ABSTRACT

Title: Quantifying the effects of sleep on cardiovascular health risk factors: A population analysis using UK Biobank data

Preliminary work from others and the investigative team has shown that sleep is correlated with heart health, and as such may be a risk factor for cardiovascular disease. The longitudinal relationship between sleep and key heart health metrics, however, is not known. To address this critical gap, we will quantify the role of sleep duration and chronotype (sleep-wake timing) on heart health. Data from the United Kingdom Biobank that includes a prospective sample of 20,000 adults with baseline and five year follow-up data will be used to: (1) quantify the cross-sectional association between sleep duration and chronotype with seven heart health metrics (dietary intake, physical activity, tobacco use, BMI, glycemic control, cholesterol, blood pressure); (2) assess the prospective predictive effects of sleep duration and chronotype on each of heart health metrics; and (3) examine moderating effects of age, sex, race, education, shift-work and residence (urban versus rural) on the relationship between sleep and heart health. A positive signal from this study would advance the field by showing that sleep is upstream to other cardiovascular risk factors and as such has the potential to leverage sleep (a universal physiological function) as a public health strategy to improve and optimize heart health.

Title: Quantifying the effects of sleep on cardiovascular health risk factors: A population analysis using UK Biobank data

Background

Up to 95% of adults' do not meet ideal heart health standards defined by the American Heart Association's (AHA) "Simple 7".[1,2] This includes adequate physical activity, healthy diet, tobacco abstinence, low fasting glucose, low cholesterol, normal body mass index (BMI), and healthy blood pressure levels (see Table 1). Failure to meet these behavioral and physiological Simple 7 metrics by the vast majority of the population bodes poorly for achieving national guidelines for a 20% improvement in cardiovascular disease (CVD) by 2020.[3] Adults who do report meeting several of these metrics including being a nonsmoker, having a BMI less than or equal to 25 kg/m^2, participating in 30 minutes or more of moderate to vigorous physical activity per day, drinking ½ a glass or more of wine every day and having a healthy diet have an 87% and a 83% lower incident risk of coronary heart disease 14 years later.[4,5] Efficacious, achievable, and sustainable approaches to achieving the Simple 7 and preventing CVD are needed. Sleep duration and chronotype (sleep-wake timing) have been independently and interactively associated with several Simple 7 metrics,[6,7] implying that without improving sleep, ideal heart health may be unachievable for many. However, the longitudinal relationships between sleep and heart health metrics is not known. Thus the potential to leverage sleep habits to improve heart health is being lost.

Adequate sleep (7-8 hours) is associated with multiple risk factors including higher levels of fruit and vegetable consumption,[8-11] lower odds of current tobacco use,[12,13] hypertension,[14] obesity, and sedentary behavior.[15,16] Adequate sleep also lowers the risk of CVD beyond what is achieved by meeting recommendations for physical activity, healthy diet, alcohol consumption and tobacco use alone.[17] However, sleep duration was not shown to predict 10-year cardiovascular risk.[18] With regard to chronotype variation in CVD risk factors, late chronotype has been associated with a higher likelihood of hypertension and lower cholesterol,[14,19] tobacco use,[20-22] poorer dietary intake,[8-11] higher BMI[23] and less weight loss,[24] and lower levels of physical activity.[25] These cross-sectional data collectively implicate sleep duration and to a lesser extent chronotype in the etiology of CVD risk factors.

Limiting progress in preventing and treating CVD risk factors (e.g., tobacco use, inactivity, poor diet) is their co-occurrence.[26,27] Seventy percent of adults who engage in one negative health behavior also engage in a second,[27] while one in five engage in three or more.[28] Combinations of negative health behaviors are thought to be multiplicative rather than additive in terms of their negative health effects.[29,30] Sustainable, efficacious strategies to addressing multiple health behaviors are unknown;[31,32] increasing the number of behaviors to be changed can decrease adherence and intervention response,[33] while even small changes can have synergistic effects on health outcome. Failure to achieve multiple health behavior change for cardiac patients can be fatal: patients who were unable to meet behavioral recommendations for tobacco use, dietary intake and physical activity had a 40% increased risk of death.[33] Determining whether sleep (e.g., long sleep, evening type) is pivotal in improving these co-occurring CVD risk factors and is predictive of Simple-7 metrics is a critical vertical step to informing multiple health behavior change programming for a multi-causal condition such as CVD.

Extending sleep has been shown to have cardiovascular benefits in small, pilot studies. Among pre- or stage 1 hypertensives (N=22), 30-minutes sleep extension significantly reduced blood pressure.[34] Sleep extension improved insulin levels,[35] decreased appetite and desire for sweets, particularly among short sleepers.[36] Sleep timing is also modifiable. Among 8 adults[37] who went from an urban setting (ambient light) to campout in the Rocky Mountains (solar light) for a 2-week period, all participants gravitated to earlier sleep-wake times. Chronotheraputic approaches to modify sleep duration and timing are plausible. This study will give empirical direction on how sleep (a universal physiological function) might be leveraged to improve heart health behaviors at the population and sub-group level as well as indicate the extent to which improvement in sleep might lower risk for CVD events (e.g., angina, stroke, myocardial infarction).

Limiting our knowledge of sleep duration and chronotype as potential upstream factors to the Simple 7 metrics is the absence of data showing the temporal relationship between sleep, the Simple 7 and CVD (e.g., incidence of angina, myocardial infarction, stroke) and whether the addition of sleep to the Simple 7 would improve the predictive capacity for these CVD outcomes. To address these critical gaps, we will use objective data from a prospective population sample (N=20,000 eligible adults) to pursue the following specific aims:

1. **To examine the effect of sleep, as measured by duration and chronotype, on the simple 7 metrics (dietary intake, physical activity, tobacco use, BMI, glycemic control, cholesterol, and blood pressure) over time.** Hypothesis 1a: There will be a direct effect of sleep at baseline with simple 7 metrics at follow-up, after adjusting for medication, family history of CVD, depression and sleep disorders, and

simple 7 at baseline. Hypothesis 1b: There will be an indirect effect of sleep at baseline on simple 7 metrics at follow-up through simple 7 metrics at baseline, after adjusting for medication, family history of CVD, depression and sleep disorders. Hypothesis 1c: There will be an indirect effect of sleep at baseline on simple 7 metrics at follow-up through sleep at follow-up, after adjusting for medication, family history of CVD, depression and sleep disorders.

2. **To examine the effect of sleep, as measured by duration and chronotype, on cardiovascular disease (CVD; 5-year incidence of angina, myocardial infarction, stroke) over time (see figure 1).** Hypothesis 2a: There will be a direct effect of sleep at baseline with CVD at follow-up, after adjusting for medication, family history of CVD, depression and sleep disorders, and simple 7 at baseline. Hypothesis 2b: There will be an indirect effect of sleep at baseline on CVD at follow-up through simple 7 metrics at baseline, after adjusting for medication, family history of CVD, depression and sleep disorders. Hypothesis 2c: There will be an indirect effect of sleep at baseline on CVD at follow-up through simple 7 metrics at follow-up, after adjusting for medication, family history of CVD, depression and sleep disorders. Hypothesis 2d: There will be an indirect effect of sleep at baseline on CVD at follow-up through sleep at follow-up, after adjusting for medication, family history of CVD, depression and sleep disorders.

3. **To compare the predictive capacity of the baseline Simple-7 metrics vs. the Simple-7 metrics + sleep on the 5-year incidence of CVD outcomes.** *Hypothesis 3*: The addition of sleep with baseline 'Simple-7' metrics, will have a significantly higher predictive capacity of each CVD outcome than the 'Simple-7" metrics alone after adjusting for medication, family history of CVD, depression and sleep disorders.

4. **To examine the moderating effects of age, sex, race/ethnicity, education, shift-work, and residence (urban versus rural) on the relationship between sleep with the 'Simple-7' metrics and sleep with CVD outcomes.** *Hypothesis 4*: The relationship between sleep and each of the Simple-7 metrics and CVD outcomes will differ (i.e., males vs. females, whites vs. non-whites, tobacco users vs. non-users, shift workers vs. non-shift workers, residence, urban vs. rural, college education vs. none, and for age).

Answering these research questions will significantly advance our understanding of the *predictive*[38] and interactive role of sleep duration and chronotype (timing) in the landscape of the AHA Simple-7 and CVD outcomes. This in turn will lay the empirical basis for developing *personalized*, *preemptive*, and *participatory* approaches to CVD and stroke prevention through sleep modification. This study, and the subsequent body of work these data will spearhead, is directly aligned with the AHA mission of building healthier lives, free of cardiovascular diseases and stroke.

Methods

Study Overview.

The proposed investigation is a longitudinal investigation of the association between sleep duration and chronotype with the "simple 7" (dietary intake, physical activity, tobacco use, body weight, glycemic control, cholesterol and blood pressure) and the CVD outcomes of angina, stroke and myocardial infarction. Data for this secondary analysis has been collected by the United Kingdom (UK) Biobank,[39] a 20-year, prospective cohort that began in 2005. The data consist of 150,000 adults aged 40-69 years at a baseline and 5-year follow-up timepoints with actigraph data for measuring sleep, physical activity and sedentary behavior.[40,41]

Data Source: The UK Biobank. The UK Biobank is a 20-year, community based prospective cohort study that began in 2005. Eligible participants were: (1) a registered user of the National Health Service, (2) aged 40-69 years, and (3) lived within a 25-mile radius of one of the UK Biobank assessment centers.[41] Between 2006-2010, 503,325 eligible and consenting adults were enrolled (5.47% response rate).[42,43] Volunteers provided informed consent and completed touchscreen questionnaires on lifestyle, environment and medical history. Blood and urine samples were obtained and height, weight and blood pressure were assessed. Web-based 24-hour dietary recall and mailed triaxial accelerometers were used to supplement self-reported diet, physical activity, and sleep assessments.[41,42] 5-year follow-up data have been collected on ~150,000 participants. For the current analysis, participants who have a history of cardiovascular disease (angina, myocardial infarction, stroke), use medication to control cardiovascular disease or are pregnant will be excluded from the analysis. Given these criteria, we expect to have data on approximately 20,000 adults for two time-points. The research team has a successful track record of working with the UK Biobank organization and has presented and published on a baseline dataset.[44-48]

Study Measures

Cardiovascular Outcomes. The cardiovascular outcomes of 5-year incidence of angina, myocardial infarction and stroke have been documented dichotomously (yes vs. no) where yes indicates if the subject had the CVD event in the last 5-years.

Sleep Variables

Sleep duration will be estimated from self-report and accelerometry. Participants responded to the query "About how many hours sleep do you get in every 24 hours? (Please include naps)." Self-reported sleep duration has been validated against sleep diaries (Spearman correlation 0.79).[49] An accelerometer (triaxial device worn on the dominant wrist) will provide objective data about rest/activity (proxy for sleep/wake) for 7 continuous days.[50] Data will be collected in 1 minute epochs and analyzed for the sleep parameter of interest, total sleep time (sleep per 24 hours). Accelerometry derived sleep duration will be coded as very short (≤4 hours), short (5-6 hours), adequate (7-8 hours), or long (≥ 9 hours).[51] Accelerometry monitored total sleep time will be the primary measure for sleep duration; self-reported total sleep time will be the secondary measure for total sleep time. *Chronotype* will be estimated from self-report and midpoint of sleep computed from accelerometry. Participants responded to the query "Do you consider yourself to be… definitely a morning person, more a morning than evening person, more an evening than a morning person, definitely an evening person." Responses will be coded as early (definitely morning), intermediate (more a morning than evening person and more an evening than morning person), or late (definitely evening).[52] Mean midpoint of sleep will be computed from accelerometry based on the mean sleep onset and sleep offset[11] and corrected as needed for shift work.[53] Midpoint of sleep has been validated against dim light melatonin onset.[54] Results will be coded as early (midpoint of sleep before 1am), intermediate (midpoint of sleep between 1am and 5:29am), and late (midpoint of sleep equal to or later than 5:30 am) chronotypes.[8]

Simple-7 Heart Health Metrics (See Table 1)

Physical activity: Accelerometry data will measure physical activity as counts per minute with light activity <1,951 counts per minute (cpm), moderate activity = 1,952–5,724 cpm, and vigorous activity = 5,724 cpm. Total minutes of light, moderate, and vigorous activity per week are computed by UK Biobank. These measures have been correlated with steady state oxygen consumption (Pearson's r= 0.88).[55] A categorical variable of ideal, intermediate and poor physical activity will also be generated.[1]

Diet: Average daily intake of fruits/vegetables and whole grains, average weekly intake of fish and sugar-sweetened beverages and use of sodium (rarely/sometimes/usually/always) over the past year will be used to generate a composite healthy diet variable. The ideal diet thresholds for fruit and vegetable consumption is ≥4.5 cups/day of fruits and vegetables, for fish it is ≥3.5oz servings/week, for whole grains it is ≥three 10z servings a day, for sugar sweetened beverage it is <4 glasses a week and for sodium (<1500mg/day or rare use of sodium). These thresholds will be used to determine ideal, intermediate and poor diet classifications.[1]

Tobacco use: Participants self-reported smoking status is recorded categorically as current smoker (smoked ≥1 cigarettes in the last month), former smoker (quit >1 year) and never smoker (never smoked a cigarette). Self-reports have been validated against serum cotinine[56] and test retest reliability has been established.[57] A categorical variable of ideal, intermediate and poor tobacco use will be generated.[1]

Body mass index (BMI): A continuous and categorical variable for BMI will be generated. BMI will be computed from measured weights and standing or seated heights (Seca 202).The sensitivity and specificity of BMI for obesity ranges from 0.39 (men) to 0.49 (women) and from 0.95 (men) to 0.99 (women), respectively.[58] A categorical variable of ideal, intermediate and poor BMI will be generated.[1]

Blood pressure: Blood pressure was measured with an automated blood pressure cuff reading (Omron HEM-7015IT). Inter-observer agreement for similar devices has been reported (-0.36 + 2.32 mmHg for systolic blood pressure, 0.02 + 2.42 mmHg for diastolic blood pressure), as well as, automated device-observer agreement (1.56+ 4.42mmHg for systolic blood pressure, 3.49 + 4.61 mmHg for diastolic blood pressure).[59] A categorical variable of ideal, intermediate and poor blood pressure will be generated.[1]

Total cholesterol: Total cholesterol levels will be measured from non-fasting venous samples aliquotted at 4^0C, and stored at -80^0C in SST vacutainers by the UK Biobank. Total cholesterol will be determined using the cholesterol oxidase (CHO) and peroxidase (POD) method (Beckman Coulter AU5800). The coefficient of variation within an assay is 1.1%. Ideal, intermediate and poor total cholesterol will also be generated.[1]

Hemoglobin A1C: Hemoglobin A1C will measure glycemic control from non-fasting blood samples [18] clotted for 25-30 minutes, centrifuged at 2,500 for 10 minutes aliquotted at 4^0C, and stored in EDTA vacutainers by the UK Biobank. Ion exchange high performance liquid chromatography (HPLC) assays will be used to determine hemoglobin A1C (Bio-Rad VARIANT II Turbo). The coefficients of variation range from 0.39% (patients with diabetes) to 0.90% (patients without diabetes) within assays and from 0.91% (patients with

diabetes) to 1.60% (patients without diabetes) between assays. A categorical variable of ideal, intermediate and poor total Hemoglobin A1C will also be generated.

Table 1: Definition of Ideal, Intermediate and Poor for the Seven Heart Health Metrics [1,60]

Metric	Ideal	Intermediate	Poor
Physical activity	> 75 minutes per week vigorous, or > 150 min/week moderate plus vigorous	1 – 74 min/week vigorous, or 1 – 149 min/week moderate plus vigorous	0 min/week
Diet	4-5 healthy diet components	2-3 healthy diet components	0 -1 healthy diet components
Tobacco use	Never smoked	Previous smoker (>1 year ago)	Current smoker or quit <1 year
BMI	< 22.9	23 to 26.9	≥ 27.0
Blood pressure	< 120.80 mm/Hg,	120 – 139 / 80 mm/Hg	> 140 / 80 mm/Hg
Cholesterol	<200 mg/dL untreated	200 to 239 mg/dL or treated to goal	≥ 240 mg/dL
Hemoglobin A1C*	< 6.0% untreated	6.0% - 6.9%	≥ 7.0%

**Note: ***an estimate for glycemic control used in place of 'Simple-7' fasting blood glucose metric.[60]

Moderating Variables: age (continuous), sex (male/female), ethnicity (coded as White, Asian/Asian British/Chinese, Black/Black British, mixed/other), attended college (coded as yes/no), shift work (yes/no), and employment (coded as employed, not-employed, or retired).

Covariates: All covariates are self-report variables that were verified using patient medical records. *Medication:* Participants responded to queries about the regular use of medication for cholesterol (yes/no), blood pressure medication use (yes/no), and diabetes medication (yes/no).[39] Self-reports are verified using medical records. Controlling for all medication use will enable the consideration of current cardiovascular disease in these analyses. *Depression status:* Participants with a diagnosis of bipoloar disorder (I or II) and/or major depression disorder were coded "Yes" for depression status (otherwise coded as "No"). *Family history of CVD:* Participants reporting any parental CVD (high blood pressure, stroke, heart disease) will be coded "Yes". *Sleep Disorders:* Participants reporting any history of narcolepsy or insomnia will be coded "Yes".

Work to be Undertaken: Data Analysis

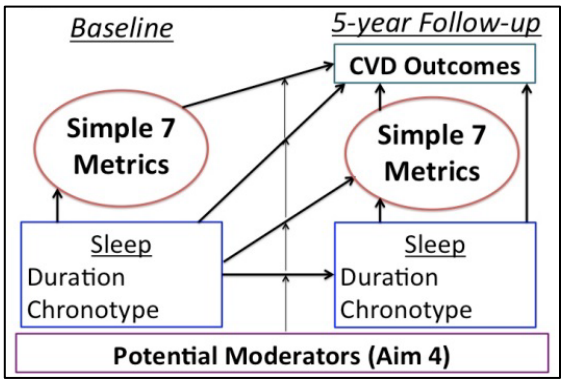
Overview: *Descriptive Statistics* including frequencies and percentages for categorical variables and estimates of central tendency (means, medians), measures of variability (standard deviations, interquartile ranges, ranges), and derived moments of skewness and kurtosis, for continuous variables, will be generated. *Structural equation modeling* (SEM) will be used to quantify the temporal relationship between sleep (duration and chronotype), with the simple 7 metrics of dietary intake, physical activity, tobacco use, BMI, glycemic control, cholesterol and blood pressure[1] (Aim 1) and the 5-year incidence of angina, myocardial infarction and stroke (Aim 2). Cox regression modeling and Receiver Operator Curves (ROC) will be used to determine the extent to which the predictive capacity of key cardiovascular outcomes can be increased with the addition of sleep indices (Aim 3). The moderating effects of age, sex, race, education, shiftwork, and urban vs. rural residence will be assessed (Aim 4). Models will be adjusted for medication, family history of CVD, depression and sleep disorders. MPlus and SAS software will be used for these analyses.[61] *Missing Data:* Items missing at random will be imputed prior to calculating final scores using conditional means, estimated with an iterated version of Buck's method.[62] Analyses will be repeated using only complete observations to assure that parameter estimates are not impacted by imputation.

Rationale for SEM: The primary analytic strategy for examining the interactive and complex relationships proposed in this study will rely on SEM. SEM tests complex multivariate models of direct (i.e. sleep on Simple 7 metrics and CVD outcomes) and indirect (i.e. sleep effect on CVD outcomes through heart health) effects, as well as being able to test overall model fit and specific pathways.[63] SEM combines a measurement model, which allows observed variables to be indicators of their underlying latent factors (i.e., latent factor of heart health as indicated by simple 7), and a structural model, that models the interrelationships among these factors and any observed variables.[63] SEM can be generalized to longitudinal models and in a cross-lagged panel model to test causal pathways that occur among sets of variables over time.[64] Each of the factors are related non-directionally, at the same time point, (sleep at baseline ↔ hearth health at baseline and sleep at follow-up ↔ heart health at follow-up), but predict their own subsequent occurrence, (sleep at baseline → sleep at follow-up and heart health at baseline → heart health at follow-up).[65] Baseline measures also predict the opposite factor's subsequent occurrence (sleep at baseline → heart health at follow-up and hearth health at baseline → sleep at follow-up).[63]

Power and Sample Size Considerations: The strategy for estimating sample size and power for the

study is based on MSEM. Klein recommended that a desirable ratio of the number of participants to the number of parameters is 20:1, and a satisfactory ratio of 10:1. The total number of parameters to be estimated is approximately 60 in each group, based upon 16 direct effects, 18 factor loadings, 5 correlations, and 20 error parameters. A proposed sample size of 1200 meets this recommendation with the ratio of participants to parameters equal to 20:1 (1200/60).[66]

Figure 1. Conceptual Overview of Aim 2



Aim 1 will examine the predictive effects of sleep duration and chronotype with the simple 7 metrics across two time points. The pathways in the structural model regressing the Simple-7 latent construct at follow-up on the latent sleep factor at baseline will be examined, adjusting for the covariates. The direct effect of baseline sleep on each of the Simple-7 metrics (H1a), the indirect effect baseline sleep on the Simple 7 metrics at follow-up through the baseline Simple-7 metrics (H1b) and the indirect effect of sleep at baseline on the Simple-7 metrics at follow-up through sleep at follow-up (H1c) will be tested

Aim 2 will examine the predictive effects of sleep duration and chronotype with 5-year incidence of CVD outcomes across two time points. The pathways in the structural model regressing the 5-year incidence of CVD outcomes (at follow-up) on the latent sleep factor at baseline will be examined, adjusting for the covariates. The direct effect of baseline sleep on the CVD outcomes (H2a), the indirect effect of baseline sleep on the CVD outcomes at follow-up through the baseline Simple-7 metrics (H2b), the indirect effect of sleep at baseline on CVD outcomes at follow-up through Simple 7 at follow-up (H1c), and the indirect effect of sleep at baseline on CVD outcomes at follow-up through sleep at follow-up (H1d) will be tested

Aim 3 will quantify the predictive capacity of the baseline AHA "Simple 7" versus the "Simple 7" + sleep (duration and chronotype) for key cardiovascular events (angina, myocardial infarction, stroke), independent of medication use, family history of cardiovascular disease, sleep disorders and depression. This aim will be assessed in 3 steps. Step 1: 3 Cox Regression models will test if the Simple 7 metrics (dietary intake, physical activity, tobacco use, body mass index, glycemic control, cholesterol, blood pressure) and chronotype are related to the 5-year incidence of each of the 3 outcomes (angina, myocardial infarction, and stroke) separately after adjusting for the covariates of medication use, family history of CVD, depression, and sleep disorders. Step 2, baseline sleep duration and chronotype will be added to each of the Cox Regression models and the pseudo-R^2, as well as log-likelihood fit statistics will be compared to see how much predictive power is added over and above the covariates and the Simple 7. Step 3, the analysis will be repeated using a single Poisson regression model of a cumulative 5-year incidence of angina, myocardial infarction and stroke combined to determine the predictive capacity of the Simple 7 for any of the cardiovascular outcomes when sleep duration and chronotype are included.

Aim 4 will identify the moderating effects of age, sex, race, education, shift-work, and residence on the relationship between sleep and each Simple 7 metric, and sleep and 5-year incidence of CVD outcomes. Moderation in SEM will be examined according to the level of measurement.[63] The continuous effect of age will be tested using the Quasi-Maximum Likelihood (QML) method.[66] Nominal moderation (sex, race, education, shift-work, area of residence) will be tested using MSEM and nested model comparisons.

Evaluation of SEM Model Fit (Aim 1 and 2): Evaluation of the overall model fit will be carried out using the chi-square goodness of fit and, as recommend by Klein and Muthén,[66] the Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), and Tucker-Lewis Fit Index (TLI). Should the proposed model fit poorly, model modification procedures, Lagrange Multiplier and Wald tests, will be applied.

Use of a Validation Sample: A training and validation sample will be used to verify findings. A training sample comprised of 80% of the cases will be used for model building, while the remaining 20% will be a hold-out sample for model validation.[67]

Outlook for the future

A positive signal from this study will lead to an RO1-level application to use a RCT methodology to test the effects of a chronobiological approach (i.e., adjustment of sleep duration and timing) to improve heart health in at-risk groups. This work addresses the strategic plan of the American Heart Association as well as several NIH institutes, including NHLBI (e.g., cardiovascular/ metabolic health outcomes), NICHD (e.g., basic social science and community factors), NIA (e.g., effects of age), NINR (e.g., interface with health, behavioral factors), NIAAA/NIDA (e.g., substance use), and NIMHD (e.g., sleep disparities).