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SCIENTIFIC INVESTIGATIONS

Short Sleep Duration Is Associated With Increased Serum Homocysteine: Insights From a National Survey

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Study Objectives: Both short sleep duration and increased serum homocysteine levels are associated with cardiovascular events. However, research on the relationship between sleep duration and serum homocysteine levels is sparse. The aim of this study is to examine the association between sleep duration and serum homocysteine levels from a national database.

Methods: In total, 4,480 eligible participants older than 20 years who had serum homocysteine data and reported sleep duration were enrolled from the US National Health and Nutrition Examination Survey of 2005 to 2006. The association between sleep duration and serum homocysteine levels was analyzed using multivariate regression models for covariate adjustment.

Results: Serum homocysteine level was lowest in individuals with a sleep duration of 7 hours and increased in those with both shorter and longer self-reported total sleep time (groups were categorized into ≤ 5 hours, 6 hours, 7 hours, 8 hours, and ≥ 9 hours). After adjustment for covariates, those in the group sleeping ≤ 5 hours had significantly higher serum homocysteine levels than the reference group (sleep duration of 7 hours). In subgroup analyses by sex, body mass index (BMI), and ethnicity, the association between short sleep duration (≤ 5 hours) and higher serum homocysteine levels persisted in women, individuals with obesity (BMI ≥ 30 kg/m^2), and non-Hispanic whites.

Conclusions: This study highlighted that short sleep duration was associated with higher serum homocysteine levels in women, individuals with obesity (BMI ≥ 30 kg/m^2), and non-Hispanic whites; this finding might suggest increased vulnerability to cardiovascular risk or other atherothrombotic events in these groups in the context of short sleep.

Keywords: cardiovascular risk, homocysteine, sleep duration

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BRIEF SUMMARY

Current Knowledge/Study Rationale: Both short sleep duration and increased serum homocysteine levels are associated with cardiovascular events; however, direct evidence of the relationship between sleep duration and serum homocysteine levels is limited. This study used a national database with large sample size, stratified by sex, obesity, and race to assess the association between sleep duration and serum homocysteine levels.

Study Impact: The current study demonstrates that an extremely short sleep duration (≤ 5 hours) is significantly associated with increased serum homocysteine levels. Notably, when relevant covariates were considered, the association between short sleep duration (≤ 5 hours) and higher serum homocysteine levels was true for women, individuals with obesity (body mass index ≥ 30 kg/m^2), and only in non-Hispanic whites, which highlights the potential vulnerability of certain groups.

INTRODUCTION

Both short and long sleep durations have been associated with morbidity and mortality.[1–3] Emerging epidemiological research shows that self-reported short sleep duration (≤ 6 hours) has increased by 31% in the United States since 1985,[4] and another study among participants from 10 countries found that long sleep duration (> 9 hours) was more widespread than was short sleep duration.[5] Thus, short and long sleep duration and the association between health outcomes have gained great importance in recent years.

Cardiovascular diseases are the leading cause of death globally. Accumulating evidence has indicated that short (< 6 hours) or long (> 9 hours) sleep duration is associated with obesity,[3,6] hypertension,[7] stroke,[8] and cardiovascular diseases.[9–11] In addition, several inflammatory markers for cardiovascular risk such as C-reactive protein, interleukin-6, and tumor necrosis factor alpha are related to extremely short or long sleep duration.[12–14]

Homocysteine is a sulfur-containing amino acid that is an intermediate product in the conversion of methionine to cysteine. The primary atherogenic mechanisms of homocysteine include increased intima thickness, platelet accumulation, elastic lamina disruption, and arterial thrombosis.[15,16] Many studies have shown a clear correlation between increased serum homocysteine levels and cardiovascular diseases and related mortality.[17–19] The relationship between sleep duration and homocysteine is not clear; one recent study investigated nutritional biomarkers and sleep conditions and found that short sleep duration might be associated with increased serum homocysteine levels.[20] However, many possible confounding factors related to cardiovascular events were not considered in the previous study.

The aim of this study was to investigate the relationship between sleep duration and serum homocysteine levels by examining data from the 2005–2006 National Health and Nutrition Examination Survey (NHANES). We hypothesized that extreme sleep durations (≤ 5 hours, ≥ 9 hours) would be associated with increased serum homocysteine levels.

METHODS

Study Populations

The individuals in this study were derived from the 2005–2006 NHANES, a national survey designed by the National Center for Health Statistics of the Centers for Diseases Control and Prevention. NHANES is a continuing series of cross-sectional and multistage population-based surveys to evaluate the health and nutritional status of United States residents. Extensive household interviews, physical examinations, and laboratory tests were collected in the datasets. The NHANES database has been released on the NHANES website and has been freely accessible for download and analysis since 1999.

Ethics Statement

The datasets from NHANES were approved by the National Center for Health Statistics (NCHS) Institutional Review Board in compliance with the revised Declaration of Helsinki. All informed consent was obtained prior to data collection.

Measurement of the Level of Serum Homocysteine

The Abbott homocysteine assay, a fully automated fluorescence polarization immunoassay from Abbott Diagnostics (Abbott Park, Illinois, United States) was used to measure the level of serum homocysteine. The transformation of homocysteine into S-adenosyl-homocysteine is catalyzed by S-adenosyl-homocysteine hydrolase in the use of added adenosine. In the following steps, the fluorescence polarization immunoassay detection system uses a specific monoclonal antibody and a fluoresceinated S-adenosyl-homocysteine analog tracer. The level of serum homocysteine was calculated by the Abbott Axsym using a machine-stored calibration curve.

Measurement of Sleep Duration

Sleep duration was assessed with the survey item, "How much sleep do you usually get at night on weekdays or workdays?" Answers were recorded, in hours, in whole numbers by the participants.

Assessment of Covariates

The associated information concerning variables, such as age, sex, race/ethnicity (Mexican American, other Hispanic, non-Hispanic white, and non-Hispanic black), body mass index (BMI), smoking, alcohol consumption, mental health service use, snorting/gasping during sleep, and medical conditions diagnosed by doctors, including sleep disorders, asthma, coronary heart disease, congestive heart failure, angina pectoris, stroke and cancer/malignancy, were collected by self-report. BMI was calculated as measured weight in kilograms divided by the square of height in meters. Smoking status was assessed with the item, "Smoked at least 100 cigarettes in life." Alcohol consumption was evaluated by the item, "Had at least 12 alcoholic drinks/1 year?"; a drink was equal to 12 ounces of beer, a 5-ounce glass of wine, or 1.5 ounces of liquor. Mental health service use was assessed by the question, "During the past 12 months, have you seen or talked to a mental health professional such as a psychologist, psychiatrist, psychiatric nurse or clinical social worker about your health?"; snorting or stop breathing was assessed with the item, "How often do you snort/stop breathing in the past 12 months?" The answers were distinguished as either a clinical problem (1–2 nights/wk to 5 or more nights/wk) or not. The sleep disorders were evaluated by the question, "Have you ever been told by a doctor or other health professional that you have a sleep disorder (eg, sleep apnea, insomnia, restless legs, and others)?"

The levels of C-reactive protein (CRP) were quantified with latex-enhanced nephelometry using a Behring Nephelometer System (Dade Behring Diagnostics, Inc, Newark, Delaware, United States). Total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured enzymatically using Roche Hitachi 717 and 912 analyzers (Hitachi, Tokyo, Japan). Total bilirubin and creatine were assessed using automated biochemical profiling (Beckman Synchron LX20; Beckman Coulter Inc, Fullerton, California, United States). Fasting glucose was measured by the hexokinase enzymatic method according to the Roche Hitachi 911 analyzers. Insulin was assessed using the enzyme-linked immunosorbent assay method with Merocodia Insulin assays (Mercodia-ab, Uppsala, Sweden). Vitamin B6 was measured using a high-performance liquid chromatographic method by National Center for Environmental Health (Centers for Disease Control and Prevention, Atlanta, Georgia, United States). Vitamin B12 and folate were assessed by the Bio-Rad Quantaphase II procedure (Bio-Rad Laboratories, Hercules, California, United States). All calculations and protocols used standardized methods with certificated accuracy according to the Centers for Disease Control and Prevention guidelines.

Statistical Analysis

Sleep duration was operationalized categorically to ≤ 5, 6, 7, 8, and ≥ 9 hours. Chi-square for trend test was used in the analysis of descriptive data. Because a 7-hour sleep duration has been associated with a lower risk of premature death,[21] we

chose 7-hour sleepers as a reference group based on previous similar studies.[9,12,22,23]

The association between sleep duration and serum homocysteine level was evaluated by multivariate linear regression with homocysteine as the dependent, continuous variable (μmol/L, natural log transformation). Three different models were used for control for various complements of potential confounders in the determination of the association of sleep duration classes with homocysteine value. Model 1 was adjusted for age, sex, and race and ethnicity. Model 2 included model 1 plus BMI, CRP, total bilirubin, total cholesterol, triglycerides, HDL, creatine, fasting glucose, insulin, vitamin B6, vitamin B12, and folate. Model 3 included model 2 plus sleep disorders, snorting/stop breathing, mental health service use, asthma, congestive heart failure, coronary heart disease, angina, stroke, cancer, smoking, and alcohol consumption.

We dichotomized the dependent variable, homocysteine, based on 75th percentile of the log homocysteine level, 9.74 μmol/L. The odds of elevated homocysteine level given different classes of sleep duration was determined by multivariate logistic regression. Three different models as previously mentioned were also used to control for potential confounders.

We stratified the population by sex (male, female), BMI (< 30, ≥ 30 kg/m^2), and ethnicity to further evaluate the association between sleep duration and serum homocysteine levels. We used both multivariate linear and logistic regression (again using three different models to control for potential confounders) to determine within which groups there was still (1) an association between sleep duration and log homocysteine level and (2) an association between sleep duration and likelihood of elevated (dichotomized at the 75th percentile) homocysteine.

All data analyses were done with the Statistical Product and Service Solutions version 18.0 for Windows (SPSS Inc., Chicago, Illinois, United States). Two-sided $P < .05$ were considered statistically significant.

RESULTS

Characteristics and Demographic Data of Participants

In total, 4,480 participants, who both had homocysteine data and reported sleep duration, were included in the NHANES dataset from 2005 to 2006. **Table 1** presents the demographic and clinical characteristics of participants categorized by different sleep duration. The mean age was 48.36 ± 18.91 years and 48% of the participants were men. In addition, men predominated the ≤ 5 hour sleep duration group and the percentage of men decreased with longer sleep duration, whereas women made up increasingly higher percentages of the longer sleep duration categories. Individuals with sleep disorders and frequent snorting/gasping also comprised a higher percentage of the ≤ 5-hour sleep duration group.

Association Between Sleep Duration and Serum Homocysteine

Participants with a 7-hour sleep length had the lowest serum homocysteine level; serum homocysteine levels increased in participants with either shorter or longer sleep duration. The results from the regression analysis between sleep duration and log serum homocysteine levels are shown in **Table 2**. Compared with the reference group (7- hour sleepers), participants with the shortest sleep duration (≤ 5 hours) had higher serum homocysteine levels. In model 1, which included an adjustment for age, sex, and race, the regression coefficient of the shortest sleep duration (≤ 5 hours) compared with the reference group (7-hour sleepers) was 0.059 ($P = .009$). After further adjustment for multiple covariates in models 2 and 3, the results remained statistically significant with higher serum homocysteine levels in the group with ≤ 5-hour sleep duration. In the fully adjusted model, compared to sleepers in the 7- hour group, individuals in the ≤ 5-hour group had log homocysteine values that were 0.046 higher.

Log homocysteine was dichotomized at the 75th percentile (9.74 μmol/L). **Table 3** presents the odds of high homocysteine at different sleep durations. Compared with the participants with 7-hour sleep length, those with ≤ 5-hour sleep length had greater odds for high homocysteine levels in all three models (model 1: odds ratio [OR] 1.962, 95% confidence interval [CI] 1.307–2.944; model 2: OR 1.854, 95% CI 1.195–2.876; and model 3: OR 1.769, 95% CI 1.131–2.766).

Association of Sleep Duration and Homocysteine After Stratification for Sex, BMI, and Ethnicity

Linear and logistic regression models adjusted for various potential confounders and separated by sex are presented in **Table 4**. In model 1 (adjusted for age and race only), women (β = .076, $P = .028$) but not men (β = .040, $P = .159$) demonstrated an association between short sleep duration (≤ 5 hours) and log homocysteine levels. Additionally, women with short sleep duration had an increased odds of elevated homocysteine (OR 2.691, $P = .010$) that was not seen in men (OR 1.357, $P = .355$). In women, the association between short sleep duration and homocysteine (both as a continuous and dichotomous outcome) persisted in fully adjusted models.

Results of linear and logistic regression models that were adjusted for various potential confounders and categorized based on BMI (≥ 30 versus < 30 kg/m^2) are presented in **Table 5**. In model 1, participants with BMI ≥ 30 kg/m^2 (β = .091, $P = .008$) demonstrated an association between short sleep duration (≤ 5 hours) and log homocysteine levels, which persisted in fully adjusted models (model 2: β = .063, $P = .033$; model 3: β = .062, $P = .039$). However, the odds of elevated homocysteine (when considered as a dichotomous outcome) were not increased in the BMI ≥ 30 kg/m^2 group (model 1: OR 1.787, $P = .161$; model 2: OR 1.646, $P = .300$; model 3: OR 1.847, $P = .226$).

We also used linear and logistic regression models adjusted for various potential confounders to investigate ethnicity-specific association between sleep duration and homocysteine levels, as shown in **Table 6**. An association between short sleep duration (≤ 5 hours) and log homocysteine level was found only in the non-Hispanic white group and persisted in the fully adjusted model (model 1: β = .089, $P = .008$; model 2: β = .072, $P = .022$; model 3: β = .068, $P = .032$). However, the odds of elevated homocysteine (when considered as a dichotomous outcome) were not increased in the non-Hispanic white group

Table 1—Characteristics of study participants.

Characteristic	Sleep Duration at Night ≤ 5 hours (n = 677)	6 hours (n = 1,008)	7 hours (n = 1,190)	8 hours (n = 1,240)	≥ 9 hours (n = 365)	Total (n = 4,480)	*P*
Continuous variables							
Age (years) **	47.90 (17.90)	48.12 (17.95)	46.97 (17.75)	49.44 (20.00)	50.81 (22.46)	48.36 (18.91)	.002
BMI (kg/m^2) ***	30.19 (7.59)	29.14 (6.76)	28.23 (5.79)	28.34 (6.40)	28.58 (8.66)	28.79 (6.76)	< .001
Homocysteine (μmol/L) **	9.12 (6.92)	8.42 (3.31)	8.28 (4.70)	8.29 (3.79)	8.67 (4.22)	8.47 (4.59)	.001
CRP (mg/dL) **	0.51 (0.70)	0.48 (0.84)	0.42 (0.86)	0.51 (0.88)	0.59 (0.89)	0.49 (0.84)	.008
Total bilirubin (mg/dL) *	0.70 (0.28)	0.70 (0.29)	0.73 (0.48)	0.69 (0.28)	0.67 (0.28)	0.70 (0.35)	.041
Total cholesterol (mg/dL)	198.54 (46.18)	198.90 (40.75)	201.70 (45.22)	202.60 (43.85)	200.66 (44.69)	200.76 (43.99)	.188
Triglyceride (mg/dL)	154.85 (130.50)	145.54 (101.07)	157.79 (141.20)	160.19 (120.64)	155.14 (94.24)	155.04 (122.21)	.066
HDL cholesterol (mg/dL)	54.01 (16.95)	54.68 (15.90)	55.36 (17.04)	55.62 (17.18)	56.29 (16.81)	55.15 (16.80)	.156
Creatinine (mg/dL)	0.95 (0.45)	0.97 (0.42)	0.92 (0.30)	0.94 (0.58)	0.92 (0.33)	0.94 (0.44)	.135
Fasting glucose (mg/dL)	106.11 (34.85)	105.07 (35.09)	101.67 (22.52)	105.96 (36.63)	107.44 (40.93)	104.84 (33.40)	.124
Insulin (μU/mL)	12.85 (11.96)	12.46 (11.34)	12.47 (18.46)	11.87 (12.27)	10.91 (9.35)	12.23 (13.69)	.532
Vitamin B6 (nmol/L) ***	61.38 (74.71)	67.99 (86.96)	76.30 (85.25)	74.89 (99.50)	61.11 (67.87)	70.55 (87.29)	< .001
Vitamin B12 (pg/mL)	637.60 (2740.25)	601.71 (1491.89)	598.25 (1465.21)	577.36 (805.89)	530.57 (407.01)	593.74 (1547.91)	.865
Folate (ng/mL) ***	12.28 (9.22)	13.60 (9.51)	13.67 (8.50)	14.52 (10.07)	15.36 (13.25)	13.82 (9.76)	< .001
Categorical Variables							
Male **	344 (50.8)	497 (49.3)	581 (48.8)	586 (47.3)	142 (38.9)	2150 (48.0)	.004
Race-ethnicity ***							< .001
Mexican American	124 (18.3)	196 (19.4)	238 (20.0)	282 (22.7)	69 (18.9)	909 (20.3)	
Other Hispanic	21 (3.1)	32 (3.2)	46 (3.9)	33 (2.7)	6 (1.6)	138 (3.1)	
Non-Hispanic white	258 (38.1)	448 (44.4)	666 (56.0)	682 (55.0)	202 (55.3)	2256 (50.4)	
Non-Hispanic black	246 (36.3)	288 (28.6)	193 (16.2)	202 (16.3)	72 (19.7)	1001 (22.3)	
Sleep disorders ***	100 (14.8)	67 (6.6)	59 (5.0)	59 (4.8)	21 (5.8)	306 (6.8)	< .001
Snorting/stop breathing ***	160 (26.6)	224 (24.3)	189 (17.1)	194 (17.2)	56 (17.0)	823 (20.2)	< .001
Mental health service use **	69 (10.2)	60 (6.0)	75 (6.3)	82 (6.6)	38 (10.4)	324 (7.2)	.001
Asthma ***	121 (17.9)	135 (13.4)	132 (11.1)	143 (11.5)	44 (12.1)	575 (12.8)	< .001
Congestive heart failure ***	38 (5.6)	31 (3.1)	20 (1.7)	44 (3.5)	15 (4.1)	148 (3.3)	< .001
Coronary heart disease	33 (4.9)	43 (4.3)	28 (2.4)	58 (4.7)	13 (3.6)	175 (3.9)	.056
Angina pectoris	32 (4.7)	31 (3.1)	29 (2.4)	34 (2.7)	11 (3.0)	137 (3.1)	.119
Stroke ***	36 (5.3)	41 (4.1)	34 (2.9)	34 (2.7)	28 (7.7)	173 (3.9)	< .001
Cancer/malignancy **	47 (6.9)	68 (6.7)	99 (8.3)	111 (9.0)	46 (12.6)	371 (8.3)	.001
Smoking	341 (50.4)	488 (48.4)	542 (45.5)	579 (46.7)	171 (46.8)	2121 (47.3)	.464
Alcohol drinking **	395 (62.9)	650 (70.0)	801 (71.7)	793 (69.0)	220 (64.7)	2859 (68.7)	.003

Continuous variables are presented as mean (standard deviation). Categorical variables are presented as number (percentage). Asterisks indicate statistical significance: * = $P < .05$, ** = $P < .01$, *** = $P < .001$. BMI = body mass index, CRP = C-reactive protein, HDL = high-density lipoprotein.

(model 1: OR 1.732, $P = .121$; model 2: OR 1.758, $P = .128$; model 3: OR 1.532, $P = .274$). We did not observe a positive association between sleep duration and homocysteine (both as continuous and dichotomous variables) among the Mexican American, other Hispanic, and non-Hispanic black groups. In addition, when homocysteine was considered a dichotomous outcome, some of the analyses (Mexican American with ≥ 9 hour sleep duration and other Hispanic group) could not be conducted because of the small sample size.

DISCUSSION

Using a large, nationally representative sample of the United States population of individuals older than 20 years, the current study demonstrates that an extremely short sleep duration (≤ 5 hours) is significantly associated with increased serum homocysteine levels. Notably, we found that the association between short sleep duration (≤ 5 hours) and higher serum homocysteine levels was associated with women and not men, obesity (BMI ≥ 30 kg/m^2), and only in non-Hispanic whites.

Direct evidence of the relationship between sleep duration and serum homocysteine levels is limited. In agreement with our findings, the study of Beydoun et al.,[20] which also used the NHANES data, found that increased homocysteine levels were related to short sleep duration. However, the aim of the previous study was to investigate the association between different kinds of nutritional biomarkers and sleep conditions; as such, many confounding factors for cardiovascular events were not included. Our study focused on the relationship between sleep duration and serum homocysteine levels, included a larger sample size, carefully conducted subgroup analyses, and controlled for important comorbid conditions and cardiovascular risk factors. Another study investigated 21 healthy older adults and 21 individuals with

Table 2—Association between different sleep duration and homocysteine level.

Models	Sleep Duration †	β (95% CI) ‡	*P*
Model 1	≤ 5 hours **	0.059 (0.015, 0.103)	.009
	6 hours	0.030 (−0.011, 0.070)	.149
	7 hours	reference	–
	8 hours	−0.024 (−0.062, 0.014)	.217
	≥ 9 hours	0.029 (−0.026, 0.084)	.301
Model 2	≤ 5 hours *	0.050 (0.009, 0.091)	.016
	6 hours	0.016 (−0.021, 0.053)	.400
	7 hours	reference	–
	8 hours	−0.008 (−0.042, 0.027)	.669
	≥ 9 hours	0.045 (−0.005, 0.095)	.078
Model 3	≤ 5 hours *	0.046 (0.005, 0.087)	.029
	6 hours	0.014 (−0.023, 0.051)	.473
	7 hours	reference	–
	8 hours	−0.009 (−0.044, 0.026)	.618
	≥ 9 hours	0.048 (−0.002, 0.098)	.061

Adjusted covariates: model 1 = age, sex, race; model 2 = model 1 + (BMI, CRP, total bilirubin, total cholesterol, triglycerides, HDL, creatine, fasting glucose, insulin, vitamin B6 & B12, folate). Model 3 = model 2 + (sleep disorders, snorting/stop breathing, mental health service use, asthma, congestive heart failure, coronary heart disease, angina, stroke, cancer) + (smoking, alcohol drinking). † = individuals with 7-hour sleep length were the reference group. ‡ = β coefficient can be interpreted as differences in the mean homocysteine comparing individuals in the other 4 groups of sleep duration to those in the 7-hour sleep length. Asterisks indicate statistical significance: * = $P < .05$, ** = $P < .01$. BMI = body mass index, CI = confidence interval, CRP = C-reactive protein, HDL = high-density lipoprotein.

Table 3—Association between different sleep duration and presence of high homocysteine level.

Models	Sleep Duration †	OR (95% CI) ‡	*P*
Model 1	≤ 5 hours **	1.962 (1.307, 2.944)	.001
	6 hours	1.114 (0.754, 1.645)	.588
	7 hours	reference	–
	8 hours	0.995 (0.687, 1.442)	.981
	≥ 9 hours	1.529 (0.908, 2.577)	.110
Model 2	≤ 5 hours **	1.854 (1.195, 2.876)	.006
	6 hours	1.019 (0.662, 1.567)	.932
	7 hours	reference	–
	8 hours	1.028 (0.685, 1.542)	.896
	≥ 9 hours	1.565 (0.884, 2.771)	.124
Model 3	≤ 5 hours *	1.769 (1.131, 2.766)	.012
	6 hours	1.012 (0.655, 1.562)	.958
	7 hours	reference	–
	8 hours	1.004 (0.667, 1.511)	.986
	≥ 9 hours	1.572 (0.886, 2.789)	.122

High homocysteine level defined as more than the 75th percentile of homocysteine level, cutoff point: 9.74 μmol/L. Adjusted covariates: model 1 = age, sex, race. Model 2 = model 1 + (BMI, CRP, total bilirubin, total cholesterol, triglycerides, HDL, creatine, fasting glucose, insulin, vitamin B6 & B12, folate). Model 3 = model 2 + (sleep disorders, snorting/stop breathing, mental health service use, asthma, congestive heart failure, coronary heart disease, angina, stroke, cancer) + (smoking, alcohol drinking). † = individuals with 7-hour sleep length were the reference group. ‡ = OR of different sleep duration for patients with and without high homocysteine level (more than the 75th percentile of homocysteine level, cutoff point: 9.74 μmol/L). Asterisks indicate statistical significance: * = $P < .05$, ** = $P < .01$. BMI = body mass index, CI = confidence interval, CRP = C-reactive protein, HDL = high-density lipoprotein, OR = odds ratio.

Table 4—Sex-specific association between sleep duration and homocysteine level.

Models	Sleep Duration †	Men		Women	
		β (95% CI) ‡	OR (95% CI) §	β (95% CI) ‡	OR (95% CI) §
Model 1	≤ 5 hours	0.040 (−0.016, 0.097)	1.357 (0.711, 2.589)	0.076 (0.008, 0.144) *	2.691 (1.268, 5.709) *
	6 hours	−0.003 (−0.056, 0.051)	1.025 (0.545, 1.927)	0.063 (0.004, 0.122)	1.209 (0.589, 2.484)
	7 hours	reference	reference	reference	reference
	8 hours	0.007 (−0.043, 0.058)	0.775 (0.408, 1.472)	−0.044 (−0.099, 0.122)	0.695 (0.317, 1.522)
	≥ 9 hours	0.078 (−0.002, 0.159)	1.591 (0.573, 4.417)	0.015 (−0.059, 0.089)	1.206 (0.411, 3.539)
Model 2	≤ 5 hours	0.021 (−0.032, 0.074)	1.144 (0.569, 2.303)	0.086 (0.026, 0.145) **	4.207 (1.717, 10.307) **
	6 hours	−0.011 (−0.062, 0.039)	0.924 (0.457, 1.869)	0.032 (−0.019, 0.084)	1.472 (0.610, 3.553)
	7 hours	reference	reference	reference	reference
	8 hours	−0.003 (−0.050, 0.045)	0.638 (0.316, 1.288)	0.013 (−0.036, 0.061)	1.216 (0.475, 3.109)
	≥ 9 hours	0.073 (−0.003, 0.148)	1.843 (0.609, 5.581)	0.045 (−0.019, 0.110)	1.084 (0.294, 3.995)
Model 3	≤ 5 hours	0.020 (−0.034, 0.074)	1.003 (0.476, 2.113)	0.075 (0.015, 0.135) *	4.565 (1.737, 11.997) **
	6 hours	−0.012 (−0.062, 0.039)	0.961 (0.462, 2.000)	0.024 (−0.027, 0.076)	1.607 (0.637, 4.053)
	7 hours	reference	reference	reference	reference
	8 hours	−0.005 (−0.052, 0.043)	0.597 (0.291, 1.226)	0.013 (−0.035, 0.062)	1.271 (0.487, 3.318)
	≥ 9 hours	0.077 (0.001, 0.154)	1.620 (0.493, 5.321)	0.044 (−0.021, 0.108)	1.014 (0.254, 4.049)

Adjusted covariates: model 1 = age, race. Model 2 = model 1 + (BMI, CRP, total bilirubin, total cholesterol, triglycerides, HDL, creatine, fasting glucose, insulin, vitamin B6 & B12, folate). Model 3 = model 2 + (sleep disorders, snorting/stop breathing, mental health service use, asthma, congestive heart failure, coronary heart disease, angina, stroke, cancer) + (smoking, alcohol drinking). † = individuals with 7-hour sleep length were the reference group. ‡ = β coefficient can be interpreted as differences in the mean homocysteine comparing individuals in the other 4 groups of sleep duration to those in the 7-hour sleep length. § = OR of different sleep duration for patients with and without high homocysteine level (more than the 75th percentile of homocysteine level, cutoff point: 9.74 μmol/L). Asterisks indicate statistical significance: * = $P < .05$, ** = $P < .01$. BMI = body mass index, CI = confidence interval, CRP = C-reactive protein, HDL = high-density lipoprotein, OR = odds ratio.

mild cognitive impairment and found that shorter sleep duration may contribute to elevated homocysteine levels and oxidative stress.[24] The other animal study found that sleep deprivation may reduce rather than enhance homocysteine levels in rats.[25] However, no related evidence was collected in human studies.

Table 5—BMI specific association between sleep duration and homocysteine level.

Models	Sleep Duration †	BMI < 30 kg/m²		BMI ≥ 30 kg/m²	
		β (95% CI) ‡	OR (95% CI) §	β (95% CI) ‡	OR (95% CI) §
Model 1	≤ 5 hours	0.041 (−0.018, 0.099)	1.815 (0.970, 3.398)	0.091 (0.023, 0.158) **	1.787 (0.794, 4.023)
	6 hours	0.025 (−0.028, 0.078)	0.838 (0.447, 1.571)	0.042 (−0.020, 0.104)	1.621 (0.768, 3.420)
	7 hours	reference	reference	reference	reference
	8 hours	−0.025 (−0.072, 0.023)	0.849 (0.470, 1.533)	−0.033 (−0.097, 0.031)	0.458 (0.175, 1.195)
	≥ 9 hours	0.038 (−0.032, 0.108)	1.030 (0.414, 2.561)	0.001 (−0.089, 0.088)	1.728 (0.500, 5.973)
Model 2	≤ 5 hours	0.035 (−0.019, 0.089)	1.670 (0.836, 3.335)	0.063 (0.005, 0.120) *	1.646 (0.641, 4.227)
	6 hours	0.005 (−0.044, 0.054)	0.906 (0.450, 1.821)	0.030 (−0.023, 0.083)	1.648 (0.669, 4.060)
	7 hours	reference	reference	reference	reference
	8 hours	−0.016 (−0.060, 0.028)	0.864 (0.443, 1.684)	−0.005 (−0.060, 0.049)	0.393 (0.124, 1.244)
	≥ 9 hours	0.058 (−0.006, 0.122)	0.976 (0.350, 2.726)	0.011 (−0.065, 0.086)	1.964 (0.459, 8.403)
Model 3	≤ 5 hours	0.029 (−0.025, 0.084)	1.545 (0.746, 3.197)	0.062 (0.003, 0.121) *	1.847 (0.684, 4.985)
	6 hours	0.003 (−0.046, 0.053)	0.938 (0.457, 1.927)	0.030 (−0.024, 0.083)	1.742 (0.682, 4.451)
	7 hours	reference	reference	reference	reference
	8 hours	−0.019 (−0.063, 0.025)	0.865 (0.438, 1.705)	−0.004 (−0.059, 0.051)	0.386 (0.119, 1.253)
	≥ 9 hours	0.063 (−0.001, 0.128)	0.874 (0.300, 2.547)	0.009 (−0.067, 0.085)	1.931 (0.436, 8.552)

Adjusted covariates: model 1 = age, sex, race. Model 2 = model 1 + (BMI, CRP, total bilirubin, total cholesterol, triglycerides, HDL, creatine, fasting glucose, insulin, vitamin B6 & B12, folate). Model 3 = model 2 + (sleep disorders, snorting/stop breathing, mental health service use, asthma, congestive heart failure, coronary heart disease, angina, stroke, cancer) + (smoking, alcohol drinking). † = individuals with 7-hour sleep length were the reference group. ‡ = β coefficient can be interpreted as differences in the mean homocysteine comparing individuals in the other 4 groups of sleep duration to those in the 7-hour sleep length. § = OR of different sleep duration for patients with and without high homocysteine level (more than the 75th percentile of homocysteine level, cutoff point: 9.74 μmol/L). Asterisks indicate statistical significance: * = $P < .05$, ** = $P < .01$. BMI = body mass index, CI = confidence interval, CRP = C-reactive protein, HDL = high-density lipoprotein, OR = odds ratio.

The aforementioned studies reported inconsistent relationships between sleep duration and homocysteine levels. One potential explanation for the different results between studies that were conducted in humans and rats is species differences. Compared with previous studies, we carefully adjusted for cardiovascular risk factors, such as BMI, CRP, lipid profile, insulin, smoking, alcohol consumption, and previous cardiovascular diseases. Adjusting for cardiovascular risk factors could strengthen the association between short sleep duration and increased serum homocysteine levels.

Many studies have reported that extremely short sleep duration (< 6 hours) is associated with cardiovascular events.[11,13,26–30] One recently published study included 4,437 participants and found that those with short sleep duration (< 6 hours) had a 29% higher risk of incident cardiovascular disease.[11] In addition, a recent systematic review and meta-analysis reviewed 153 studies and found that a short sleep duration is significantly associated with cardiovascular diseases, coronary heart diseases, and all-cause mortality.[26] Another Dutch population-based cohort study with 10 to 15 years of follow-up found a 15% higher risk of total cardiovascular diseases and a 23% higher risk in coronary heart diseases in short sleepers (≤ 6 hours), and no association was found in long sleepers (≥ 9 hours).[27] Furthermore, several studies reported that short sleep or sleep deprivation may play a role in inflammation, thus increasing the risk of cardiovascular diseases.[13,28–30] Our study found that short sleep duration was associated with increased levels of serum homocysteine. Because increased homocysteine levels are considered an independent risk factor for cardiovascular diseases,[17,18] further studies are needed to better understand the relationships among short sleep duration, homocysteine levels, and cardiovascular events.

In this study, we have several noteworthy findings. We used 9.74 μmol/L, the 75th percentile value of the serum homocysteine levels, as a cutoff point and found that participants with short sleep duration (≤ 5 hours) had a higher risk of increased serum homocysteine levels (OR 1.769). Currently, the actual cutoff value of serum homocysteine levels for predicting cardiovascular risk remains unclear. Humphrey et al. conducted a meta-analysis and found that each increase of 5 μmol/L in homocysteine levels increases the risk of coronary heart disease events by approximately 20%.[31] A study of Chinese participants reported a cutoff value of 9.47 μmol/L for cardiovascular events and 11.84 μmol/L for all-cause death.[32] Veeranna et al. classified homocysteine levels into three groups (< 10, 10–14.9, and ≥ 15 μmol/L) and found that the highest homocysteine levels (≥ 15 μmol/L) significantly predicted cardiovascular events.[33] In general, although these studies used different homocysteine cutoff values, our findings still indicated that people with short sleep duration (≤ 5 hours) have almost double the odds of being in the highest quartile of homocysteine levels than 7-hour sleepers.

Our subgroup analyses revealed that associations between homocysteine and sleep duration were specific to certain groups. We found that only women had a significant association between short sleep duration (≤ 5 hours) and increased serum homocysteine levels. In general, the level of plasma homocysteine is higher in men than in women and increases with age.[34] Notably, many studies have found that women show a stronger association than men between sleep duration and cardiovascular diseases.[7,22,35,36] Two large population-based studies in Finland and the United Kingdom found that sleep duration was only an independent risk factor for cardiovascular mortality and morbidity in women.[37,38] Other direct evidence showed that

Table 6—Ethnicity specific association between sleep duration and homocysteine level.

Models	Sleep Duration †	Mexican American		Other Hispanic	
		β (95% CI) ‡	OR (95% CI) §	β (95% CI) ‡	OR (95% CI) §
Model 1	≤ 5 hours	0.009 (−0.093, 0.110)	2.206 (0.497, 9.790)	0.062 (−0.136, 0.260)	–
	6 hours	0.036 (−0.054, 0.127)	0.791 (0.177, 3.533)	0.100 (−0.080, 0.280)	–
	7 hours	reference	reference	reference	–
	8 hours	−0.034 (−0.115, 0.048)	1.678 (0.427, 6.598)	−0.022 (−0.220, 0.176)	–
	≥ 9 hours	−0.030 (−0.152, 0.092)	–	−0.048 (−0.371, 0.274)	–
Model 2	≤ 5 hours	0.026 (−0.062, 0.114)	1.005 (0.156, 6.471)	0.100 (−0.094, 0.295)	–
	6 hours	0.023 (−0.057, 0.104)	0.442 (0.068, 2.850)	0.035 (−0.137, 0.208)	–
	7 hours	reference	reference	reference	–
	8 hours	−0.001 (−0.074, 0.072)	2.294 (0.419, 12.548)	0.033 (−0.178, 0.245)	–
	≥ 9 hours	0.022 (−0.085, 0.129)	–	−0.042 (−0.345, 0.261)	–
Model 3	≤ 5 hours	0.034 (−0.058, 0.127)	0.788 (0.080, 7.729)	0.144 (−0.084, 0.372)	–
	6 hours	0.024 (−0.059, 0.107)	0.451 (0.050, 4.049)	−0.008 (−0.207, 0.191)	–
	7 hours	reference	reference	reference	–
	8 hours	0.001 (−0.076, 0.075)	1.791 (0.269, 11.920)	−0.012 (−0.238, 0.215)	–
	≥ 9 hours	0.025 (−0.084, 0.134)	–	−0.156 (−0.520, 0.208)	–
Models	**Sleep Duration †**	**Non-Hispanic White**		**Non-Hispanic Black**	
		β (95% CI) ‡	**OR (95% CI) §**	**β (95% CI) ‡**	**OR (95% CI) §**
Model 1	≤ 5 hours	0.089 (0.023, 0.154) **	1.703 (0.869, 3.335)	0.023 (−0.060, 0.107)	2.268 (0.808, 6.365)
	6 hours	0.015 (−0.043, 0.072)	0.802 (0.421, 1.525)	0.031 (−0.052, 0.114)	2.178 (0.789, 6.016)
	7 hours	reference	reference	reference	reference
	8 hours	−0.049 (−0.099, 0.002)	0.580 (0.314, 1.072)	0.008 (−0.082, 0.098)	0.652 (0.189, 2.249)
	≥ 9 hours	0.044 (−0.029, 0.117)	1.477 (0.614, 3.554)	0.058 (−0.072, 0.188)	2.886 (0.512, 16.269)
Model 2	≤ 5 hours	0.072 (0.010, 0.133) *	1.758 (0.850, 3.637)	0.009 (−0.065, 0.083)	1.865 (0.574, 6.065)
	6 hours	0.021 (−0.032, 0.075)	1.007 (0.492, 2.060)	−0.007 (−0.081, 0.066)	1.329 (0.410, 4.310)
	7 hours	reference	reference	reference	reference
	8 hours	−0.030 (−0.077, 0.017)	0.653 (0.329, 1.295)	−0.011 (−0.091, 0.068)	0.339 (0.078, 1.481)
	≥ 9 hours	0.059 (−0.009, 0.127)	1.542 (0.607, 3.918)	0.038 (−0.076, 0.153)	2.854 (0.358, 22.723)
Model 3	≤ 5 hours	0.068 (0.006, 0.130) *	1.532 (0.713, 3.291)	0.012 (−0.065, 0.089)	2.455 (0.664, 9.070)
	6 hours	0.017 (−0.037, 0.071)	0.920 (0.437, 1.938)	−0.007 (−0.081, 0.068)	1.853 (0.513, 6.691)
	7 hours	reference	reference	reference	reference
	8 hours	−0.035 (−0.082, 0.012)	0.609 (0.304, 1.221)	−0.009 (−0.090, 0.073)	0.329 (0.065, 1.678)
	≥ 9 hours	0.064 (−0.004, 0.132)	1.530 (0.581, 4.026)	0.023 (−0.094, 0.141)	3.495 (0.381, 32.057)

Adjusted covariates: model 1 = age, sex. Model 2 = model 1 + (BMI, CRP, total bilirubin, total cholesterol, triglycerides, HDL, creatine, fasting glucose, insulin, vitamin B6 & B12, folate). Model 3 = model 2 + (sleep disorders, snorting/stop breathing, mental health service use, asthma, congestive heart failure, coronary heart disease, angina, stroke, cancer) + (smoking, alcohol drinking). † = individuals with 7-hour sleep length were the reference group. ‡ = β coefficient can be interpreted as differences in the mean homocysteine comparing individuals in the other 4 groups of sleep duration to those in the 7-hour sleep length. § = OR of different sleep duration for patients with and without high homocysteine level (more than the 75th percentile of homocysteine level, cutoff point: 9.74 μmol/L). Mexican American with ≥ 9-hour sleep duration and Other Hispanic group were not able to evaluate the OR because of small sample size. Asterisks indicate statistical significance: * = $P < .05$, ** = $P < .01$. BMI = body mass index, CI = confidence interval, CRP = C-reactive protein, HDL = high-density lipoprotein, OR = odds ratio.

homocysteine levels, as measured by an immunoassay, showed a positive association with cardiovascular events in women but not in men.[39] Although the aforementioned evidence supports our sex-specific results, serum homocysteine levels have been found to vary across the menstrual cycle and are lower in premenopausal and pregnant women than in postmenopausal women.[40,41] It has also been reported that short sleep duration correlates with irregular menstrual cycles.[42] We were not able to evaluate individual menstrual cycle phases, and this characteristic may confound the results that were found among women. However, our findings can still remind women to consider the risks of short sleep duration.

We found that only participants with obesity (BMI ≥ 30 kg/m^2) presented a significant association between short sleep duration (≤ 5 hours) and increased serum homocysteine levels. Because of the high prevalence of sleep apnea in participants with obesity, we controlled for sleep disorders and snorting/gasping during sleep; however, the results remained unchanged. Many studies have demonstrated that obesity itself is highly associated with short sleep duration,[3,6,26] and investigations of the relationship between obesity and serum homocysteine levels have provided inconsistent results. Uysal et al. investigated 55 participants with obesity and found that serum homocysteine levels did not differ between healthy control patients and individuals with obesity who were free from atherosclerosis and impaired renal function.[43] Similarly, nonsignificant associations were also found in a study with 25 participants.[44] Another study with a larger sample size of 205 individuals with obesity observed moderately increased serum homocysteine levels, and stressful events were hypothesized to be mediators between obesity and homocysteine levels. Thus, short sleep duration may be a stressful event for individuals with obesity,

and further investigations into the risk factors for increased homocysteine levels and their associations with cardiovascular events are needed.

This research provided evidence of significant differences in the associations between sleep duration and serum homocysteine levels among different racial groups. Among Mexican American, other Hispanic, non-Hispanic white, and non-Hispanic black groups, only non-Hispanic whites presented a significant association between short sleep duration (≤ 5 hours) and increased serum homocysteine levels in every adjusted model. The evidence for an association between sleep duration and serum homocysteine levels by race/ethnicity is limited. Several studies have reported that serum homocysteine levels vary by race/ethnicity, nutritional support, and environments,[45,46] whereas other studies have found no ethnic differences in the association of homocysteine levels and cardiovascular events.[47,48] In general, white race is supposed to confer a lower risk of cardiovascular diseases compared with other ethnic groups,[49,50] but the association between sleep duration and ethnicity is not clear. Our findings indicate that short sleep duration might be a specific risk factor for non-Hispanic whites regarding cardiovascular events. Though we could not enroll equal numbers for each racial group and the other Hispanic group had a limited sample size, our study still brought to light the potential for racial differences. More studies will be needed to improve our understanding of ethnic differences in the association between sleep duration and homocysteine levels.

The strengths of the study include its use of a national survey with a large sample size and its use of a multiple-model approach to control pertinent confounding factors. In the subgroup analyses, groups characterized by sex, obesity, and race were also carefully assessed. Finally, we describe several reasonable putative explanations that may have implications for clinical practice.

Nonetheless, the study has several limitations. First, this is a cross-sectional observational study in which sleep duration and serum homocysteine levels were assessed only at one time; thus, we could not make causal inferences. Second, sleep duration was evaluated by self-reported questionnaires, which are subject to recall bias. In addition, sleep duration was assessed with the question "How much sleep do you usually get at night on weekdays or workdays?", without assessment of naps, sleep-wake schedule, and weekend sleep information. The lack of naps, sleep-wake schedule, and weekend sleep information is another point requiring further consideration. For example, frequent daytime naps in some patients might be suggestive of unrecognized sleep-disordered breathing, or patients who had sleep-wake schedule irregularities might increase sleep need with resultant sleep extension on weekends. Patients with those symptoms could not be appropriately assessed in our study. Third, the quality of sleep was not directly evaluated in the NHANES database. Therefore, self-reported accounts of diagnosed sleep disorders and the symptom of frequent snorting/gasping were used as a surrogate. Fourth, there are several important unmeasured confounding factors that may influence these results. For instance, recent psychosocial stress and individual lifestyles may influence sleep duration, and these were not assessed in our analyses. Finally, our findings may not be generalizable to different ethnic populations, as baseline homocysteine levels might vary by ethnicity, nutritional support, and environment. Hence, our results obtained for the serum homocysteine levels should be carefully interpreted.

CONCLUSIONS

This study shows that an extremely short sleep duration (≤ 5 hours) was closely associated with increased serum homocysteine levels in women, those with obesity, and in non-Hispanic whites. Very short sleep duration might negatively affect homocysteine metabolism and potentially lead to increased risk for cardiovascular diseases. Further longitudinal investigations concerning the effect of sleep deprivation on homocysteine alteration might help provide a better understanding of the pathogenesis of cardiometabolic risk.

ABBREVIATIONS

BMI, body mass index
CRP, C-reactive protein
HDL, high-density lipoprotein
NCHS, National Center for Health Statistics
NHANES, National Health and Nutrition Examination Survey

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DISCLOSURE STATEMENT

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