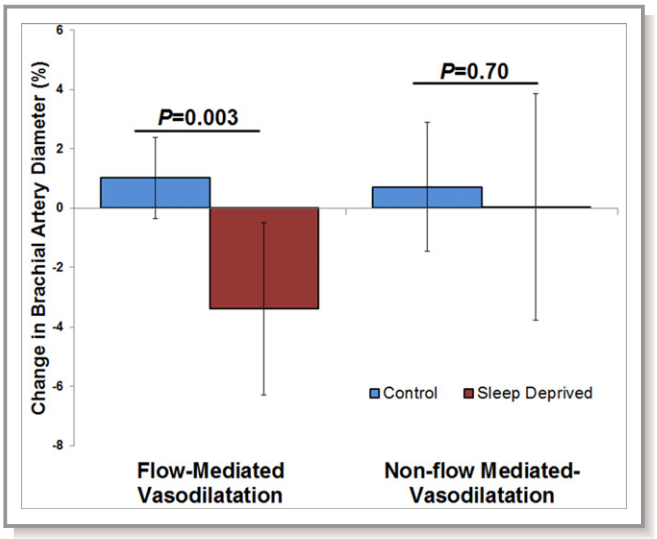

Figure. Effect of sleep restriction on endothelial function—endothelial function, assessed by percentage change in flow-mediated dilatation (FMD), was impaired by sleep restriction while non-flow-mediated vasodilatation (NFMD) was not. Those randomized to sleep restriction showed an impairment in FMD (8.6±4.6% during the acclimation phase vs 5.2±3.4% during the experimental phase, *P*=0.01) whereas no change was seen in the control group (5.0%±3.0 during the acclimation phase vs 6.73±2.9% during the experimental phase, *P*=0.10) for a between groups difference of −4.40% (95% CI −7.00 to −1.81%, *P*=0.003). In contrast, no change was seen in NFMD.

Blood pressure in the sleep-deprived group went from an average of 105.8±9.2/62.9±9.7 in the acclimation phase to 107.3±11.2/60.0±8.6 mm Hg in the experimental phase (*P*=0.090/*P*=0.13) whereas blood pressure in the control group went from 110.0±6.4/65.6±5.5 in the acclimation phase to 106.3±4.9/61.3±5.9 mm Hg (*P*=0.020/*P*=0.04), for a group difference of 3.08 (95% CI −6.49 to 12.66), *P*=0.49)/−0.46 (95% CI −7.19 to 6.27, *P*=0.88).

Heart rate in the sleep-deprived group went from 58.3±7.8 to 61.4±7.9 beats per minute (*P*=0.07) and from 56.0±6.7 to 60.6±9.8 beats per minute in the control group (*P*=0.22), for a non-significant between group difference of −0.89 (95% CI −7.6 to 5.8, *P*=0.78).

There was no significant correlation between change in FMD and change in caloric intake (*r*=−0.30, unadjusted *P*=0.48) or concentrations of insulin, glucose, ghrelin or the adipokines adiponectin, or leptin. No significant associations were seen between FMD and inflammatory markers including hsCRP, IL-6, TNF-α, I-CAM, V-CAM, CCL-2, or CCL-5 or ET-1 (Table 2).

Discussion

We found that moderate sleep restriction during an 8-day period is associated with a significant impairment in flow-mediated vasodilatation. The magnitude of impairment seen in this study with sleep restriction is similar to that reported in people who smoke, or have diabetes, or who have coronary artery disease,[24,28,29] and helps further our understanding of the cardiovascular risks association with sleep deprivation. The potential public health impact of this relationship may be enormous given the high prevalence of voluntary sleep restriction.[11]

Endothelial function describes the physiologic role of the vascular endothelium in maintaining vascular homeostasis and vascular tone and modulating thrombosis, inflammation, vascular growth, and remodeling.[30] FMD is a measure of endothelial function, and impaired FMD, indicative of endothelial dysfunction, appears to link a variety of cardiovascular risk factors with the development[12,13] and progression[31–33] of atherosclerotic vascular disease. Impaired endothelial function is a systemic disease, and forearm endothelial dysfunction correlates with endothelial dysfunction in other vascular beds, including those of the heart.[31] Endothelial dysfunction may be an important physiologic mechanism to explain the link between sleep duration and total mortality,[1–5] cardiovascular mortality,[7–10] and cardiovascular events,[6] and its use has been advocated as a tool for assessment of novel cardiovascular risk factors.[34]

Consistent with prior studies, we found that sleep deprivation impaired FMD.[14–16,35] The magnitude of endo-

Table 2. Correlation Between Change in FMD and Blood Markers in Sleep Deprived Subjects

	r	Unadjusted *P* Value*
Caloric intake	−0.30	0.48
Glucose	0.68	0.06
Insulin	0.05	0.90
hsCRP	0.82	0.01
IL-6	0.52	0.19
TNF-α	−0.47	0.24
I-CAM	−0.04	0.92
V-CAM	−0.04	0.92
CCL-2	0.21	0.69
CCL-5	0.27	0.51
ET-1	0.08	0.85
Cortisol	0.20	0.63
Adiponectin	0.04	0.93
Leptin	−0.62	0.10
Ghrelin	0.16	0.70

CCL indicates Chemokine (C-C motif) ligand; ET-1, endothelin-1; FMD, flow mediated dilatation; hsCRP, high sensitivity C-reactive protein; I-CAM, intercellular adhesion molecule-1; IL-6, interleukin-6; TNF-α, tumor necrosis factor- α; V-CAM, vascular adhesion molecule-1.
*An unadjusted *P*-value of <0.003 is required to achieve a family wide *P*-value of 0.05 after adjustment for multiple comparisons.

thelial function (absolute reduction in FMD of 4.4%) is similar to that from a single 24-hour work shift (absolute reduction in FMD of 3.8%)[14] and a study of chronic stress with sleep restriction (absolute reduction in FMD of 3.7%).[15] However, contrary to the prospective study of Sauvet and colleagues,[16] we did not find that sleep deprivation led to impaired NFMD; our data thus suggest that partial sleep deprivation selectively results in a defect in nitric oxide production but does not attenuate smooth muscle response to nitric oxide.[36] It has been suggested that the effects of sleep loss on FMD are independent of changes in sympathic activity and blood pressure. Whether this difference in our studies is due to the specific arterial bed studied, sleep deprivation model, or other factors is unclear. We believe it may be due to the acute and total sleep deprivation protocol used by Sauvet et al, which differ from the sustained partial sleep deprivation we used in order to more closely simulate the prevalent "real life" type of sleep deprivation.

Indeed, the relatively life-like sleep restriction model is an important strength of our study as is the comprehensive sleep monitoring which confirms with good precision the exact duration of sleep and wakefulness in each of our subjects. Furthermore, the inclusion of normal, healthy individuals free of established cardiovascular risk factors helps avoid potential confounders. Finally, our method of endothelial function testing is robust and validated, having been studied across a wide range of populations and disease states, is predictive of vascular risk[6–10] and provides valuable insight into pathophysiologic mechanisms linking inadequate sleep to cardiovascular disease.

Our study has several potential limitations that need to be considered. This was a relatively small study, and we only had data from 15 subjects for FMD and NFMD analyses. There was an imbalance in FMD during the acclimation phase at baseline. Even for randomized studies like this, there will generally be some level of imbalance at baseline. Though not rising to the level of statistical significance (P=0.09), this imbalance is a weakness of the study. Sleep duration was proportionally reduced by delaying bedtime while keeping the awakening time consistent. While this allowed us to study endothelial function at the same time of day in all individuals and the proportional reduction in sleep time may help attenuate individual variability in sleep time, it is unclear to what extent our results may have been influenced by any circadian misalignment induced by our protocol. On the other hand, this pattern of sleep restriction, coupled with circadian misalignment mimics real-life sleep restriction circumstances, including shift work.[37] Sleep restriction also led to an increase in caloric intake without a compensatory change in activity energy expenditure and a trend toward weight gain.[21] Both sleep restriction and circadian disruption appear to be related to metabolic abnormalities that may promote obesity,[38,39] and our group has previously shown that weight gain of ≈4 kg due to overeating is associated with impaired FMD. There was no clear correlation between change in caloric intake and change in FMD and our study was too short to show changes in fat mass, suggesting that the endothelial dysfunction observed in this study was more likely due to sleep disturbance than metabolic derangements. It seems plausible that a longer-term exposure to sleep restriction would have resulted in even greater endothelial dysfunction due to the combined effects of sleep deprivation and weight gain.

Furthermore, participants were relatively young and healthy, and it is unclear if similar results can be expected in older individuals with established cardiovascular disease risk factors. On the other hand, it is this young population that is most commonly sleep deprived[40,41] and who may face decades of sleep disturbance. These individuals may conceivably be at greater risk for an epidemic of future cardiovascular risk.

In conclusion, substantial partial sleep restriction in healthy individuals leads to impaired endothelial function. Further studies will need to examine the more fundamental mechanisms that link sleep deprivation to endothelial dysfunction.

Author Contribution

Dr Calvin participated in study design, study supervision, data generation and interpretation, and drafting and writing of the report, and has full access to the data and will vouch for the integrity of the data analysis. Dr Covassin participated in data interpretation and drafting and writing of the report, and has full access to the data and will vouch for the integrity of the data analysis. Dr Kremers participated in data interpretation, statistical analyses, and drafting and writing of the report. Dr Adachi participated in study design, data generation, and drafting and writing of the report. Dr Macedo participated in study design, data generation, and drafting and writing of the report. Dr Albuquerque participated in study design, data generation, and drafting and writing of the report. Mr Bukartyk participated in study design, data generation and drafting and writing of the report. Ms Davison participated in study design, study supervision, and drafting and writing of the report. Dr Levine participated in study design, data generation, and drafting and writing of the report. Dr Singh participated in study design, data generation, and drafting and writing of the report. Dr Wang participated in data generation, and drafting and writing of the report. Dr Somers participated in study design, study supervision, data generation and interpretation, and drafting and writing of the report and has full access to the data and will vouch for the integrity of the data analysis.

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