Stress, Salt Excretion, and Nighttime Blood Pressure
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Study Title: Stress, Salt Excretion, and Nighttime Blood Pressure (SABRE)
Principal Investigator: Daichi Shimbo, MD
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1. Study Purpose and Rationale

Blood pressure (BP) has a *diurnal* rhythm; it is normally highest during the daytime period and lowest during the nighttime period (BP dipping). The *diurnal* pattern of BP over a 24-hour period can be assessed using ambulatory BP monitoring (ABPM). Evidence indicates that an abnormal *diurnal* pattern of BP on ABPM, defined by reduced BP dipping or elevated nighttime BP, is associated with an increased risk of cardiovascular disease (CVD) events.

Psychological stress occurs when an individual perceives that the environmental demands exceed his/her adaptive capacity. An individual's response to events that are representative of this overload, such as perceived stress and negative affect including anger, hostility, depression, vital exhaustion, and symptoms of posttraumatic stress disorder, are associated with reduced BP dipping and/or higher nighttime BP. Exposure to environmental factors which tax an individual's ability to cope, including lower socioeconomic status, job strain, and perceived racism, are also associated with reduced BP dipping and/or higher nighttime BP. This study will examine the disruption of the normal *diurnal* pattern of sodium excretion by psychological stress as a novel biological mechanism underlying an abnormal *diurnal* pattern of BP.

During the daytime period, the normal response to sodium intake is increased BP, which leads to increased urinary sodium excretion. Most of the sodium that a person consumes is excreted during the daytime period, and a minimum is excreted during the nighttime period. It has been hypothesized that when urinary sodium excretion does not occur during the daytime period, urinary sodium excretion is shifted toward the nighttime period. Increased urinary sodium excretion at night is accompanied by an increase in nighttime BP which in turn facilitates sodium excretion.

Exposure to an acute stressor using a mental stress task normally increases urinary sodium excretion in the laboratory setting. However, there is substantial variability between individuals in the degree of stress-induced sodium excretion. Individuals with lower stress-induced sodium excretion who experience psychological stress during the daytime may exhibit a shift of urinary sodium excretion from the daytime to nighttime periods due to an inability to excrete additional sodium during the daytime. This abnormal *diurnal* pattern of urinary sodium excretion may, in turn, be associated with a smaller decline in BP from the daytime to nighttime periods, leading to reduced BP dipping and elevated nighttime BP. The overall goal of the proposed study is to examine the associations between psychological stress, the *diurnal* pattern of sodium excretion, and the *diurnal* pattern of BP. The aims of the study are:

> **Primary Aim 1**: To examine the association between urinary sodium excretion after provoked psychological stress and the diurnal pattern of sodium excretion.
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> **Primary Aim 2**: To examine the association between the diurnal pattern of sodium excretion and the diurnal pattern of BP.

Two secondary or exploratory aims that will also be investigated:

> **Secondary Aim**: To examine whether the association between urinary sodium excretion after provoked stress and the *diurnal* pattern of sodium excretion is modified by ecological momentary levels of perceived stress, experienced during the daytime period.

Exploratory Aim: To determine the sociodemographic, behavioral, and psychological traits, chronic stress, and biological stress-related factors that are associated with lower stress-induced sodium excretion. Identification of these factors will help determine who is at risk for having a differential sodium excretion response to psychological stress.

2. Study Design

The study will be conducted both in the laboratory and in the naturalistic environment with a multi-ethnic sample of 211 adult community participants from upper Manhattan who do not have a history of *CVD*, *diabetes, chronic kidney disease, difficulty with blood draws,* or another *major medical condition* and are not taking antihypertensive medication. During a Laboratory Visit, urinary sodium excretion in response to mental stress tasks will be examined. Urinary volume, electrolytes, creatinine, and endothelin-1 will be measured at baseline, post-stress, and recovery; salivary cortisol will be measured at baseline and post-recovery; the urinary albumin-to-creatinine ratio will be measured at baseline; plasma angiotensin II and epinephrine/norepinephrine will be measured at baseline and post-stress, and a basic metabolic panel, a lipid panel, and a Hemoglobin A1c assessment will be used at the baseline only. Blood pressure and heart rate will be measured continually during the Laboratory Visit (every five minutes) and for the following 24 hours (every 30 minutes).

In the naturalistic environment, during the 24-hour period following the Laboratory Visit, the diurnal pattern of sodium excretion (measured during the daytime and nighttime) will be assessed by measuring urine volume and the urine concentrations of electrolytes and creatinine. Heart rate will be monitored using ABPM and ecological momentary assessments (EMA) of perceived stress will be surveyed.

The study has high clinical significance for improving our understanding of how psychological stress, sodium excretion and its *diurnal* pattern, and the *diurnal* pattern of BP are linked, and has the potential for identifying a treatment target to reduce the CVD risk associated with reduced BP dipping.

3. Study Procedures

a. Participant Engagement

Interested individuals will review an online information sheet and provide consent to participate in an electronic eligibility screen. This form will provide the contact information of the primary investigator, clinical research coordinator, and IRB office to ensure that all questions about the study, form, and participant's rights are answered before consent for screening is provided. Consent must be provided before access to the eligibility screen is allowed.

Individuals who pass the initial eligibility screen will be asked to provide contact information so that they may be contacted by a research coordinator by phone or email using a participant-specified contact method and time. Participants who pass the initial screen will also be asked to provide their gender. This is done to ensure that gender distribution in recruitment and enrollment can adhere to the targeted proportions. During this contact, the coordinator will ensure that potential participants understand the study, are eligible to participate, and can communicate in English satisfactorily. After all participant questions are answered and eligibility has been reconfirmed, an Enrollment Visit will be scheduled. If the Enrollment Visit cannot be scheduled at this time, the research coordinator will contact the participant at another specified time to do so. When scheduling the Enrollment Visit, the coordinator will ask the participant not to smoke within one hour before the scheduled visit time.

For the Enrollment Visit, participants will come to the Shimbo Hypertension Lab (Tower 1) at the Columbia University Medical Center between 9 AM and 5 PM. To start, the research coordinator will explain the study procedures to participants in detail. The coordinator will then present the participant with a physical copy of the study consent form, review this form verbally, and answer any questions that

the participant may have. A HIPAA Authorization form will also be provided to the participant. The provision of consent on both forms is necessary for further study participation. If consent is provided, demographic information will also be collected at this time. Participants will also be asked to provide a home address so coordinators can provide an estimation of transportation costs that can be reimbursed in future visits. Then, the clinical research coordinator will measure participant blood pressure three times with one-minute intervals between measurements. Measurements will be made using an Omron HEM-907XL device. These measurements will be used to ensure that participants are not in violation of the study's eligibility criteria concerning hypertension. The coordinator will also ensure that participants have readily available veins for blood draws during the Laboratory Visit. A copy of blood pressure readings will be provided to participants. Next, the "ISQ: Psychometric Evaluation" will be administered as a short survey to ensure that participants do not experience insomnia, another condition precluding participation in the study. If the participant does not meet eligibility criteria for either blood pressure or insomnia, then the participant is compensated and the visit will end.

If the participant remains eligible, then the "Life Events Checklist" and "PTSD Checklist" will be provided separate from the other measures to ensure that the completion of these two questionnaires does not alter stress felt during the Laboratory Visit. Stress- and demographic-related measures will be presented to participants using Qualtrics opened on a provided laptop, and the insomnia survey will be administered as a paper form. The participant's weight, height, hip and waist circumference, gender, age, normal activity levels, and dietary limitations will then be recorded on a Bionutrition Center form. In addition, a list of foods provided by the Bionutrition Center will be provided and the participant asked whether they agree to eat all of the foods on the checklist. This information will be recorded for research use and forwarded to the Bionutrition Center to prepare the meals and snacks that will be consumed in the three days prior to the Laboratory Visit.

Finally, the research coordinator will provide the participant with a home polysomnography device and instruct him or her in its use. This tool is used to detect sleep apnea, a condition that will preclude participation in the study. An instruction sheet will be provided to participants with the device for their reference. This device will be used by the participant during the night after the Enrollment Visit. Collected data will be analyzed when the device is returned to the Shimbo Hypertension Lab (Tower 1) within 1 week.

The dates of the Pre-Laboratory and Laboratory Visits may be scheduled during the Enrollment Visit or via phone or email in the following days. Within one week of the Enrollment Visit, the home polysomnography device must be returned to the clinical research coordinator. After returning the device, participants will also be informed as to whether they are eligible to participate in the remainder of SABRE. If eligible, the Pre-Laboratory and Laboratory Visits will be scheduled at this time. Regardless of whether participants experience sleep apnea, they will be provided with a form that details their measured AHI (Apnea-Hypopnea Index) and the AHI cutoffs that represent mild, moderate, and severe obstructive sleep apnea.

If sleep apnea (mild, moderate or severe) is identified in a study participant, the participant will be informed that such a diagnosis warrants further evaluation and possible treatment by a physician. The participant will also be provided with a summary of the assessment results upon request (a sample report is included for IRB review). The participant will have the option to bring the report to a physician they already have a treatment relationship with or, if they do not have a primary care physician, then research staff will offer to refer the participant to a primary care physician at Columbia University Medical Center/Columbia Doctors.

If participants do not experience sleep apnea and are fully eligible for the study, he or she will then work with the clinical research coordinator to schedule the Pre-Laboratory and Laboratory Visits or

confirm the dates that were proposed at the Enrollment Visit. Additionally, the clinical research coordinator will make the necessary preparations for these visits, including informing the Bionutrition Center about the need for prepared meals, scheduling an examination room in the Irving Institute for Clinical and Translational Research (CTSA), and preparing materials for the visit.

Within eight weeks of the Enrollment Visit, the Pre-Laboratory Visit will occur. At this time, the participant will be provided with meals and snacks prepared by the Irving Institute Bionutrition Unit. These meals will be packed into a portable cooler for participant convenience. In addition, the participant will have been provided with the link to the SABRE Online Measures one week prior to the Pre-Laboratory Visit. The research coordinator will check in with the participant at this time to verify they have completed the questionnaire by this time and respond to any questions that may arise. These measures ask about different aspects of life and personality, and they may be completed at any time before the Pre-Laboratory Visit.

At the end of the Pre-Laboratory Visit, participants will be informed that fees associated with parking or transportation to their desired destination with the cooler of food can be reimbursed during the final visit, provided they pay in cash and keep the receipt.

Starting the morning after the Pre-Laboratory Visit, participants will be asked to eat only this specially prepared food for the next 3 days. No additional foods or drinks may be consumed other than water, plain decaf or mint or chamomile tea without sugar, milk or other additives, and up to one cup, or approximately 8 fluid ounces, of black coffee/tea per day. The meals and snacks consumed each day will contain the same total amount of salt. The diet that will be provided to participants is not a dietary intervention designed to lower the amount of sodium in a person's diet, but rather to keep the amount of dietary sodium fixed during the study visits. As such, the amount of dietary sodium (as well as potassium) are considered to be a moderate amount, which is consistent with the average amount a US adult is taking. Please see https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/1112/Table_1_NIN_GEN_11.pdf. The participant will eat this food for the three days following the Pre-Laboratory Visit and leading up to the Laboratory Visit. The research assistant will contact him or her every day to make sure there are no issues. The Laboratory Visit will be scheduled 4 days after the Pre-Laboratory Visit. For example, if the Pre-Laboratory Visit takes place on Thursday, the Laboratory Visit will take place on Monday.

For the Laboratory Visit, participants will come to the Shimbo Hypertension Lab (Tower 1) at the Columbia University Medical Center. Appointments will be scheduled between 8 AM and 9 AM in the morning four days after the Pre-Laboratory Visit. This visit should be attended in a fasting state (without eating or drinking anything more than small sips of water after midnight of the day before. Participants will also be asked to not exercise for 12 hours before the visit and to not smoke within the hour prior to the visit. At 30 minutes before the appointment, the participant will be asked to drink 8 ounces of Poland Spring water that will be provided prior to the appointment.

The research coordinator will escort the participant to the adult outpatient unit of the Irving Institute for Clinical and Translational Research (CTSA) on PH-10 where the Laboratory Visit will be conducted. The participant will be seated in a comfortable chair in an examination room with a private bathroom for the entire laboratory session. First, he/she will be asked to use the bathroom to void their bladder, if possible. The participant will then return to the exam room chair and drink about one cup of bottled spring water. At this time, the research coordinator will attach a SpaceLabs OnTrak Ambulatory Blood Pressure Monitor (ABPM) blood pressure cuff to his/her upper arm. This device will automatically inflate and measure blood pressure every five minutes for the duration of the Laboratory Visit, excluding time spent in the bathroom and times when blood is drawn. The participant will then rest by watching a movie for 30 minutes. This movie will show ocean scenery and aquatic wildlife to relax patients before

blood, saliva, and urine are collected at the baseline time point. No narration, camera motion, or potentially stressful events are used or shown in the video. After this rest period, the participant will be asked about the level of stress he/she is feeling using a Visual Analog Scale (VAS). A sample of saliva will be collected by the research coordinator. The participant will again use the bathroom where he/she will be asked to urinate into a collection container. He/she will return to the exam room chair and a blood sample of about 31.5 milliliters, or 7 teaspoons, will be drawn. If the phlebotomist is not available to draw the participant's blood within the exam room, participants will be escorted to the phlebotomist within the CTSA for this service.

Next, the participant will drink about one cup of bottled spring water. During the next 10 minutes, a research assistant (separate from the research coordinator) known as the stress inducer will read a mental stress script and conduct two stress tests. First, the participant will be shown a series of words for five minutes and a research coordinator will ask him/her to name the color of each (Stroop Color-Word Conflict Task). Then, for another five minutes, the participant will be asked to complete a mental arithmetic task, comprising of a series of subtractions and additions (Mental Arithmetic Task). The stress inducer will adjust the difficulty of the task appropriate for each participant. The stress inducer will ask the participant to work as quickly and accurately as possible for both tasks.

Immediately after the math task, the participant will be asked about the level of stress he/she is feeling using a VAS measurement. Then, the participant will use the bathroom, where he/she will be asked to urinate into a collection container. He/she will return to the exam room chair and another blood sample of about 24.5 milliliters, or 5.5 teaspoons, will be drawn. If the phlebotomist is not available to draw the participant's blood within the exam room, participants will be escorted to the phlebotomist within the CTSA for this service.

Next, the participant will drink about one cup of bottled spring water and remain inactive during for a post-stress recovery period. During this time, participants will rest naturally, allowing for elevated heart rate, increased blood pressure, and other stress symptoms to recover. Twenty minutes following the end of the stress induction, a saliva sample will be collected. After 30 minutes of recovery, the participant will be asked about the level of stress he/she is feeling for a final time, using a VAS measurement. Then, he/she will use the bathroom and asked to urinate into a collection container one final time.

Before leaving the Laboratory Visit, the participant's non-dominant arm will be fitted with a new standard ambulatory blood pressure monitor (ABPM). A research coordinator will explain how to use the device, provide one detailed instruction sheet and one concise instruction sheet, and provide a paper form where the times at which participants fall asleep, wake up, take naps, and remove the device may be listed. Participants will also receive a cooler containing two urine collection bottles, which are to be used following the Laboratory Visit. The participant will also receive instructions on the two bottles for collecting daytime and nighttime urine, respectively, over the next 24 hours. One, labelled Day, should be used during the day following the Laboratory Visit. The other, labelled Night, should be used if the participant wakes up to urinate during the night and for the first instance of urination after waking up the next day. The participant will be instructed to continue their normal daily and nightly routines, but they must limit their alcohol intake to one drink during the 24 hour period. The Laboratory Visit may take up to 3 hours.

The clinical research coordinator will ensure that, at 5 random times throughout the day and evening, the participant will be prompted by text message to respond electronically to 5 short questions through Qualtrics. The questions will ask about how participants feel in that moment.

After 24 hours, participants will return the ABPM device, the cooler containing both urine collection bottles, and the cooler containing any remaining food from the diet to Shimbo Hypertension Lab (BB-210 or Tower 1). They will complete a final 5-question survey through Qualtrics, identical to the ones they received via text that will ask them about the emotions they were feeling that morning prior to the visit. Participants will be provided the option of having Lyft concierge rides coordinated for them while traveling with the cooler, our research team will coordinate this service. Alternatively, transportation may be provided at the discretion of the Principal Investigator on a case-by-case basis. Patient satisfaction surveys will be provided at this time if participants are interested in completing one.

b. Blood, Urine, and Saliva Analysis

During each Laboratory Visit, three samples of urine and two samples of saliva will be collected. In addition, blood will be drawn twice. On the following day, two additional samples of urine, one for the previous day and one for the previous night, will be returned to the Shimbo Hypertension Lab (Tower 1) by the participant.

Saliva will be collected using a salivettes tube and salivary cortisol will be measured at the baseline and recovery time points. The first blood draw of 31.5 milliliters will be used to test for a basic metabolic panel (which includes glucose and creatinine), a lipid panel (including cholesterol, low density and high density lipoproteins, and triglycerides), Hemoglobin A1c, Angiotensin II, and Catecholamines. The second blood draw of 24.5 milliliters will be tested for Angiotensin II and Catecholamines. An approximate total of 56 mL of blood will be collected. The urine collected during the laboratory study will be tested for sodium, potassium, chloride, creatinine, Endothelin-1, and volume. The Albumin to Creatinine ratio in urine will also be measured at the baseline time point. Urine collected during the ambulatory session will also be tested for sodium, potassium, chloride, creatinine, and volume.

c. Providing Results to the Participant

After completing the study, the participant will be given the following results: total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, hemoglobin A1c, glucose, and creatinine. Please see attached SABRE Blood Draw Results_REPORTING TEMPLATE. The participant will also be provided the results from the ABPM. Please see ABPM REPORT_EXAMPLE and ABPM REPORTING TEMPLATE. The participant will be given the option to not receive these results (i.e. opt-out). The participant will be invited to share the results with their health care provider. If they do not have a health care provider, the research staff will offer to refer the participant to a primary care physician at Columbia University Medical Center/Columbia Doctors.

4. Statistical Procedures

Data Preparation: All variables will be reviewed for outliers and internally inconsistent values. Once a valid, final data set is compiled, variable distributions will be evaluated for non-normality and data transformations will be applied as needed. Winsorization may be employed to reduce the effect of extreme, but valid, observations on data analyses. Although transformed data will be used for primary inferential analyses, the description of data will feature the original (untransformed) metric for ease of interpretation.

Missing Data: Given the reasons for missing data (i.e., laboratory error or equipment failure), missing data for our primary variables will likely conform to a "missing completely at random" (MCAR) pattern. However, we will use the less restrictive "missing at random" (MAR)-analytic techniques (e.g. mixed effects regression, multiple imputation) for the analyses. For those who are missing a small number of items used to construct a psychosocial scale, we will use the EM algorithm in SAS' PROC MI procedure to estimate the expected scale score for these participants conditional on their responses to all the other

items; this is the best estimate, assuming MAR, of their scores. Although we anticipate few missing data for the primary variables (i.e. stress-induced sodium excretion, *diurnal* patterns of sodium excretion and BP) as 100% of the participants in the SABRE pilot study had complete data, we have conservatively allowed for approximately 5% (N=*11*) of the *211* enrolled participants to have some missing data. A minimum sample size of *200* participants without missing data will provide adequate power for testing our primary hypotheses.

Fidelity Checks: VAS stress ratings will be collected before and after laboratory stress induction. VAS stress ratings will be monitored during the conduct of the study to ensure that the manipulation (i.e. mental stress tasks) is having its intended effects. If the stress tasks are not having their intended effects, Dr. Shimbo will work with the research staff to bolster the dose of the experimental manipulation that is administered. The focus of these fidelity checks will be on the magnitude of the pre-/post-stressor change in VAS ratings rather than on the statistical significance of the effects (which may be limited by sample size). The research coordinator will be blinded to the VAS stress ratings.

Analytical Approach for Each Aim:

Primary Aim 1: Standard regression analysis will be used. We will estimate the unadjusted relation of awake-to-sleep ratio of urinary sodium excretion rate to change in urinary sodium excretion rate with stress. Next, we have *a priori* selected the following covariates for adjustment in a multiple regression model: age, sex, race/ethnicity (non-Hispanic whites, non-Hispanic blacks, Hispanics, and other), and body mass index. Age, sex, and race/ethnicity are standard covariates in studies of hypertension given their well-known associations with BP. Body mass index is included as a covariate since it is associated with both lower stress-induced sodium excretion and lower day-to-night ratio of urinary sodium excretion rate. The statistical significance of the coefficient for the change in urinary sodium excretion rate with stress in this adjusted model will constitute the primary test of the hypothesis. Sensitivity analyses will be performed to assess whether the strength of the association changes when additional covariates are included. Higher clinic SBP/DBP and parental history of hypertension are associated with lower stress-induced sodium excretion. Fasting blood glucose and 24-hour creatinine clearance are associated with lower daytime-to-nighttime ratio of urinary sodium excretion rate. Each of these will be evaluated as candidate covariates by selecting those that are associated with the outcome (awake-to-sleep ratio of urinary sodium excretion rate) at $p<0.10$. Finally, secondary analyses will use generalized additive models (GAM) to explore potential non-linearity of the relationship, using PROC GAM in SAS.

Primary Aim 2: The same approach for Hypothesis 1 will be used for the analysis of this hypothesis. SBP dipping and DBP dipping will be the primary and secondary outcomes, respectively. The *a priori* covariates that will be included in adjusted models are: age, sex, race/ethnicity, body mass index, 24-hour sodium excretion, 24-hour potassium excretion, 24-hour creatinine clearance, FENa, current smoking, alcohol consumption, and fasting blood glucose. In addition to age, sex, and race/ethnicity (which were chosen because they are standard covariates in hypertension studies), the other measures were chosen as covariates as they may confound the association between the *diurnal* pattern of sodium excretion and the *diurnal* pattern of BP. Sensitivity analyses will be performed after additional covariates are included in the adjusted model. Prior studies have suggested that physical activity and fasting glucose are associated with the diurnal pattern of BP. Each of these measures will be evaluated as candidate covariates using the same approach described above for Primary Aim 1.

Sample Size and Minimum Detectable Effect Sizes for Primary Aims: The sample size for this study was selected to ensure 80% power to detect a bivariate/partial correlation between the primary predictor and outcome of r=0.20 or greater; this is conservative, but consistent with our preliminary data in which the correlation between the change in urinary sodium excretion rate with stress and awake-to-sleep

ratio of urinary sodium excretion rate (Primary Aim 1) was 0.25, and the correlation of awake-to-sleep ratio of urinary sodium excretion rate and SBP dipping (Primary Aim 2) was 0.30. The minimum sample size required to detect this effect size with power 80% is 194 with valid data for the three primary measures (change in urinary sodium excretion rate with stress, awake-to-sleep ratio of urinary sodium excretion, and SBP dipping). We are targeting a final minimum sample size with complete data of 200.

Secondary Aim: The ecological stress level for the awake period during which participants' sodium excretion is monitored will be defined as the mean perceived stress level across the Ecologic Momentary Assessment (EMA) reports. Ecological stress is conceptualized as a moderating variable. Controlling for the effects of this measure and stress-induced sodium excretion, we will test their multiplicative interaction term; an extension of the regression model used to test Primary Aim 1. A positive interaction effect would provide the first empirical evidence supporting the conclusion that the hypothesized tendency for those with lower stress-induced sodium excretion rate to have a lower daytime-to-nighttime ratio of urinary sodium excretion rate is stronger among those who experience higher stress during the day of monitoring. Although a moderator is not required to be associated with the outcome, we will examine the association between mean perceived stress and daytime-to-nighttime ratio of urinary sodium excretion rate. In the SABRE pilot study, the correlation coefficient for this association was -0.42.

Exploratory Aim: Univariable and multivariable linear regression analyses will be performed to assess which factors are independently associated with lower stress-induced sodium excretion. All models will adjust for age, sex, and race/ethnicity. Variables entered into subsequent multivariable regression models will include other social determinants (place of birth, years living in the US, primary language spoke in the home, education attainment, employment status, health insurance status, marital status, household income, household size, social cohesion and safety, social support), behavioral factors (body mass index, current smoking, alcohol consumption, physical activity, recreational drug use), psychological traits and other psychosocial constructs, chronic stress factors (related to work, marriage, finances, caregiving, discrimination; perceived stress; and childhood adversity), and biological stress measures (baseline angiotensin II, epinephrine and norepinephrine, BP, heart rate, endothelin-1, and cortisol; and change in these measures with stress). Given the large number of predictors being examined, we will apply a false discovery rate of 0.05 (rather than ignore the inflated risk of Type I errors or use the excessively conservative Bonferroni correction), and interpret the results as "hypothesis generating" rather than "confirmatory".

Additional Exploratory Analyses: Social determinants including place of birth, years living in the US, etc. will be evaluated as candidate covariates by selecting those that are associated with the outcomes in Primary Aims 1 and 2 at $p<0.10$. The analyses for Primary Aim 2 will be repeated using mean sleep SBP and mean sleep DBP instead of SBP dipping and DBP dipping, respectively. Some investigators consider mean sleep BP to also be a measure of the diurnal pattern of BP. The analyses for the Secondary Aim will be repeated using the average of the EMA reports for each negative affect (angry/hostile, aggravated/irritated, sad/blue/depressed, and anxious/tense/nervous) instead of perceived stress to determine whether negative affect moderates the association between lower stress-induced sodium excretion and an abnormal diurnal pattern of sodium excretion. This analysis will be repeated using a composite negative affect score derived by averaging the EMA reports of all 4 negative affect ratings. We will evaluate multiplicative interaction terms of sex and separately race/ethnicity with stress-induced sodium excretion to test for group differences in its association with the diurnal pattern of sodium excretion and with the diurnal pattern of sodium excretion to test for group differences in its association with diurnal pattern of BP.

5. Risks
There may be risks or discomforts associated with taking part in this study.

There may be minimal discomfort during and after the Laboratory Visit due to the blood pressure arm cuffs that are used in clinical blood pressure measurements and ambulatory blood pressure monitoring. The discomfort is temporary and minor. The ambulatory monitor takes measurements every 30 minutes. This may also cause disturbances to sleep.

Risk of Blood Draw
The risks of having blood drawn are soreness and/or a black and blue mark at the site where the blood is drawn. Sometimes, participants feel uncomfortable at the time of the blood draw. Occasionally, participants feel lightheaded or faint. There is also a small risk of infection whenever blood is drawn.

Risk from Sensitive Questions
This research study involves psychological testing. The questions being asked may be sensitive and personal in nature. It is possible that answering some questions may cause stress. Participants will be instructed to immediately inform the study coordinators if they become upset or anxious. The study staff will then stop performing psychological testing.

Risk of Breach of Confidentiality
A risk of taking part in this study is the possibility of a loss of confidentiality. Loss of confidentiality includes having personal information shared with someone who is not supposed to see or know about your information. The study team plans to protect participant confidentiality.

6. Benefits
Participants will not receive personal (direct) benefit from taking part in this research study. However, the information collected from this research may help others in the future.

7. Alternatives
Participants may choose not to take part in this research study.

8. Data and Safety Monitoring
The research team will monitor the study to prevent any risks or dangers that may occur and will appropriately report any adverse events according to IRB policy.

If a participant feels upset or experiences emotional distress, a member of the research team will be available to talk to them and discuss appropriate care. He/she will be offered a period of rest from study activities, and he/she will also be reminded that participation may be discontinued willingly at any time. Further, should any psychological issues arise, clinical psychologists on staff at the Shimbo Hypertension Lab (BB-210 and Tower 1) will also be available to evaluate the participant. If the participant needs further attention, he/she will be escorted to the emergency room for further evaluation. If there is no immediate risk, the participant will remain until the end of the study visit and be discharged. Participants will then be referred to their primary care doctor and as appropriate, the Columbia University Psychiatry Referral Line for further management.

Participants will be provided with copies of their own blood pressure and apnea-hypopnea index (AHI) measurements after this data is collected at the Enrollment Visit or during the following night.

The average blood pressure reading will be accompanied by a group of recommendations from the American Heart Association regarding actions that should be taken for abnormal values. If the average BP (systole ranges/diastole ranges) is high normal, or 120-139/80-90 mmHg, it will be recommended that participants ask a physician about lifestyle changes and be rechecked within a year. If the average

BP is mildly elevated, or 140-159/90-99 mmHg, it will be recommended that participants meet with their doctors to confirm this reading and receive instruction within a month. If the average BP is high, or 160-179/100-109 mmHg, it will be recommended that participants meet with their doctors for a follow-up within a week. If the average BP is very high, or 180-209/110-119 mmHg, it will be recommended that participants meet with their doctors for a follow-up within a week. If the average BP is equal to or greater than 210/120, participants will be escorted to the emergency room for immediate care.

The measured AHI value will be accompanied by a list of AHI ranges that correspond to mild, moderate, or severe sleep apnea. If the participant's measured AHI falls outside of the normal range, the participant will be informed that such a diagnosis warrants further evaluation and possible treatment by a physician, and the form itself will reiterate that they inform their regular physician of these results and ask for further guidance. If the participant does not have a primary care physician, then research staff will offer to refer the participant to a primary care physician at Columbia University Medical Center/Columbia Doctors for follow-up.