|  |  |  |  |
| --- | --- | --- | --- |
| Introduction | Points Allowed | Comment | Points Allocated |
| Thorough Literature Review | 10 | Good but maybe also talk about the confounders you will adjust for (how they are associated with the variables of primary interest. | 9 |
| Literature is synthesized (not a summary of each paper) | 10 | Well done | 10 |
| Organized into a structured argument justifying the need for this research study | 10 | Yes, nicely done! | 10 |
| Originality of research question (does it really add to the literature) | 10 | Excellent! | 10 |
| Citations appropriate & in software | 5 | Yes | 5 |
| All sections of a methods section present | 10 | Yes | 10 |
| Data collection description includes all necessary detail and cites reference (in endnote) | 10 | A bit more description of the study design, sampling, data collection methods, participation rate, would be good | 9 |
| Measures described well | 10 | Good, see comments in text | 10 |
| Statistics section includes all necessary information | 10 | Good, but I am not sure I know the mediation analysis method you are using. We can discuss. | 10 |
| Information well organized, concise but sufficiently comprehensive | 10 | Excellent | 10 |
| Grammar & spelling correct | 5 | Good | 5 |
|  |  |  |  |
| GRADE |  | Really nice paper, I think likely publishable | 98% |

Sleep duration and quality as mediators of socioeconomic disparities in inflammatory burden

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# Introduction

Sleep deficiency, which includes insufficient and poor quality sleep, represents a growing public health problem in the United States. Nearly 30% of adults in the U.S. report sleeping 6 or fewer hours per night,1 20% report excessive daytime sleepiness, and 20-30% experience insomnia symptoms.2 A growing body of literature links poor sleep duration and quality to a number of health outcomes, including all-cause mortality,3,4 incidence of type 2 diabetes,5,6 hypertension,7 coronary heart disease,8 and stroke9. Specifically, meta-analyses support a “U-shaped” association in which both short and long sleep (generally <6 and >8 hours, respectively) are related to elevated all-cause mortality risk.3,4

An important factor linking sleep and many chronic diseases may be low-grade, systemic inflammation, commonly measured by plasma concentrations of the immune markers interleukin-6 (IL-6), fibrinogen, tumor necrosis factor-a, and c-reactive protein (CRP). The most extensively studied biomarker of inflammation is c-reactive protein (CRP), an acute phase reactant (chemicals accompanying inflammatory pathway activation), for which high sensitivity assays are widely available.10 Based on Mendelian randomization studies, CRP itself is unlikely to be a causal risk factor of metabolic syndrome11 or ischemic vascular disease,12 although limited human experimental evidence suggests it has an etiologic role in atherosclerosis.13 CRP has a complex role in inflammation and its primary function may be anti-inflammatory;14 nonetheless, it is useful biomarker corresponding to general, potentially subclinical risk.

CRP is best characterized in relation to cardiovascular disease (CVD), as it is a strong predictor of cardiovascular events.15-17 Extensive experimental and observational evidence ties inflammatory processes marked by CRP to atherogenesis, the primary pathogenic process underlying coronary heart disease (CHD).18CRP is also a potent risk factor for all-cause mortality,19 and is associated with incidence of metabolic syndrome,16 colorectal cancer,20 and end stage renal disease,21 indicating inflammation may be an underlying pathogenic process shared by many chronic diseases. Short (<6 hours) and poor quality sleep have been shown to affect inflammation in experimental studies22,23 and to be associated with CRP and IL-6 in observational studies.24-26 Sleep restriction induces changes in glucose tolerance, thyrotropin concentration, evening cortisol concentrations, and sympathetic nervous activity, alterations which have implications in inflammation.27

A central challenge in public health is tackling socioeconomic disparities in health outcomes. Graded, inverse associations between socioeconomic status (SES) and a broad array of health outcomes, including CVD, diabetes, hypertension, a number of cancers, and all-cause mortality, have been extensively documented by a long history of research.28,29 More recently, a number of studies have observed socioeconomic disparities in inflammatory burden, with CRP, IL-6, and fibrinogen being consistently elevated in lower SES categories.30-33

Commonly hypothesized pathways for the impact of SES on inflammation are health status, behavioral (smoking, physical activity) and psychosocial (stress etc.) factors,31,34 and sleep may represent an underexplored link in this causal chain. Sleep restriction35 and poor quality sleep36,37 have been found to be more prevalent among individuals of low SES. Low income and low education are associated with adverse social and environmental conditions that impede adequate sleep38 and a growing number of lower-paid jobs involve precarious shift work and non-standard hours.39 Sleep is a modifiable risk factor for which efficacious non-pharmacological interventions exist.40 However, to our knowledge, no study has examined whether sleep mediates the relationship between SES and inflammation. The purpose of this study was to assess the role of duration and quality of sleep as a potential mediator between SES measures such as income and education, and inflammatory burden marked by plasma CRP.

# Methods

## Datasets

We used data from the continuous National Health and Nutritional Examination Survey (NHANES), an ongoing cross-sectional survey of the civilian non-institutionalized population in the United States. Data were collected by the National Center for Health Statistics and the Centers for Disease Control and Prevention (CDC) and involve a questionnaire, physical exam, and laboratory measures. Detailed descriptions of the survey methodology and operations are published elsewhere.41 In brief, approximately 5000 people were recruited each year using a stratified, multistage, probability sample. Individuals agreeing to participate completed a computer-assisted interview conducted by trained personnel, with physical and laboratory examinations conducted at the Mobile Examination Centers (MECs).

We used questionnaire, physical exam, and laboratory data from 3 waves spanning 2005-2010. We selected all respondents aged 18 years and older, who had complete data for CRP and answered questions on sleep duration and quality. We excluded individuals who had CRP concentrations greater than 10 mg/L, which indicate acute infection.17 We also excluded pregnant women, which demonstrate elevated and/or unstable CRP.17

## Measures

### Exposure Variables

In addition to raw family income, NHANES also reports the poverty income ratio (PIR), a ratio of family income to federal poverty level (FPL), which was chosen for this analysis because it takes into account family size and more accurately represents available financial resources. We categorized PIR as poor (below FPL), nearly poor (100-199% FPL), and middle and high income (≥200% FPL), according to the CDC’s Healthy People 2020 guidelines (<https://www.healthypeople.gov/2020/disparities-user-guide>).

In addition to family income, the most commonly used measure of SES, we also used highest educational level achieved, a measure that is more stable throughout the life course and a stronger predictor of inflammation than income.42 NHANES assesses education with the question, “What is the highest grade or level of school [you have/spouse has] completed or the highest degree [you have/s/he has] received?”, with the options “Less than 9th Grade”, “9-11th Grade (Includes 12th grade with no diploma)”, “High School Grad/GED or Equivalent”, “Some College or AA degree”, “College Graduate or above”.

### Mediator Variables

Sleep quality was operationalized according to the method used by Bansil et al.:7 participants were characterized as having poor sleep quality if they reported 5 or more episodes in the previous month of one or more of the following events: (i) having trouble falling asleep; (ii) waking up during the night and having trouble getting back to sleep; (iii) waking up too early in the morning and being unable to get back to sleep; (iv) feeling unrested during the day, no matter how many hours of sleep he/she had; or (v) feeling excessively or overly sleepy during the day. Sleep duration was characterized according to the question, “How much sleep [do you/does SP] usually get at night on weekdays or workdays?”, and categorized as <6, 6, 7, 8, or >8, with the reference level set to 7 hours to capture any “U-shaped” association, similarly to other population-based studies.25

### Outcome Variables

C-reactive protein is measured from blood collected during the physical exam and processed, stored, and shipped to a Johns Hopkins University laboratory. Plasma CRP concentrations are quantified by high sensitivity assay using latex-enhanced nephelometry, with a lower limit of detection of 0.1 mg/L.41 We categorized CRP into two clinically relevant categories: <3 and ≥3mg/L, representing normal and elevated inflammation, respectively.17

### Confounding Variables

We considered a number of potential confounders of the relationship between sleep and CRP, based on being a potential common cause (or proxy to a common cause) of both sleep and CRP. Analyses were adjusted for age (in 5-year categories), sex (male or female), race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, Other Hispanic, and Other Race including Multi-Racial), physical activity (number of times per week exercising enough to sweat or breathe hard), smoking in the past 30 days, obesity (body mass index (BMI) >= 30 kg/m2, measured in the physical exam), psychosocial stress (number of days in the past 30 when mental health was not good), use of birth control pills or hormone replacement therapy, and use of sleep medications often or almost always (5 or more times per month).

We separately considered potential confounders of the relationship between SES and CRP, which included only age. Although previous studies of this relationship have adjusted for serious chronic conditions,31,32 we chose to consider this variable a potential collider and not adjust for it. Final models were adjusted for all variables considered confounders of the SES → CRP and/or sleep → CRP relationships.

## Data Analysis

We summarized all variables in the population overall as well as stratified by sleep duration and sleep quality. All variables are presented as unweighted n, as well as weighted percent for categorical variables, and weighted mean ± sd for continuous variables that are approximately normally distributed, or median [interquartile range (IQR)] for non-normally distributed variables. Associations were tested using chi-square tests for categorical variables and t-tests or ANOVA for continuous variables.

### Assessment of Mediation

We sought to assess whether sleep duration and quality were intermediate variables in the causal pathway between SES and inflammation. Therefore, data analysis focused on assessment of total indirect effect (TIE),43 or the proportion of inflammation that would be prevented if SES did not cause poor sleep.43 We used the counterfactual approach detailed by Hafemen et al.,43 with adjusted risk differences based on predicted probabilities from logistic regression using marginal standardization, a straightforward extension of simple standardization.44 After fitting a regression model to the sample data accounting for the survey design, we computed predicted probabilities based on artificial data sets with the exposure, SES, set to each category other than the reference level, and for each SES value, one dataset set to each level of the mediator (sleep). All artificial datasets were generated to represent the predicted population distribution of the included covariates based on the survey weights. The final model contained all potential confounders of the SES 🡪 inflammation relationship, as well as the sleep 🡪 inflammation relationship, and contained terms for education, income, sleep duration, and sleep quality. Confidence intervals for indirect effects were calculated using the bootstrap method with 1000 replications.45 Statistical significance was determined for all tests at α<0.05. All analyses were conducted in SAS v. 9.4 and adjusted for the complex survey design.

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