Study Title: Impact of Sleep Restriction on Cardiometabolic Risk Factors in Pre- and Postmenopausal Women

Short Title: Impact of Sleep Restriction in Women

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SPECIFIC AIMS

The overall goal of this project is to test the hypothesis that long-term, sustained SR, in women, will lead to increased cardio-metabolic risk. This will be characterized by increases in visceral adiposity, unhealthy lifestyle behaviors (poor dietary quality and low physical activity) and cardio-metabolic risk factors (blood pressure, glucose intolerance) relative to HS. We expect these changes to be worse in pre- compared to post-menopausal women.

Specific Aim 1. To assess the effects of SR on cardio-metabolic risk profile, relative to HS.

Hypothesis 1: Prolonged SR will lead to increased blood pressure and reduced glucose tolerance relative to HS.

Specific Aim 2. To assess the effects of SR on adiposity, relative to HS, over a 6-week period.

Hypothesis 2: Prolonged SR will lead to increased visceral adiposity, assessed by magnetic resonance imaging (MRI), relative to HS.

Specific Aim 3. To assess the effects of SR on diet and physical activity level.

Hypothesis 3a: Prolonged SR will lead to reduced physical activity relative to HS.

Hypothesis 3b: Diet quality will be reduced with SR relative to HS, as reflected by increased intake of snacks, fat, and simple sugars.

Exploratory Hypothesis: The effects of prolonged SR on adiposity and cardio-metabolic risk factors will be greater in pre-menopausal women relative to post-menopausal women.

RESEARCH DESIGN & METHODS

Study population & Sample size determination

We will enroll 80 participants and expect 50 to complete the study. This drop-out rate accounts for participant non-compliance and withdrawal from the study. In our previous *Sleep Study*, our completion rate was 90% (27/30). We expect a larger drop-out rate in the proposed study due to the longer duration of each treatment phase, and also, some participants may be dismissed from the study from their inability to achieve and maintain the 1.5 h average weekly SR.

In this proposed study, each participant will undergo 2 study phases, and each response variable will be measured *at least* once per phase. Thus, for each response measure we will have at least 50 x 2 paired observations. Based on preliminary data analysis, we have estimated an effect size (mean paired difference) of 0.69 ± 0.36 g for fat mass and an effect size (mean paired difference) of 0.91 ± 0.65 g for weight gain. This translates to very large effect sizes of 1.92 (fat mass) and 1.4 (weight gain) for paired t-tests. With these effect sizes we will have >80% power for paired t-tests with our expected sample size and will have sufficient power to test for differences between pre- and post-menopausal women.

Eighty pre- and postmenopausal women, age 20-65 y [i] with an overweight BMI (25-29.9 kg/m^2) [ii] will be recruited from the New York City area. These women will come from the pool of participants in the Population Science project in this Center application. Pre-menopausal women will not be on oral contraceptive medications and post-menopausal will not be on hormone replacement therapy. In addition, women recruited to participate in this study will habitually sleep 7-9 h/night, will be free of any current and past sleep and psychiatric disorders, including eating disorders (ex. anorexia, bulimia, night eating syndrome), and will not have type 2 diabetes or CVD (**Table 2**) [iii]. We will exclude women taking medication to reduce blood pressure and cholesterol levels. Smokers (defined as currently smoking any cigarettes or ex-smokers <3 y), non-day and rotating shift workers, persons who plan to travel across time zones within 4 weeks of the study, and those with a history of drug and alcohol abuse, drowsy driving, or excessive caffeine (>300 mg/d) or alcohol intakes >2 drinks/day) will also be excluded. Further, we will not enroll women who have had a recent weight change or who actively participated in a diet or weight loss program in the previous 3 mo, and those with a neurologic condition that may disrupt the procedures. Women who are pregnant or <1 y post-partum, and those with contra-indications for MRI scanning will be excluded [iv]. All participants will have normal scores on the

[i] See Amendment 1b
[ii] See Amendment 1c
[iii] See Amendment 1d
[iv] See Amendment 1a

Pittsburgh Quality of Sleep Questionnaire (1) (global score <5) and Epworth Sleepiness Scale (2) (score <10), no indication of sleep apnea (Berlin Questionnaire) (3), sleep disorders (Sleep Disorders Inventory Questionnaire) (4), depression (Beck Depression Inventory II) (5), significant delayed or advanced sleep phase (Composite Scale of Morningness/Eveningness) (6), and involuntary sleep movement, by self-report. Individuals who take daytime naps, have unstable sleep, restrained eaters and those with abnormal scores on the Three Factor Eating Questionnaire will be excluded (7). These stringent inclusion/exclusion criteria will ensure that women who likely have nighttime disturbances, such as young mothers and women with early morning or late-night commitments will not be enrolled.

To further ensure the safety of our study participants and the general population, women will be prevented from driving any vehicle during the period of SR. All women will be asked to agree to this by signing on a separate line in the consent form.

Table 1. Inclusion and exclusion criteria for the study*

Inclusion	Exclusion
Age 20-65 y	Smokers (any cigarettes or ex-smoker <3 y)
All racial/ethnic groups	Neurological, medical or psychiatric disorder, diabetics
Body mass index 25-29.9 kg/m^2	Eating and/or sleep disorders
Sleep 7-9 h in bed/night with no daytime nap	Contraindications for MRI scanning
Normal scores on: Pittsburg Quality of Sleep Questionnaire Epworth Sleepiness Scale Berlin Questionnaire Sleep Disorders Inventory Questionnaire Beck Depression Inventory Composite Scale of Morningness/ Eveningness Three Factor Eating Questionnaire	Travel across time zones within 4 wk
	History of drug and alcohol abuse
	Shift worker (or rotating shift worker)
	Caffeine intake >300 mg/d
	Oral contraceptive use or hormone replacement therapy
	Heavy equipment operators Commercial long-distance drivers

**The table includes original eligibility criteria. See Section 1 of "Amendments" section for changes to eligibility criteria and justification.*

Overview of study design

This study will be a randomized, crossover, outpatient SR study with 2 phases of 6 weeks each. Sleep duration in each phase will be the participant's regular bed- and wake-times during the HS phase and HS minus 1.5 h in the SR phase. During the HS phase, participants will be asked to follow a fixed bedtime routine based on their screening sleep schedule. During the SR phase, participants will be asked to keep their habitual wake time constant but delay their bedtime to achieve a reduction of 1.5 h in total sleep time. A delay in bedtimes was chosen rather than advancing wakeup time because it likely most closely reflects differences in sleep timing behavior between short and normal sleepers. Spaeth and colleagues have also shown that restricting sleep to the latter portion of the night is associated with increased food intake and weight gain relative to HS (8). We also considered providing fixed bed- and wakeup times for all women but decided to provide individualized bedtimes and wakeup times to reduce variability in our sample.

On the first day of each study phase (baseline), participants will come to the Clinical Research Resource (CRR) of the Irving Center for Clinical and Translational Research (CTSA) at CUMC in the morning after an overnight, 12 h fast. Participants will have anthropometric measurements taken and will then be taken to the department of Radiology to undergo magnetic resonance imaging (MRI) scanning to assess body composition. They will then return to the CRR for an oral glucose tolerance test. Participants will begin the fixed bedtime routine that night. These baseline measurements will be

repeated at endpoint, 6 weeks later. Body weight and waist circumference will be measured weekly and fasting blood samples will be taken bi-weekly during adherence check visits [v].

Recruitment and blinding

Women who fit the general inclusion/exclusion criteria for this study will be identified from the Population Science project and invited to participate in this project. Women who express an interest in participating will undergo a preliminary phone screening. During this phone interview, more details of the study will be provided to the prospective participant and additional information will be obtained to determine potential eligibility. Women who pass the phone screening and are interested in participating in the study will be scheduled for an in-person screening. At this second screening point, eligibility will be ascertained. From there, sleeping behavior will be established. This will be done with actigraphy over a 2-week period.

Two weeks prior to randomization and during the washout period, participants will wear an actigraph (Micro Motion Logger Sleep Watch, Ambulatory Monitoring, Inc., Ardsley, NY) [vi] and keep a sleep diary to verify sleep duration and sleep-wake schedule. In addition to achieving an average sleep of 7-9 h/night, by wrist actigraphy, participants will only be enrolled if they achieve 7 h of sleep for at least 10 of the 14 nights of screening and have <4 nights with <6 h of sleep [vii]. At the time of randomization, a urine pregnancy test and drug screen will be performed. Participants will be asked to abstain from caffeine and alcohol intake for 24 h prior to the start of each study phase. Between study phases, if participants have not returned to baseline sleep patterns, an additional 2-4 week washout period will be provided. In general, washout periods will be 6 weeks in duration. This washout length will ensure that women are in the same phase of their menstrual cycle at the start of each experimental phase (**Fig. 3**).

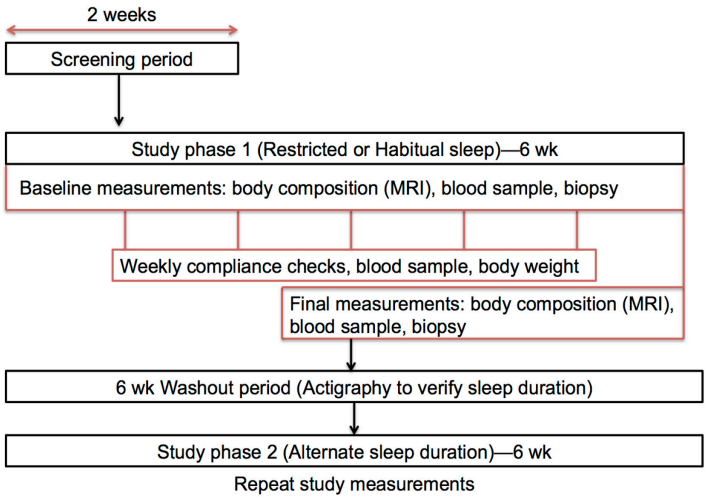

Figure 3. Study protocol. Participants will undergo a 2-wk screening to assess eligibility prior to randomization to their first study phase: restricted or habitual sleep. At baseline, body composition will be assessed and blood samples will be taken. Compliance with the sleep protocol will be assessed weekly, along with weight measurements. A blood sample will be taken bi-weekly. Baseline measurements will be repeated at endpoint (6 wk). Participants will undergo a 6-wk washout period before undergoing phase 2.

Participants will be blinded to the purpose of the study to ensure that they do not alter their habits from knowledge that SR may cause alterations in food intake and physical activity, resulting in weight gain. Participants will be told that the purpose of the study is to assess the effects of mild SR on mood and cognitive and physical performance. Questionnaires and performance tests will be administered at baseline and bi-weekly to maintain blinding.

Details of the study procedures

Ensuring adherence to the sleep protocol.

We have a well-defined strategy for ensuring adherence to the sleep protocol in this outpatient setting. Participants will be given written instructions on study procedures and investigator phone calls will be made as reminders of the bedtimes and wakeup times on a weekly basis [viii]. To verify that participants adhere to the sleep protocol, they will be asked to keep a sleep diary and wear an activity monitor 24 h/day. Participants will wear a Motionlogger actiwatch on their wrist (Ambulatory Monitoring, Inc, Ardsley, NY) [ix], which will serve to track their sleep duration, sleep timing, and ambient light. This watch is water resistant and therefore does not need to be removed to shower or swim. To further ensure compliance with the sleep protocol, participants will be required to come to the research center on a

[v] See Amendment 2c
[vi] See Amendment 2a
[vii] See Amendment 1e
[viii] See Amendment 2b
[ix] See Amendment 2a

weekly basis. This weekly visit will serve several purposes: (1) to download data from the actiwatch (verify sleep duration); (2) to charge the watch; (3) to obtain weekly diaries (verify bedtimes and wakeup times) and other questionnaires; (4) to obtain body weight, waist circumference, and blood pressure measurements and blood samples (bi-weekly only). Participants will be told a priori, as part of the screening process, that they must adhere to the sleep protocol for continued participation in the study and that adherence will be checked weekly. Non-adherent participants will be terminated from the study. Adherence will be defined as having bedtimes and wake times within 30 min of scheduled times at least 5 days/week and achieving an average of >7 h of sleep each week with no more than one night of sleep <7 h during the HS phase and achieving an average sleep reduction of 1.5 h from screening sleep duration each week with no more than 1 night with <1.5 h of restriction during the SR phase. Daytime naps will not be permitted. Participants will receive weekly incentives, in the form of a gift card, for their continued adherence to the sleep protocol.

Blood sampling procedures.

Fasting blood samples will be taken at baseline, 3 weeks, and endpoint of each sleep phase for the measurement of metabolic risk factors: glucose, insulin, and inflammatory markers [x]. An oral glucose tolerance test will be performed at baseline and endpoint as well. Two baseline blood samples (8 mL) will be obtained at 10 and 5 min before glucose ingestion (-10 and -5). At time 0, participants will consume 75 g of glucose in 300 mL solution orally. Subsequent blood samples (10 mL) will be obtained at 15-, 30-, 60-, 90- and 120-min post-intake. These procedures will be performed by a certified nurse in the CRR at CUMC.

Details of the outcome measures

Blood pressure measurements.

Blood pressure will be measured weekly with the participant sitting, with their legs uncrossed, for at least 5 min. Measurements will be taken twice from the non-dominant arm using an appropriately sized cuff. In addition, participants will wear a blood pressure monitor for 24 h at baseline, 3 weeks, and 6 weeks of each intervention [xi].

Body composition measures.

Anthropometric measurements will be obtained at the New York Obesity Research Center Human Phenotyping/Body Composition Core Laboratory. A single trained technician will obtain all measurements for this study using standard procedures in the laboratory. Height, weight, and body circumferences (waist and hip) will be measured in duplicate with the participant wearing only a hospital gown and no shoes.

Whole-body MRI will be carried out with a T1-weighted, fast spin-echo sequence, with a 210 ms TR and a 17 ms TE using a 1.5T General Electric system (6X Horizon, Milwaukee, WI). A field of view of 48 cm and a 256 x 256 matrix will be used. Participants will be positioned in a supine position with their arms stretched overhead in the scanner. The scanner will be landmarked at the L4-L5 intervertebral disc and images from L4-L5 to the top of the fingerprints and from L4-L5 to the end of the toes. Images will have a slice thickness of 1 cm and will be spaced 4 cm apart for the upper and lower body but 2 cm apart for the visceral area, demarcated as 10 cm below to 20 cm above L4-L5. Shen and colleagues have reported that estimate errors in assessing total visceral adipose tissue and subcutaneous adipose tissue increased with increasing distance between images (9). The percent difference between estimated and actual volumes for visceral adipose tissue was 3.14% with the 2-cm inter-slice interval vs 9.70% with the 5-cm inter-slice interval, corresponding values for subcutaneous adipose tissue were 0.26 and 0.85%. Since a primary outcome of interest is visceral adipose tissue, we chose to perform the image acquisition with a 2-cm inter-slice interval rather than the typical 5 cm.

Cardio-metabolic risk assessment.

All samples will be analyzed in the Hormone and Metabolite Core laboratory of the NYORC by skilled technicians [xii]. Insulin will be assayed using a radioimmunoassay (RIA; Linco Research Products Inc., St. Charles, MO; mean inter-assay CV of 4.5% (10, 11). Tumor necrosis factor-α will be assessed with

[x] See Amendment 2d

[xi] See Amendment 2d

[xii] See Amendment 2f

an ELISA (R&D Systems, Inc., Minneapolis, MN), using 200 uL sera. The manufacturer indicates that the tumor necrosis factor-α assay has an approximate minimal detectable concentration of 0.12 pg/mL. Interleukin-6 will be assessed with an ELISA (R&D Systems, Inc., Minneapolis, MN) using 200 uL sera. High-sensitivity C-reactive protein will be assessed with an ELISA (ALPCO Diagnostics, Windham, NH). Serum samples will be diluted 1:100 prior to use.

Lifestyle behavior assessments.

We will also assess physical activity level by waist actigraphy (GT3X+ Actigraph LLC, Pensacola, FL) throughout each study phase [xiii]. We hypothesize that total energy expenditure and physical activity level will be lower during the period of SR compared to HS.

Throughout the study, participants will self-select their food intake in a free-living situation. Food intake will be assessed at baseline and endpoint by 3-day food records (2 weekdays and one weekend day). Energy and macronutrient intakes from the food records will be assessed using the University of Minnesota Nutrition Data System for Research (NDSR, Minneapolis, MN).

Statistical analysis plan

Exploratory data analysis for all response variables will be performed using R, SAS and other software. As part of the analysis, we will compute descriptive statistics for each variable. In particular, the proportion, mean, variance, standard error and the range of continuous variables and frequency and model for discrete variables will be computed. Descriptive statistics will be computed for each specific sleep. In addition, correlation coefficients between the variables will be computed and scatter plots will be checked for linear relationships. The correlations and scatter plots help assess the degree of collinearity among the variables, which will be useful for the interpretation of regression-based linear mixed model analyses discussed later. The data will be tested for normality, and if needed an appropriate transformation will be used on the raw data to make the normal approximation better.

As we are collecting data from two phases for each subject, we will test for phase effect by using phase main effect term in an initial version of each linear model. If the term is not found to be significant then we will rebuild the linear model without the term.

Linear Mixed Model Analyses. We will fit a linear mixed model to analyze the data. Treatments (sleep: SR vs. HS) will be used as fixed effects and subject will be used a random effect. The other independent variables (covariates) will be age, baseline BMI, and race/ethnicity. If required anywhere in the analysis plan, p-values will be adjusted for multiple comparisons.

Specific Aim 1. To assess the effects of SR on cardio-metabolic risk profile, relative to HS. (*Hypothesis 1:* Prolonged SR will lead to increased blood pressure and reduce glucose tolerance relative to HS.) We will perform a repeated measure linear mixed model analyses separately with each of blood pressure and glucose tolerance measures (each in a separate model) as the response and sleep (SR vs. HS), time, sleep-time interaction and the covariates as independent variables, with data from all time points combined. Subject will be used as a random effect. Additionally, we will also use the change from baseline to week-6 in each of blood pressure and glucose tolerance measures as response variables in separate regressions, where sleep (SR vs. HS) and the covariates will be used as the independent variables. Paired one-sided t-test and Wilcoxon test will be used to determine whether the SR is associated with increased blood pressure and reduced glucose tolerance vs. HS.

Specific Aim 2. To assess the effects of SR on adiposity, relative to HS, over a 6-week period. (*Hypothesis 2:* Prolonged SR will lead to increased visceral adiposity, assessed by MRI, relative to HS.) We will perform a repeated measure linear mixed model analyses separately with each visceral adiposity variable (as observed by MRI) (each in a separate model) as the response and sleep (SR vs. HS), time, sleep-time interaction and the covariates as independent variables, with data from all time points combined. Subject will be used as a random effect. Additionally, we will also use the change from baseline to week 6 in each adipose tissue variable as response variables in separate regressions, where sleep (SR vs. HS) and the covariates will be used as the independent variables. Paired one-sided t-test and Wilcoxon test will be used to determine whether the SR is associated with increased adiposity variable relative to HS.

[xiii] See Amendment 2a

Specific Aim 3. To assess the effects of SR on diet and physical activity level. *(Hypothesis 3a:* Prolonged SR will lead to reduced physical activity relative to HS.) We will perform a repeated measure linear mixed model analyses separately with each physical activity measure (each in a separate model) as the response and sleep (SR vs. HS), time, sleep-time interaction and the covariates as independent variables, with data from all time points combined. Subject will be used as a random effect. Additionally, we will also use the change from baseline to week-6 in each physical activity measure as response variables in separate regressions, where sleep (SR vs. HS) and the covariates will be used as the independent variables. Paired one-sided t-test and Wilcoxon test will be used to determine whether the SR is associated with reduced physical activity measures relative to HS. (*Hypothesis 3b:* Diet quality will be reduced with SR relative to HS, as reflected by increased intake of snacks, fat, and simple sugars.) We will perform a repeated measure linear mixed model analyses separately with each diet quality measure (snacks, fat, and simple sugars intakes, each in a separate model) as the response and sleep (SR vs. HS), time, sleep-time interaction and the covariates as independent variables, with data from all time points combined. Subject will be used as a random effect. Additionally, we will also use the change from baseline to week-6 in each diet quality measure (snacks, fat, and simple sugars intakes) as response variables in separate regressions, where sleep (SR vs. HS) and the covariates will be used as the independent variables. Paired one-sided t-test and Wilcoxon test will be used to determine whether the SR is associated with reduced diet quality measure (i.e., increased snacks, fat, and simple sugars intakes) relative to HS.

Exploratory Hypothesis: The effects of prolonged SR on adiposity and cardio-metabolic risk factors will be greater in pre-menopausal women relative to post-menopausal women. We will first compute the effect of SR on adiposity and cardio-metabolic risk factors. This will be computed as the increase in SR from the baseline value of the SR-phase on each measure of adiposity and cardio-metabolic risk factors for each woman. Then these differences will be compared between pre- and post-menopausal women by means of a linear model analysis, one-sided unpaired t-test and Wilcoxon test. The comparison by linear models will be done by using the (SR vs. baseline) difference as the outcome (separately for each of adiposity and cardio-metabolic risk factors) and menopausal-status (pre- vs. post) and the covariates as independent variables. Paired one-sided t-test and Wilcoxon test will be used to determine whether the menopausal-status (greater in pre- vs. post-, to be used as the two groups for the tests) is associated with greater average difference (SR vs. baseline) in adiposity and cardio-metabolic risk factors.

ETHICAL ASPECTS OF THE PROPOSED RESEARCH

Protection of human subjects

All personnel involved in this project, including fellows who will be trained as part of the Center grant, will undergo human subjects research training. Courses include ethical considerations of human subjects research and health information privacy rules. Research will not be performed until approval from our Institutional Review Board is obtained. All participants will be informed of the study procedures during the screening and consenting process and will be given the opportunity to ask questions about the study. Furthermore, participants will be informed that they can withdraw from the study at any time without jeopardizing their future care at our medical center.

All data will be kept on file coded (de-identified) with a study number and without any specific identifying personal health information. Study data will be stored on password-protected, encrypted end-point devices. All paper files will be stored in locked cabinets. Imaging data will be stored (de-identified) in a secure computer database that is protected by firewalls and passwords. MRI data will be transferred to the New York Image Reading Center via secure servers. Only personnel immediately involved in the study will have access to study files and documents.

Potential risks & risk management

The major potential risks in this study are related to blood drawing, arterial line placement, and MRI as well as sleepiness, difficulty concentrating, or feeling drowsy as a result of SR.

Risks associated with SR. Participants may feel sleepy and irritable during the SR phase. They may feel drowsy and less attentive. Because of the risks associated with drowsy driving, participants will not

be permitted to drive during the SR period. This should not hinder participant recruitment since this study will be conducted in NYC, where mass transit system is extensive and widely used.
Risks associated with evaluations. During the evaluation we might uncover unanticipated psychiatric and medical information. In that case we will discuss those incidental findings with the participant and will recommend appropriate follow-up.
Risks associated with blood drawing. At the screening visit and weeks 3 and 6, routine venipuncture will be performed for screening laboratory studies, and on the oral glucose tolerance test days, an antecubital vein catheter will be inserted in one arm for multiple blood sampling. Drawing blood and inserting an intravenous line into an arm vein are safe and standard medical procedures. Sometimes a bruise will occur at the puncture site and rarely a blood clot or infection will occur in the vein. Certain individuals may feel light-headed during venipuncture. The volume of blood collected during this study will be approximately 10 mL per time point and approximately 66 mL for the oral glucose tolerance test (approximately 142 mL per 6-week period; 284 mL over the entire study). This is not expected to have any serious negative effects on a study participant. The risks of bruising, clotting, and infection will be minimized by having venipuncture performed by trained and experienced personnel under sterile conditions. To avoid injury due to fainting, the antecubital vein catheter will be inserted when the subjects are recumbent. The oral glucose tolerance test will be performed in the Clinical Research Resource with nursing staff and the study physician will be available on site. Fasting blood draws not associated with venal catheters will be performed by trained personnel in the CRR of the CTSA at CUMC.
Risks associated with MRI scanning. There are no significant risks associated with the use of MRI except the risk of flying object in the magnetic field. The MRI personnel are trained and qualified in the area of patient safety concerns. All ferrous materials must be excluded from the study. To date, no harmful effects from the use of clinically approved MRI equipment have been reported. There have been no reproducible results concerning potential adverse effects of static magnetic fields. MRI scanning involves the use of a magnet and radio frequency waves (much like an ordinary short-wave radio). There are no known risks or adverse effects resulting directly from exposure to magnetic fields and radio frequency signals used in this study, other than the potential risks associated with the scanning procedure summarized below.

MRI uses a strong magnetic field to create images of the body. Because of the strong magnetic field, the greatest risk is that a metal object could be pulled into the scanner and hit someone. To reduce this risk, everyone near the magnet will remove all metal from their clothing or pockets when in the scanning environment. The door to the scan room will remain closed during the exam for the participant's safety. There are no known risks or adverse effects resulting directly from exposure to MRI. However, participants who have a pacemaker or metal objects in their body such as shrapnel or metal in the eye should not have the scan performed. Technologists or investigators will answer any questions or concerns participants may have before entering the magnet room.

Some people may feel confined and experience anxiety in the scanner. We will exclude individuals with claustrophobia. The scan can be stopped at any time if the participant becomes uncomfortable in the scanner. The scanner produces tapping sounds during operation, which may reach very loud levels. To minimize any discomfort from this noise, participants will be given disposable earplugs to reduce the noise levels but will still allow voice communication with the scanner operator. In extremely rare cases, a magnet can lose its magnetism, in which case cooling fluids may be released noisily through escape valves and may collect in gas form in the scan room. The gas is not harmful as long as fresh air is available. In this very remote event, participants will immediately be brought out of the magnet room. Some subjects may experience muscle twitches or tingling sensation and/or a slight increase in body temperature during some types of scan activity. These are very unlikely under current guidelines.

Potential benefits to the subject and to others

There is no direct benefit to the subject participating in this project. The potential benefit to others is the information gained by determining the role of SR on cardio-metabolic health markers. If successful, future work using this methodology will increase our understanding of how sleep affects behaviors and tissues/metabolites related to CVD. The risks associated with the study are moderate. Because this study may not have direct benefits to the individual participant, subjects will be offered payment for their

participation. Subjects will have the opportunity to receive compensation upon completion of various parts of the study.

AMMENDMENTS TO THE PROTOCOL (Listed in chronological order within sections)

1. Eligibility criteria modifications:
    a. History of fainting was added as an exclusion criteria.
    b. The upper age limit was removed from eligibility criteria to enhance recruitment of postmenopausal women.
    c. Inclusion criteria for weight status was extended in both directions to include women with BMI 30-33 kg/m^2 as well as 20-24.9 kg/m^2; women with BMI 20-24.9 kg/m^2 were required to have elevated familial risk for CVD (family history [1 or more parent with] of type 2 diabetes, cardiovascular disease, or hypertension) to be considered for participation.
    d. Women with stable (>3 mo) controlled hypertension (but not resistant hypertension) or diabetes (HbA1c <7%) or stable use of cholesterol-lowering medication were considered for study participation in order to enhance enrollment of postmenopausal women.

       [*Note: No accrued participants reported any of the above. All met initial eligibility criteria related to cardio-metabolic health.]*
    e. Women who slept 7 or more hours per night for at least 70% of screening time (at least 14 days) were eligible for this study. Previous requirement stated 10/14 nights with 7 or more hours of sleep/night **and** average sleep of 7 or more hours during the 14 days. This amendment removed the requirement for average sleep duration of 7 hours/night or more. This was to enhance enrollment of postmenopausal women who naturally have shorter sleep duration than premenopausal women.

       [*Note: All accrued participants met the original, more stringent sleep-based criteria*.]
2. Study procedure modifications:
    a. The tri-axial accelerometry device as well as its placement (on body) and duration of use were modified. The Actigraph GT3X+ (Actigraph, Pensacola, FL) replaced the Motionlogger. This device was worn on the non-dominant wrist continuously during screening and for the full duration of each study phase to ensure compliance with the sleep duration requirements. In addition, hip-worn actigraphy (originally planned to be assessed via Actigraph GT3X+) was eliminated, since physical activity and inactivity can be derived from the wrist-worn device.
    b. Rather than providing weekly phone-call reminders of sleep assignments, study staff provided participants with written target nightly target bed and waketime schedules each week. Email reminders were sent throughout each study phase to complement weekly in-person meetings.
    c. Fasted blood draws occurred at Baseline, Week 3, and Endpoint study visits, rather than bi-weekly, to reduce participant burden.
    d. An additional fasted blood draw was added at the Week 4 study visit of each phase.
    e. Frequency of ambulatory blood pressure monitoring was reduced to one endpoint measure to reduce participant burden and in response to participant complaints about this measure.
    f. Blood samples were analyzed by the Biomarkers Core Laboratory of the Irving Institute for Clinical and Translational Research.

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