**Intro**

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 (Ram 2010), 20% report excessive daytime sleepiness, and 20-30% experience insomnia symptoms (Roth 2007). A growing body of literature links sleep duration and quality to a number of health outcomes, including all-cause mortality (Gallicchio 2009, Cappuccio 2010), as well as incidence of type 2 diabetes (Cappuccio 2010, Ayas 2003), hypertension (Gangwisch 2006), coronary heart disease (Ayas 2003), stroke (Yaggi 2005). 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 (Cappuccio 2010, Gallicchio 2009).

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). ~~Extensive experimental and observational evidence ties inflammatory processes to atherogenesis, the primary pathogenic process underlying coronary heart disease (CHD) (Libby 2002).~~ The most extensively studied biomarker of inflammation is c-reactive protein (CRP), an acute phase reactant (biochemicals accompanying inflammatory pathway activation), for which high sensitivity assays are widely avaiable (Roberts 2001). ~~Based on Mendelian randomization studies, CRP itself is unlikely to be a causal risk factor of metabolic syndrome (Timpson 2015) or ischemic vascular disease (Zacho 2008), although limited human experimental evidence suggests it has an etiologic role in atherosclerosis (Bisoendial 2005). CRP has a complex role in inflammation and its primary function may be anti-inflammatory (Marnell 2005); nonetheless, it is useful biomarker corresponding to general, potentially subclinical risk.~~

CRP is best characterized in relation to cardiovascular disease, as it is a strong predictor of cardiovascular events (Ridker 1998, 2003). It is also a potent risk factor for all-cause mortality (Marsik 2008), and is associated with incidence of metabolic syndrome (Ridker 2003), colorectal cancer (Erlinger 2004),and end stage renal disease (Arici 2001), 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 studies (Meier-Ewert 2004, Vgontzas 2004) and to be associated with CRP and IL-6 in observational studies (Prather 2013, Jackowska 2013, Hall 2014). Sleep restriction induces changes in glucose tolerance, thyrotropin concentration, evening cortisol concentrations, and sympathetic nervous activity, alterations which have implications in inflammation (Spiegel 1999).

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 (Adler 1999, CDC 2013). Further, a number of studies have observed socioeconomic disparities in inflammatory burden, with CRP, IL-6, and fibrinogen being consistently elevated in lower SES categories (Phillips 2009, Kershaw 2010, Matthews 2016, Schmeer 2016, Stepanikova 2017).

Commonly hypothesized pathways for the impact of SES on inflammation are health status, behavioral (smoking, physical activity) and psychosocial (stress etc.) factors (Grunewald 2009, Kershaw 2010), and sleep may represent an underexplored link in this causal chain. Sleep restriction (Piccolo 2013) and poor quality sleep (Patel 2010, Mezick 2008) 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 sleep (Gradner 2010) and a growing number of lower-paid jobs involve shift work and non-standard hours (Alterman 2012). Sleep is a modifiable risk factor for which efficacious non-pharmocological interventions exist (Montgomery 2004, Montgomery 2003). 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.

Cardiovascular disease is the number one killer in many parts of the developed world, representing XX deaths in the United States per year. The disease remains difficult to prevent, its causes not fully understood, and expensive to treat. Moreover, people of lower socioeconomic status (SES) are at heightened risk for incidence and mortality from cardiovascular disease. This health disparity has been extensively characterized, but its mechanisms have not been fully elucidated.

Atherosclerosis is implicated in the pathogenesis of 99% of coronary heart disease (CHD), the most common type of CVD. Atherosclerosis begins to develop early in life with development of arterial plaque, and clinical symptoms are usually not present until much later, making early identification and preventative strategies critical. Extensive experimental and observational evidence links inflammatory processes to atherogenesis, which makes protein markers of inflammation an important diagnostic indicator of subclinical early atherosclerosis. The most extensively studied biomarker of inflammation is c-reactive protein (CRP), an acute phase reactant (biochemical accompanying inflammatory pathway activation), and one for which high sensitivity assays are readily available. Other important biomarkers used to identify a chronic inflammatory state are interleukin-6, fibrinogen, white blood cell count, and tumor necrosis factor alpha (TNF-α). Peripheral plasma levels of these markers predict CHD and all-cause and CHD-related mortality. All these markers are elevated in individuals with low socioeconomic status (Grunewald 2009 intro (get real reference)) and are potent risk factors for all-cause mortality (Marsik 2008).

While experimental evidence implicates CRP as a causal agent in atherosclerosis (Bisoendial 2006), Mendelian randomization studies, conversely, indicate it is unlikely to be causal in metabolic syndrome (Timpson 2015) or ischemic vascular disease (Zacho 2008). CRP has a complex role in inflammation, performing both pro- and anti-inflammatory functions (Marnell 2005); nonetheless, its correspondence to generalized and subclinical early cardiovascular risk makes it an important biomarker for study of cardiovascular mediators.

Commonly hypothesized pathways for the link between low SES and elevated plasma CRP, fibrinogen, and IL-6 are behavioral (smoking, diet, and physical activity) and psychosocial stress (Grunewald 2009, Kershaw 2010). However, no studies, to our knowledge, have explored sleep quality and duration as a possible mediator of this relationship. Sleep quality and duration have been shown experimentally to affect inflammation in most but not all studies (intro of Prather 2013), with a number of explanatory mechanisms. Specifically, long sleep (>8 hours per night) and short sleep (<6 hours per night) are associated with elevated CRP, as well as CVD-related and all-cause mortality. Additionally, people with lower socioeconomic status have elevated rates of long and short sleep.

The purpose of this study was to assess the relative contribution of duration and quality of sleep to SES-related disparities in markers of inflammation, in contrast to health behaviors such as diet, exercise, and smoking, and psychosocial stress, in a population-representative sample of adults.

**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 are available at the study website ([www.cdc.gov/nchs/nhanes.htm](http://www.cdc.gov/nchs/nhanes.htm)). 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, as previous literature has suggested that levels this high indicate acute infection. We also excluded pregnancies, as CRP concentrations can be elevated and/or unstable during pregnancy.

Measures

*Exposure Variables*

In addition to family income, the most commonly used measure of SES, we also used highest educational level achieved to represent SES. Educational level is more stable throughout the life course and has been shown to be a stronger predictor of inflammation than income (Dinwiddle 2016). NHANES measures 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”. 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).

*Mediator Variables*

Sleep quality was operationalized according to the method used by Bansil et. Al (2011). 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? Sleep duration in hours per night was categorized as <6, 6, 7, 8, or >8, similarly to other population-based studies (Hall 2014).

*Outcome Variables*

C-reactive protein is measured from blood specimens 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 (National 2012). We categorized CRP into two clinically relevant categories: <3 and ≥3mg/L, representing normal and elevated inflammation, respectively (Ridker 2003).

Data Analysis

We summarized all variables used in the analysis in total as well as stratified by the mediators, sleep duration and sleep quality. All variables are presented as unweighted n, as well as weighted percent for categorical variables, and weighted meansd for continuous variables that are approximately normally distributed, or median [interquartile range (IQR)] for non-normally distributed variables. Crude associations with the mediators were tested using chi-square tests for categorical variables and t-tests or ANOVA for continuous variables.

*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; these included age and BMI. Although previous studies of this relationship have adjusted for serious chronic conditions (Kershaw 2010, Matthews 2016), 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.

*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 examining the primary research question focused on assessment of total indirect effect (TIE) (Hafeman 2009), or the proportion of the effect of SES on inflammation that is explained by sleep. This quantity, in the counterfactual framework, is equivalent to the proportion of inflammation that would be prevented if SES did not cause poor sleep (Hafeman 2009).

In order to test the hypothesis of mediation, we used the counterfactual approach detailed by Hafemen et al. (2009), with risk differences based on predicted probabilities from logistic regression using marginal standardization, a straightforward extension of simple standardization (Muller 2014). 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 additional included covariates based on the survey weights. Confidence intervals for risk differences were calculated using the bootstrap method (Greenland 2004, Localio 2007). Statistical significance was determined for all tests at < 0.05. All analyses were conducted in SAS v. 9.4 and adjusted for the complex survey design.