Institutional Review Board
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DATE:	June 25, 2025
TO:	Jody Greaney, PhD
FROM:	University of Delaware IRB
STUDY TITLE:	[2056784-16] Accelerated vascular aging in midlife as a mechanism linking daily stress to cognitive decline
SUBMISSION TYPE:	Continuing Review/Progress Report
ACTION:	APPROVED
APPROVAL DATE:	June 25, 2025
EXPIRATION DATE:	July 18, 2026
REVIEW TYPE:	Full Committee Review

Thank you for your Continuing Review/Progress Report submission to the University of Delaware Institutional Review Board (UD IRB). The UD IRB has reviewed and APPROVED the proposed research and submitted documents via Full Committee Review in compliance with the pertinent federal regulations.

As the Principal Investigator for this study, you are responsible for and agree that:

- All research must be conducted in accordance with the protocol and all other study forms as approved in this submission. Any revisions to the approved study procedures or documents must be reviewed and approved by the IRB prior to their implementation. Please use the UD amendment form to request the review of any changes to approved study procedures or documents.
- Informed consent is a process that must allow prospective participants sufficient opportunity to discuss and consider whether to participate. IRB-approved and stamped consent documents must be used when enrolling participants and a written copy shall be given to the person signing the informed consent form.
- Unanticipated problems, serious adverse events involving risk to participants, and all non-compliance issues must be reported to this office in a timely fashion according with the UD requirements for reportable events. All sponsor reporting requirements must also be followed.

Oversight of this study by the UD IRB REQUIRES the submission of a CONTINUING REVIEW seeking the renewal of this IRB approval, which will expire on July 18, 2026. A continuing review/progress report form and up-to-date copies of the protocol form and all other approved study materials must be submitted to the UD IRB at least 45 days prior to the expiration date to allow for the required IRB review of that report.

If you have any questions, please contact the UD IRB Office at (302) 831-2137 or via email at hsrb-research@udel.edu. Please include the study title and reference number in all correspondence with this office.

HUMAN SUBJECTS PROTOCOL
University of Delaware

Protocol Title:
Accelerated vascular aging in midlife as a mechanism linking daily stress to cognitive decline

Principal Investigator
Name: Jody Greaney, PhD
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Advisor (if student PI):
Name:
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Other Investigators:
Name: David Almeida, PhD
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Investigator Assurance:

By submitting this protocol, I acknowledge that this project will be conducted in strict accordance with the procedures described. I will not make any modifications to this protocol without prior approval by the IRB. Should any unanticipated problems involving risk to subjects occur during this project, including breaches of guaranteed confidentiality or departures from any procedures specified in approved study documents, I will report such events to the Chair, Institutional Review Board immediately.

1. **Is this project externally funded?** **X** YES ☐ NO

If so, please list the funding source: NIH (COBRE Pilot Project)

2. Research Site(s)

X University of Delaware

X Other (please list external study sites): The Pennsylvania State University

Is UD the study lead? **X** YES ☐ NO (If no, list the institution that is serving as the study lead)

3. **Project Staff**
Please list all personnel, including students, who will be working with human subjects on this

protocol (insert additional rows as needed):

NAME	ROLE	HS TRAINING COMPLETE?
Jody Greaney, PhD	PI	yes
David Almeida, PhD	Co-I	yes
Megan Wenner, PhD	Co-I	yes
Freda Patterson, PhD	Co-I	yes
Keith Bredemeier, PhD	Co-I/Psychiatric Clinical Oversight	yes
Charles Webb, PhD	Licensed Clinical Psychologist/Psychiatric Clinical Oversight	yes
Jennifer Graber, EdD, APRN, PMHCNS-BC	Research Nurse/Psychiatric Clinical Oversight	yes
Caren Coffy-McCormick, DNP, PMHCNS-BC, MSN, RN	Research Nurse/Psychiatric Clinical Oversight	yes
Wendy Nichols, RN	Research Nurse	yes
Katie Hibbert, RN	Research Nurse	yes
Virginia Nuckols, PhD	Postdoctoral Fellow	yes
Shannon Mayberry, MS	Project Manager	yes
Aaron Autler, MSW, LSW	Graduate Student	yes
Madison Evering, BS	Graduate Student	yes
Allyson Schwab, BS	Graduate Student	yes
Clarisse Hunt, BS	Graduate Student	yes
Navyasree Vadlamudi	Undergraduate Student	yes
Joy Mochache	Undergraduate Student	yes
Fatema Javed	Undergraduate Student	yes

Note: Undergraduate Student Roles
All undergraduate student interns/volunteers involved in this project have completed all required trainings for handling biological specimens for humans, and have been trained by the PI and graduate students for each specific procedure (e.g., centrifugation, pipetting, sample storage). They will not perform any procedure that is invasive (e.g., microneurography) or requires a high degree of technical skill (e.g., Doppler ultrasonography). In addition, they will have limited direct interaction with participants. Undergraduate student interns/volunteers mostly function in a supporting role and assist all aspects of the study [e.g., controlling the data acquisition computer, which is a task that requires being present for the duration of the experiment (~4 hrs) but does not require undergraduate students (usually) to ever physically touch the participant or speak to them].

4. **Special Populations**
Does this project involve any of the following:

Research on Children?

no

Research with Prisoners?

no

If yes, complete the Prisoners in Research Form and upload to IRBNet as supporting documentation

Research with Pregnant Women?

no

Research with any other vulnerable population (e.g. cognitively impaired, economically disadvantaged, etc.)? please describe

Adults with major depressive disorder (MDD; unmedicated)

We have extensive experience in recruiting and enrolling adults with MDD in research studies. We have robust standard operating procedures in place to ensure the safety of participants. In no case will a participant be denied necessary medical or psychiatric treatment for reasons related to study participation. We will enroll and test unmedicated adults with MDD (no patients with MDD who are currently treated with antidepressant pharmacotherapy will be asked or allowed to discontinue to participate). We have multiple screening and crisis protocols in place to ensure patient safety and compliance with study procedures. Our crisis intervention plan allows for rapid referral, including immediate referral for acute psychiatric issues (described below and in accompanying documents). This crisis protocol will be triggered if participants express *1)* active current suicidal ideation with intent or a specific plan, *2)* active current suicidal behavior with a preparatory attempt, or *3)* at the discretion of the research team. The presence of suicidal ideation without intent will not trigger any additional assessments, as this is considered low acute risk. In the past 4 years, our laboratory has screened 178 adults for inclusion in similar ongoing studies, with only two instances that required triggering the crisis intervention protocol (~1% of screenings trigger the crisis protocol). As a demonstration of our laboratory's experience with this patient population, a list of publications is included below:

1. **Greaney JL**, Saunders EFH, and Alexander LM. Short-term salicylate treatment improves microvascular endothelium-dependent dilation in young adults with major depressive disorder. Am J Physiol Heart Circ Physiol, 322(5): H880-H889, 2022. *selected for APSselect award
2. **Greaney JL**, Darling AM, Mogle J, and Saunders EFH. Microvascular β-adrenergic receptor-mediated vasodilation is attenuated in adults with major depressive disorder. Hypertension, 79(5): 1091-1100, 2022.
3. Grotle AK, Darling AM, Saunders EFH, Fadel PJ, Trott DW, and **Greaney JL**. Augmented T-cell mitochondrial reactive oxygen species in young adults with major depressive disorder. Am J Physiol Heart Circ Physiol, 322(4): H568-574, 2022.
4. **Greaney JL**, Darling AM, Turner JR, Saunders EFH, Almeida DM, and Mogle J. COVID-19-related daily stress processes in college-aged adults: influence of depressive symptom severity. Front Psychol, 2021.
5. Darling AM, Akins JD, Richey RE, Saunders EFH, Brothers RM, and **Greaney JL**. The influence of current depressive symptomology on cerebrovascular function in young adults with major depressive disorder. J Affect Disord, 295: 513-521, 2021.
6. **Greaney JL**, Dillon GA, Saunders EFH, and Alexander LM. Peripheral microvascular serotoninergic signaling is dysregulated in young adults with major depressive disorder. J Appl Physiol, 128(1):100-107, 2020.
7. **Greaney JL**, Koffer RE, Saunders EFH, Almeida DM, and Alexander LM. Self-reported everyday psychosocial stressors are associated with greater impairments in endothelial function in young adults with major depressive disorder. J Am Heart Assoc 8(4), 2019.

8. **Greaney JL**, Saunders EFH, Santhanam L, and Alexander LM. Oxidative stress contributes to microvascular endothelial dysfunction in men and women with major depressive disorder. Circ Res 124(4): 564-574, 2019. *accompanied by editorial highlight

5. **RESEARCH ABSTRACT** Please provide a brief description in LAY language (understandable to an 8th grade student) of the aims of this project.

Major depressive disorder (MDD) is a highly recurrent mood disorder characterized by persistently depressed mood and/or anhedonia (loss of interest or pleasure) that causes significant functional impairments. MDD affects ~15% of adults across their lifespan and is a leading cause of global disability and disease burden, surpassing both cardiovascular disease (CVD) and cancer. In addition to its debilitating effects on mood and behavior, MDD is also directly linked to the excessive and premature development of CVD and neurocognitive decline. As such, determining the mechanisms through which MDD increases future chronic disease risk can provide critical insight into strategies for earlier and more targeted therapeutic intervention.

We and others have demonstrated that peripheral endothelial dysfunction, mediated in part by a loss of nitric oxide (NO) bioavailability and function, is evident in young/middle-aged adults with MDD but otherwise free of CVD risk factors. However, the upstream mediators remain unclear. Inflammation and mitochondrial redox homeostasis are known to regulate NO bioavailability, and intriguingly, both are disrupted in MDD. Importantly, psychosocial stress can also trigger proinflammatory cytokine production and mitochondrial dysfunction. However, whether and how daily stress influences inflammation and mitochondrial redox balance, and the degree to which this translates to vascular dysfunction, in adults with MDD remains unclear. Daily stress is also associated with impairments in current cognitive performance and increased risk of future cognitive decline, but the mechanisms remain incompletely understood.

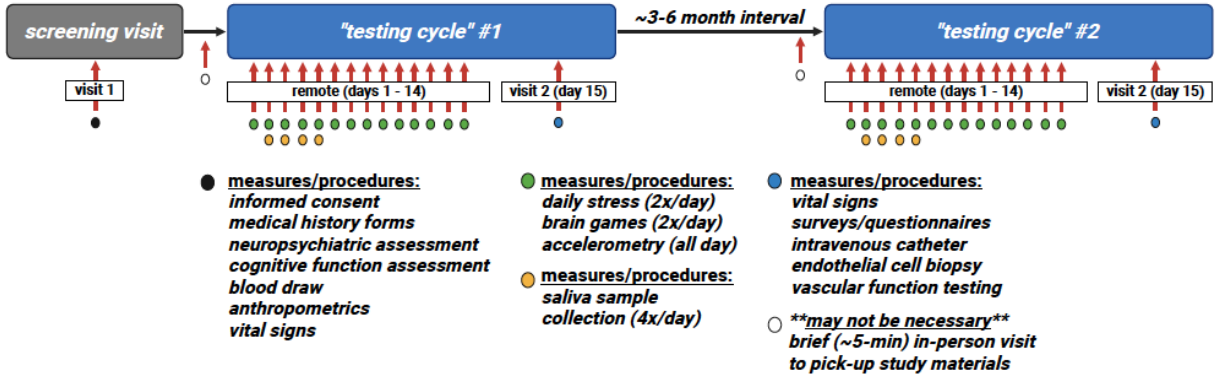
Aim 1: To determine if the links between daily stress and *1)* proinflammatory cytokine production and *2)* mitochondrial redox balance are sensitized in young-to-middle aged adults with MDD.
Aim 2: To determine the link between daily stress and peripheral endothelial function in young-to-middle aged adults with MDD.
Aim 3: To explore the complex interactions between daily stress, redox-immunological-vascular function, and daily cognitive function in young-to-middle aged adults with MDD.

6. **PROCEDURES** Describe all procedures involving human subjects for this protocol. Include copies of all surveys and research measures.

This is a cross-sectional study. Two subject groups will be recruited: *1)* non-depressed healthy adults (HA) will have no evidence of current or lifetime history of major psychiatric illness and 2) unmedicated adults with MDD will meet the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) diagnostic criteria for MDD.

Below is a detailed description of all techniques and measurements to be utilized and/or obtained, as well as a complete description of the experimental protocol (see schematic illustration below). First, all participants will complete an in-person screening/familiarization visit (~1.5 hours) to determine eligibility. Thereafter, each participant will complete two 15-day measurement bursts (hereafter referred to as “testing cycles”). Micro-longitudinal measurement burst/testing cycle designs consist of repeated sequences of closely spaced assessments that provide multiple time scales, allowing for the examination of processes unfolding within an individual over short time periods (e.g., days) as well as how these change over longer time intervals (e.g., months). Each 15-day testing cycle consists of 1) an ambulatory assessment of daily stress processes and cognitive function for 14 consecutive days (~2-3 hours in total), 2) daily wrist accelerometry for 14

consecutive days (no additional time burden), 3) daily saliva collection for 4 consecutive days (~1 hour in total), and 4) an experimental visit to assess vascular endothelial function (~6 hours). The two cycles will be separated by ~3-6 months; the lack of standardization of the timing between cycles is purposeful, as it will allow us to begin to more precisely disentangle the temporal patterning of the proposed relations both within- and between-subjects. This protocol is illustrated below:



Pre-Screening: Initial Interview/Survey to Determine Eligibility

Interested people may contact us. The script of the study information (see attached document "Study Information") will be provided verbally (if initial contact is by phone) or in writing (if initial contact is by email). During this initial contact, we also conduct a basic interview/survey to determine whether the participant is likely to meet study eligibility criteria (see attached document "Pre-Screening Survey"). Responses will either be recorded by a study team member (if initial contact is by phone) or electronically via REDCap (if initial contact is by email). Based on historical experience in our laboratory, we anticipate that the vast majority of these initial interviews will occur electronically; therefore, the attached Pre-Screening Survey is formatted for electronic administration. If the initial contact is by telephone, these interview questions will be read verbatim to participants; such telephone interviews will occur in a private office space and written responses will be documented.

This interview includes basic demographic information [name, phone number, email address, age and date of birth, preferred contact method, sex and gender, etc.] and a brief assessment of general health [height/weight, medications taken regularly, health history (e.g., any known diseases), history of tobacco use, etc.]. During this interview, potential participants will also complete the PROMIS Emotional Distress-Depression Short Form (these questions are embedded within the pre-screening survey). This scale can be administered over the phone or electronically. A raw PROMIS score > 18 is indicative of mild depressive symptoms. The PROMIS is used to "pre-screen" adults for depressive symptoms and thus limits the burden of scheduling and conducting screening visits in patients who are ultimately not likely to meet all eligibility criteria. Given the relative ease of recruiting non-depressed healthy adults into our research study, conducting the PROMIS prior to the on-site screening visit allows us to further target the patient population of interest. *This initial interview to determine eligibility presents no more than minimal risk of harm to subjects and involve no procedures for which written consent is normally required outside of the research context.*

If, after completion of this initial interview to determine study eligibility, the subject appears to meet inclusion criteria and is interested in enrolling, we will schedule a screening visit (see below for details). If enrolled, the interview responses will be coded with a unique subject number. Pre-screening 'failures' will be tracked for date of contact and reason for ineligibility; however, if not

enrolled, all pre-screening information will be de-identified and destroyed.

Screening/Familiarization Laboratory Visit
Note: All participants who attend this laboratory visit, regardless of continued enrollment or subject group, will be given materials on local resources for counseling/psychiatric care and crisis/suicide hotline information (created and published by the UD Center for Counseling and Student Development; this document outlines resources available to both students and non-students and includes resources available on- and off-campus), as well information published by the National Institute of Mental Health regarding symptoms of depression, anxiety, and suicidality (see attached document "Mental Health Resources"). If, at any time, a participant expresses active suicidal or homicidal ideation with intent to harm, an approved crisis protocol for immediate clinical evaluation will be implemented (see attached document "Crisis Protocol"). This safety plan was developed in consultation with Jennifer Graber, EdD, APRN, PMHCNS-BC and licensed clinical psychologist Keith Bredemeier, PhD. As psychiatric mental health advanced practice registered nurses, both Dr. Graber and Ms. Coffy-McCormick will provide psychiatric clinical oversight for the management of adults with MDD.

1. The participant electronically signs the informed consent before screening procedures begin using REDCap's eConsent framework. During the eConsent, the participation will take control of the screen so that they can digitally initial and sign the consent using their finger. After the participant signs the consent, they will receive a digital copy of the signed consent via email. Paper versions will be available for any participants who prefer a physical copy. Signed paper consents will be scanned and uploaded to REDCap; the hard paper copy will be shredded.
2. Participants provide contact information (see attached document "Contact Information") and complete a standardized general health history (see attached document "Medical History Form").
3. Because of the heterogeneity in the hormonal milieu of participants in the proposed age ranges, females and males will complete separate questionnaires to assess symptoms of reproductive aging (see attached documents "Females.Gyno+Meno" and "Males.AMS").
4. The investigator measures height (stadiometer), weight (digital scale), waist circumference (tape measure), seated blood pressure (Welch Allyn Connex), seated heart rate (Welch Allyn Connex), and temperature (Welch Allyn SureTemp Plus).
5. Women will provide a urine sample for a pregnancy test.
6. Dr. Greaney or a trained investigator administers the Mini International Neuropsychiatric Interview (MINI) to determine depression status (see attached document "MINI). The MINI has 16 sections and takes ~30 minutes to complete. During the neuropsychiatric interview, participants will be advised that they are able to stop the interview at any time or not answer questions if preferred.
    - The investigators provide all participants, regardless of neuropsychiatric interview responses or subject group assignment, with materials on local resources for counseling/psychiatric care and crisis/suicide hotline information (created and published by the UD Center for Counseling and Student Development; this document outlines resources available to both students and non-students and includes resources available on- and off-campus), as well information published by the National Institute of Mental Health regarding symptoms of depression, anxiety, and suicidality (see attached document "Mental Health Resources").
    - If a participant indicates a current or lifetime history of major psychiatric illness aside from MDD, we will provide them with the Mental Health Resources document, strongly encourage them to follow-up with a clinician, and exclude them from the study.
7. Dr. Greaney or a trained investigator administers the Columbia-Suicide Severity Rating Scale (C-SSRS) to assess suicidality (see attached document "C-SSRS Triage"). This scale was developed in partnership with the National Institute of Mental Health and has been validated

across many settings, including for use in biomedical research. It is an assessment tool that evaluates suicidal ideation and behavior and will help establish a subject's immediate risk of suicide and will be used as a criterion to evaluate acute suicide risk. Training is available before administering the C-SSRS (see attached document "C-SSRS.greaney completion certificate"). The C-SSRS takes <5 minutes to administer. Per existing Clinical Practice Guidelines, the presence of active current suicidal ideation with intent ("yes" to item 4) or a specific plan ("yes" to item 5) or active current suicidal behavior with a preparatory attempt ("yes" to item 6) will trigger the Crisis Intervention Protocol (see attached document "Crisis Protocol"). *The presence of suicidal ideation <u>without</u> intent will not trigger any additional assessments, as Clinical Practice Guidelines consider this to be low acute risk*.

8. Trained research personnel administer a battery of standardized cognitive function assessments on a tablet device. This assessment takes ~30 minutes. This will entail several computerized tests that assess major cognitive subdomains, including processing speed (e.g., Pattern Comparison Processing Speed Test), executive function (e.g., Dimensional Change Card Sort Test; Flanker Inhibitory Control and Attention Test), working memory (e.g., List Sorting Working Memory Test), episodic memory (e.g, Picture Sequence Memory test), and language (e.g., Picture Vocabulary Test; Oral Reading Recognition Test). These measures capture key cognitive processes known to impact daily function. Participants will also complete the NIH Patient-Reported Outcomes Measurement Information System (PROMIS) to assess self-reported cognitive function (see attached document "PROMIS Cognition").
9. A research nurse or trained laboratory personnel will perform <u>standard venipuncture</u> to obtain a blood sample (100 ml/~6.7 tbsp). The researchers do not perform genetic analyses on the blood, nor do they look for the presence of disease (e.g., HIV). Participants will be asked to be fasted for 12-hours (with the exception of water) before the blood draw. Blood samples will be sent to Labcorp Inc for the analysis of metabolic panel, complete blood count with differential, lipid panel, and HbA1c. Each blood sample container is labeled with two identifiers, the subject initials+study number and the subject's date of birth, as required by Labcorp (e.g., Subject: ABC 01234, DOB 10/14/65) and placed in a locked drop-box. Labcorp is contacted and a courier retrieves the sample from the drop-box on the same day. In case the subjects present any abnormal lab results from any of the bloodwork, we will inform them about their results and strongly encourage them to follow-up with their primary-care physician. No medical diagnosis will be provided. Some blood will also be stored in a -80°C freezer (in the laboratory space designated for freezers and refrigerators on the 2nd floor of the Tower at STAR) to allow for batched analyses of substances of interest [inflammatory cytokines, indices of redox balance, peripheral blood mononuclear cells, stress hormones, and sex hormones (estradiol, progesterone, follicle stimulating hormone, luteinizing hormone, and testosterone)] to avoid variability. All samples will be coded with a unique code for each subject. To provide the most flexibility to participants in terms of time constraints regarding scheduling, this procedure may occur at either the screening visit or the first experimental visit.
10. If the subject meets study eligibility/inclusion criteria and remains interested in continuing with enrollment, we will schedule the first testing cycle (ambulatory assessments and experimental visit). Participants will be familiarized with, and practice, the experimental measurements/procedures described in detail below:
    - Participants will receive detailed instructions on how to access and complete the daily surveys and will practice using the Gorilla experiment builder on their personal mobile device (this will be used to administer the daily cognitive tasks described below) and/or the Metricwire mobile app on a laboratory-provided smartphone. Depending on scheduling of "testing cycle #1" and the availability of associated equipment, some participants may be asked to complete an additional, but very brief (<5 min), in-person visit to pick up this equipment prior to Day 1 of the ambulatory assessments. Participants may also be asked to complete this brief in-person prior to Day 1 of "testing cycle #2."
    - Participants will be fitted for a wrist accelerometer (MotionLogger, Actigraph) that will be

worn every day during the 14-day assessment period and used to objectively measure daily sleep and activity/sedentary behaviors. They will also be instructed regarding its use (described below). Depending on scheduling of "testing cycle #1" and the availability of associated equipment, some participants may be asked to complete an additional, but very brief (<5 min), in-person visit to pick up this equipment prior to Day 1 of the ambulatory assessments. All participants will also be asked to complete this brief in-person prior to Day 1 of "testing cycle #2."

- Participants will also be instructed in and provided a home saliva collection kit (described below). Depending on scheduling of "testing cycle #1", some participants may be asked to complete an additional, but very brief (<5 min), in-person visit to pick up this equipment prior to Day 1 of the ambulatory assessments. All participants will also be asked to complete this brief in-person prior to Day 1 of "testing cycle #2."

Screening 'failures' will be tracked for date of screening and reason for ineligibility/exclusion; however, if not enrolled, all screening information and data will be de-identified and destroyed.

Ambulatory Assessment of Daily Stress Processes and Cognitive Function (Days 1-14)
Note: Participants will complete the ambulatory assessments described below using either Qualtrics (accessed via the The Pennsylvania State University institutional contract but completed using any of their personal devices with internet connectivity; e.g., smartphone, tablet, etc.) or the Metricwire mobile app on a laboratory-provided smartphone (see attached document "Metricwire Instructions").

1. Participants are familiarized with the organization, flow, and types of questions in the ambulatory assessments prior to participation. They will practice using the Gorilla experiment builder on their personal mobile device at the screening/familiarization laboratory visit.
2. Participants will complete a morning assessment and an evening assessment each day for 14 consecutive days (Days 1-14). Beginning on the first day of the ambulatory assessments, participants will receive pre-scheduled alert notifications via text/email twice per day (~9a and ~7p), prompting them to complete a short morning survey (2-3 min) and a slightly longer evening survey (5-7 min). These notifications are programmed and generated by Qualtrics or Metricwire and include direct access to the associated survey.
3. The morning survey asks questions about sleep, morning outlook, and anticipatory stress and positive experiences (see attached document "Daily Stress Script"). The investigators do not monitor answers to these surveys on a daily basis; instead, these data are compiled at study completion.
4. The evening survey asks questions about the participant's daily experiences (stressors, mood, physical symptoms), and memory lapses (see attached document "Daily Stress Script"). The investigators do not monitor answers to these surveys on a daily basis; instead, these data are compiled at study completion.
5. Within both the morning and evening surveys, some questions will ask participants to select a single option, some questions will ask participants to check the boxes of as many options as are relevant to their answer, some questions involve drop down menus, and some questions involve a slider scale.
6. After each survey, participants will complete three brief objective cognitive tasks or "brain games" (see attached document "Daily Cognition Tasks"). The cognitive tasks will be administered using either Gorilla experiment builder (accessed via a link at the end of each Qualtrics survey) or directly within the Metricwire mobile app. If using Qualtrics, participants will click on the link to directly access the tasks in Gorilla experiment builder. This link will include a unique identifier connecting the participant's survey results to their Gorilla task results. Direction screens are provided before each game.
    - In the *paired associates* task, participants see words paired with numbers and have to recall the number paired with each word.

- In the *forward number span* task, participants see a series of numbers (from 3-7 digits long) and have to enter the numbers in the order they appeared. In the *backward number span* task, participants again see a series of numbers (from 2-6 digits long) and have to enter the numbers in reverse order.
- In the *spatial n-back* task, participants see 4 squares that change color. When the same square changes color twice in a row, they have to click a button on the screen.

7. During the same 14 days, participants will also wear an accelerometer watch (MotionLogger Actigraph) on their non-dominant wrist during all waking and sleeping hours to objectively assess sleep and activity/sedentary behavior. They will be instructed in its use, including removal of the wristwatch for any water-based activities (e.g., showering, bathing, swimming, etc.).
8. On Day 15 (i.e., the day immediately after completion of the 14-day ambulatory assessments), participants will return to the laboratory for the experimental visit. They will return the accelerometer at this visit.

Daily Saliva Collection (Days 3-6)

1. Participants will be asked to provide saliva samples for 4 consecutive days, beginning on Day 3 of the ambulatory assessments (described above) and continuing until Day 6. These samples will be tested for stress hormones (e.g., cortisol) and indices of mitochondrial function (e.g., cell-free mitochondrial DNA). The home saliva collection kit contains collection instructions (see attached document "Saliva Collection Instructions"), all collection materials, and a saliva collection worksheet (see attached document "Saliva Collection Worksheet") and is provided in a small portable cooler for ease of transportation and storage. All samples are coded and do not contain personal identifiable information.
2. Participants will collect their saliva 4 times per day (once upon awakening and before getting out of bed, once 30 minutes after getting out of bed, once at lunchtime, and once in the evening). Participants will record the date and time of each collected saliva sample. They will collect a total of 16 saliva samples over the 4-day sampling timeframe.
3. To collect saliva, participants will use a salivette, which includes a cotton swab that they will put in their mouth for 2 minutes. After 2 minutes, they will place the cotton swab back in the salivette tube.
4. Participants will store these samples in their refrigerator and will return them to the researchers at the experimental visit. The samples will then be frozen and stored until analysis.

Experimental Laboratory Visit (Day 15)

1. Vital signs (oral temperature, heart rate, and blood pressure) are measured upon arrival to the lab. Women who are premenopausal submit urine for a pregnancy test if they have not been tested within the 2 weeks prior to the experimental visit.
2. Participants complete the Patient Health Questionnaire-9 (PHQ-9) to assess depressive symptom severity. The PHQ-9 has 9 questions (see attached document "PHQ-9"). Each question is scored on a Likert scale. The total score can range from 0 to 27.
3. Participants complete the Generalized Anxiety Disorder-7 (GAD-7) item scale to assess anxiety symptom severity. The GAD-7 has 7 questions (see attached document "GAD-7"). Each question is scored on a Likert scale. The total score can range from 0 to 21.
4. Participants complete the Big Five Inventory to assess neuroticism, openness to experience, conscientiousness, extraversion, and agreeableness (see attached document "Big Five Inventory"). Participants are provided with 44 personality characteristics that may or may not apply to them. On a Likert scale (strongly disagree to strongly agree), participants rate the extent to which they agree or disagree with each statement.
5. Participants complete the Life Events Checklist to assess exposure to any major stressful life events (episodic in nature, with identifiable onset) and other sources of chronic and persistent psychosocial stress (stressors that are demanding, distressing, and ongoing) over the past 12

months (see attached document “Life Events Checklist”).

6. Participants complete the Pittsburgh Sleep Quality Index (see attached document “PSQI”), a 10-item measure of sleep quality and sleep habits.
7. Participants complete the International Physical Activity Questionnaire to assess habitual physical activity (IPAQ; see attached document “IPAQ”).
8. A research nurse will insert an intravenous (IV) catheter into an antecubital vein of the dominant arm to obtain a blood sample (100 ml/~6.7 tbsp). The researchers do not perform genetic analyses on the blood, nor do they look for the presence of disease (e.g., HIV). Blood samples will be stored in a -80°C freezer (in the laboratory space designated for freezers and refrigerators on the 2nd floor of the Tower at STAR) to allow for batched analyses of substances of interest [inflammatory cytokines, indices of redox balance, peripheral blood mononuclear cells, stress hormones, and sex hormones (estradiol, progesterone, follicle stimulating hormone, luteinizing hormone, and testosterone)] to avoid variability. All samples will be coded with a unique code for each subject. The code-key will be kept in the PI’s lab in a locked cabinet. Only PI and authorized personnel will have access to the file. No other students or faculty will have access. After successful analysis, blood will be discarded into a container labelled with a biohazard sign and stored in secondary waste containers.
9. A research nurse will also perform an endothelial cell biopsy, during which they will collect some cells from the walls of the vein. Two sterile J-wires will be inserted through the IV catheter and gently moved back-and-forth inside the blood vessel. One wire is used at a time. After removal, the wires are transferred to a conical tube containing endothelial dissociation buffer. The cells are rinsed, recovered by centrifugation, and then fixed to coverslips and stored in a -80°C freezer (in the laboratory space designated for freezers and refrigerators on the 2nd floor of the Tower at STAR) to allow for batched analyses of proteins that regulate vascular function and redox balance. All samples will be coded with a unique code for each subject. The code-key will be kept in the PI’s lab in a locked cabinet. Only PI and authorized personnel will have access to the file. No other students or faculty will have access.
10. Flow-mediated dilation (FMD) of the brachial artery to assess peripheral vascular function. To provide the most flexibility to participants in terms of time constraints regarding scheduling, this procedure may occur at either the screening visit or the experimental visit. During this procedure:
    - We tape 3-5 ECG leads to the chest to measure heart rate throughout the experiment (BioAmp, AD Instruments).
    - A blood pressure cuff will be wrapped around the upper arm to obtain blood pressure via a standard automated oscillometric device (BP Monitor, Welch-Allyn). In addition, beat-by-beat blood pressure will be obtained via finger photoplethysmography (NOVA, Finapres). Blood pressure is measured continuously throughout the experiment.
    - We place a small cuff around the forearm, just below the elbow.
    - Above the elbow, we use a Doppler ultrasound probe covered in ultrasound gel to image the brachial artery. The ultrasound measures blood vessel size and blood velocity.
    - After a 3-minute resting measurement, we tightly inflate the cuff for 5 minutes to occlude blood flow to the forearm.
    - After the cuff deflates, we continue to image the artery for ~5 minutes. We may repeat this measure several times.
11. Pulse wave analysis (PWA) and pulse wave velocity (PWV) to assess arterial stiffness. To measure PWA, a standard blood pressure cuff is placed on the subject’s upper arm. The cuff will inflate/deflate to determine brachial artery blood pressure; after 5 seconds, the cuff will inflate again and automatically capture the PWA waveform. To measure PWV, a blood pressure cuff is placed on the subject’s upper leg. A pen-like probe (applanation tonometer) will be placed against the skin over the carotid artery to continuously measure the pulse wave while the blood pressure cuff on the upper leg is inflated and then deflated. To provide the most flexibility to participants in terms of time constraints regarding scheduling, this procedure

may occur at either the screening visit or the experimental visit.

12. Intradermal Microdialysis to assess the mechanistic regulation of microvascular function. During this procedure:
    - We tape 3-5 ECG leads to the chest and abdomen to measure heart rate throughout the experiment.
    - A blood pressure cuff will be wrapped around the upper arm to obtain blood pressure via a standard automated oscillometric device. Blood pressure is measured repeatedly throughout the experiment.
    - We insert microdialysis probes:
        - We place a tight band around the non-dominant forearm so we can visualize veins.
        - For each microdialysis site, we make pairs of pen-marks on the arm ~2.5 cm (1 inch) apart and away from veins. We remove the tight band. The microdialysis tubing enters and exits the skin at the marks.
        - We clean the arm with povidone iodine and alcohol and place an ice bag on the site for 5 minutes to numb the skin.
        - Then we insert a thin needle into the skin at each entry mark. The needle's tip travels between the layers of skin for ~2.5 cm (1 inch) and exits the skin at the matching exit-mark.
        - We thread the microdialysis tubing through the needle and then withdraw the needle leaving the tubing in the skin.
        - We prepare up to 3 microdialysis sites.
        - Any hyperemia related to the insertion subsides in about 60 minutes. During this time lactated Ringers or a site-specific pharmacological agent listed below perfuses the microdialysis tubing:
            - lactated Ringers
            - lactated Ringers + mitoTempol
            - lactated Ringers + Tempol
    - We use laser Doppler flowmetry to measure cutaneous microvascular blood flow:
        - We tape thin fiber optic laser Doppler flowmeter probes and their holders on the forearm over each microdialysis site.
        - The thin probe measures skin blood flow with a weak laser light. We measure skin blood flow continuously throughout the experiment.
        - We can control the temperature of the holders.
    - At the start of the experiment, we obtain ~20 minutes of baseline measurements. During this time, the temperature holders are set to thermoneutrality (33°C/91.4°F).
    - Then, we increase the temperature of the holders to 42°C/107°F (0.1°C/sec).
    - After skin blood flow stabilizes (~45 min), we switch the fluid in all probes to L-NAME.
    - After skin blood flow stabilizes a second time (~45 min), we switch the fluid in all probes to sodium nitroprusside. At the same time, we warm the probe's holder to 43°C (108°F) for ~30 minutes to cause maximal dilation.
        - If a participant is asked to repeat this procedure, it will occur during a separate laboratory visit.
13. At the conclusion of the experiment, we remove the microdialysis tubing from the skin and place sterile bandages over the site. If the subject desires, we place a bag of ice on the site for 10 min to reduce bruising that may occur. The investigators complete an assessment of each microdialysis site and complete the Microdialysis Discharge Form.
14. We measure blood pressure and heart rate before the subject leaves the laboratory.

Subject Participation: A subject may decide not to participate in a particular experimental measurement or procedure or may decide not to participate in the second testing cycle; therefore, in these situations, this portion of the protocol will not be completed. However, all other measurements and procedures will be performed. This will not affect the scientific value of the

subject's participation, as each experimental measurement and procedure provides important and, in most cases, independent information.

Pharmacological Agents: *The chemicals used for intradermal microdialysis in this study are scientific tools, some of which are not FDA-approved dispensable drugs, and they do not have expiration dates. We purchase most of these chemicals in solid form. The chemicals are stable for years when stored according to manufacturer's instructions. Nonetheless, we purchase most of the chemicals in small vials that we typically exhaust within one to several weeks. Once mixed, we use stock solutions of the drugs within a week if stored in the refrigerator. We use solutions within 6 months if they are frozen. Final dilutions of solutions prepared for an experiment are used within a couple of minutes or hours of preparation. All pharmacological agents are prepared immediately prior to use and wrapped in foil to prevent photodegradation.*

All proposed investigational agents have been previously used in human subjects research via intradermal microdialysis.

- Lactated Ringers
  - Role: vehicle control for other investigational substances
  - Form: liquid
  - Source: VWR, Moore Medical, McKesson

- N^G-nitro-L-arginine methyl ester (L-NAME)
  - Role: analog to the amino acid L-argining; non-specific inhibitor of nitric oxide synthase; prevents blood vessels from dilating
  - Form: powder
  - Source: Calbiochem, EMD Millipore, Tocris
  - Concentration: 15 mM

- mitoTempol
  - Role: scavenger of mitochondrial-derived superoxide
  - Form: powder
  - Source: Sigma-Aldrich
  - Concentration: 1 mM

- Tempol
  - Role: non-specific scavenger of superoxide
  - Form: powder
  - Source: Sigma-Aldrich
  - Concentration: 10 μM

- Sodium Nitroprusside
  - Role: nitric oxide donor; causes maximal endothelium-independent dilation of blood vessels
  - Form: powder
  - Source: USP
  - Concentration: ≤ 100 mM

7. **STUDY POPULATION AND RECRUITMENT**

Describe who and how many subjects will be invited to participate. Include age, gender and other pertinent information.

- 18 – 55 yrs

- Males and females
  - 60 non-depressed healthy adults will have no evidence of current or lifetime history of major psychiatric illness, assessed by the MINI and self-report
  - 60 unmedicated adults with MDD will meet the DSM-5 criteria for MDD, assessed by the MINI and self-report; participants with co-morbid anxiety, stress, and trauma-related disorders will be included if MDD is the primary diagnosis
  - 30 selective serotonin reuptake inhibitor (SSRI)-treated (current monotherapy) adults with MDD will meet the DSM-5 criteria for MDD, assessed by the MINI and self-report; participants with co-morbid anxiety, stress, and trauma-related disorders will be included if MDD is the primary diagnosis

Subjects will be recruited from the University of Delaware and surrounding local communities. We use recruiting methods that will reach a large percent of the general population. We advertise for subjects, and interested persons contact us. We post recruitment flyers in various campus (e.g., student union, classroom building bulletin boards, dormitory bulletin boards, campus eateries, etc.) and community (e.g., coffee shops, bookstores, local businesses, etc) locations. We will obtain permission to provide recruitment flyers to local medical and wellness practices (family physicians, mental healthcare providers/specialists, etc.) and other locations specifically targeted to recruit patients with depression (e.g., Center for Counseling & Student Development). Additionally, the text from the fliers may be utilized in "word of mouth" recruiting if the opportunity presents itself. Recruitment may also occur via online social media resources (e.g., Instagram, Twitter, Google, Craig's List, NextDoor, Facebook, ResearchMatch.com, etc.), as well as in campus newsletters and local newspapers/mailings.

Attach all recruitment fliers, letters, or other recruitment materials to be used. If verbal recruitment will be used, please attach a script.

Advertisements

- Study Information.svcMDD.20240118
- CHS ad
- gen.recruit.ad.bookmark
- gen.recruit.ad.buisinesscard
- recruit general.unMDD_1
- recruit general.unMDD_2
- CPP Recruitment EDDM Postcard
- svcMDD.research match.20240701

Describe what exclusionary criteria, if any will be applied.

The exclusion criteria are applied to all groups. We will not enroll non-English-speaking individuals. This protocol involves the use of numerous questionnaires, including a detailed neuropsychiatric interview with the investigators. In addition, participants need to understand English to follow instructions and comply with procedures conducted during the screening and experimental visits.

Subjects will be excluded at the discretion of PI/collaborating psychiatrist/examining clinician or for any of the following reasons:

- Psychiatric illness aside from MDD (e.g., bipolar disorder, schizophrenia, eating disorders), assessed by the MINI and self-report
- Active current suicidal ideation with intent or a specific plan, assessed by the Columbia-Suicide Severity Rating Scale
- Active current suicidal behavior with a preparatory attempt, assessed by the Columbia-Suicide

Severity Rating Scale
- Active substance dependence, assessed by the MINI
- Current or recent (within 8 wks) use of medications that could conceivably alter neural-cardiovascular-immunological function [including (but not limited to) antihypertensives and HMG-CoA reductase inhibitors] or psychoactive or psychopharmacological drugs aside from SSRIs [including (but not limited to) antidepressants, antipsychotics, benzodiazepines, mood stabilizers, sedatives/hyponotics, dopaminergic agents, stimulants, buspirone, and triptans]
- Changes or alterations in medication status (starting a new, additional, or different medication or changing the dose of a current medication)
- Unstable or diagnosed chronic clinical disease, including cardiovascular, metabolic, renal, hepatic, autonomic, autoimmune, or dermatological disease (e.g., hypertension, heart disease, diabetes, hyperlipidemia, psoriasis)
- Body mass index <18.5 or >35 kg/m^2
- Tobacco or nicotine use, including vaping and electronic cigarettes
- Pregnancy (including a positive urine pregnancy test) or breast-feeding
- Lack of access to a device with internet connectivity
- Known allergies to pharmacological agents/drugs

Describe what (if any) conditions will result in PI termination of subject participation.

Subjects will be terminated from participation if it is believed that such participation endangers their welfare or for an inability to comply with study procedures.

8. **RISKS AND BENEFITS**

List all potential physical, psychological, social, financial or legal risks to subjects (risks listed here should be included on the consent form).

Neuropsychiatric Interview and Depressive Symptom Severity/Suicidality Assessments

- General: There is risk that adults with MDD will experience clinical worsening.

  Mitigation: We have robust standard operating procedures in place to ensure the safety of participants. In no case will a participant be denied necessary medical or psychiatric treatment for reasons related to study participation. No patients with MDD who are currently treated with antidepressant pharmacotherapy will be asked or allowed to discontinue to participate. We have multiple screening and crisis protocols in place to ensure patient safety and compliance with study procedures. Psychiatric mental health advanced practice registered nurses and a licensed clinical psychologist will provide psychiatric clinical oversight for the management of adults with MDD. Our crisis intervention plan allows for rapid referral, including immediate referral for acute psychiatric issues (described below and in accompanying documents). This crisis protocol can be triggered at any time and at the discretion of the research team. If a participant has worsening of MDD but no immediate safety concern, we will recommend that the patient seek psychiatric consultation. If participants have questions regarding clinical care, they will be referred to a mental healthcare provider. If a participant does not have a mental healthcare provider, the investigators will help them to identify local resources. All participants will be given materials on local resources for psychiatric care and suicide hotline information. To ensure safe participation, subjects will be asked to identify an emergency contact and provide contact information so that the study team may contact these individuals in case of an emergency.

- MINI/PHQ-9/PROMIS: For the purposes of this study, we use these tests to identify potential subjects who have MDD (or not) and to identify for exclusion those who have psychiatric illness

other than depression. These interviews have been extensively validated in multiple clinical populations and are based on standard diagnostic criteria. Answering questions related to mental health and past experiences may be stressful and uncomfortable for participants. Some subjects may be disturbed if the test recommends their inclusion as an adult with depression.

Mitigation: Subjects are reminded that they may decline to answer the questions and leave the study at any time. We remind them that the test is not intended to be a diagnosis or healthcare recommendation but is used as a tool to identify people who may have particular experiences or forms of psychological distress useful for the purposes of this study. Research interviews will be interrupted if subjects become too distressed or object to answering questions. If, in the judgment of the study team or the PI, the patient has worsened to a degree that further participation would put the patient at risk, the subject will be discontinued from the study and encouraged to seek appropriate clinical care. Also, at any point during the study, any subject meeting DSM-5 criteria for any exclusionary psychiatric diagnoses (detailed above) will be discontinued from the study and encouraged to seek appropriate medical care. All participants are highly encouraged to seek follow-up with a healthcare provider and the study team will assist with identification of and communication with treating providers as needed. The investigators also will provide the subjects with a detailed explanation of the neural-cardiovascular physiology of depression-related cardiovascular disease. Furthermore, the investigators will provide subjects with their screening data so that they may forward this information to a health care provider.

- C-SSRS: Answering questions related to mental health and past experiences may be stressful and uncomfortable for participants.

  Mitigation: All suicidal behaviors are taken seriously, and it is important to assess the risk of suicide carefully when working with individuals with MDD. Many people may admit to fleeting thoughts of death or briefly wishing for death; these thoughts need to be considered in context of the subject's overall history, along with a consideration of other risk factors for suicide. Due to these reasons, as part of informed consent process, subjects will be asked to identify an emergency contact person and provide their contact information so that the study team may contact these individuals in case of an emergency. We extensive experience in working with individuals with elevated risk of suicide and maintaining the safety of individual subjects. We have developed strict safety guidelines for this study, including detailed crisis protocols that can be implemented at any time for acute psychiatric issues. This safety plan was developed in consultation with our collaborating psychiatric mental health advanced practice registered nurses, who will provide general psychiatric clinical support for this project. This crisis protocol will be triggered if participants express *1)* active current suicidal ideation with intent or a specific plan, *2)* active current suicidal behavior with a preparatory attempt, or *3)* at the discretion of the research team. The presence of suicidal ideation without intent will not trigger any additional assessments, as this is considered low acute risk. *For immediate assistance during an acute crisis in the laboratory, 911 will be called.*

Interviews/Surveys and Clinical Evaluations

- Initial Determination of Eligibility: We use this pre-screening interview to grossly determine whether a potential subject is a possible candidate for the study. The initial interview gathers minimally invasive personal healthcare information. We have used similar interviews in the past with potential participants.

  Mitigation: Only trained study personnel administer this interview. The participants may decline to answer questions or participate. All data are collected using secure online platforms approved by the University of Delaware. We keep the completed forms confidential and secure.

Only approved staff may access the results.

- Medical Screening and Health History: There are no known risks with obtaining height, weight, waist circumference, heart rate, or blood pressure or collecting medical health history information. Subjects may be uncomfortable giving medical information or being measured.

  Mitigation: The clinical evaluation and medical health history are performed by trained staff. The participants may decline to answer questions or participate in measurements. The researchers conduct screenings professionally and privately.

- Ambulatory Assessments of Daily Stress Processes and Cognitive Function: These surveys help us to interpret the physiological data we record during the experiment. Subjects may feel shy or disturbed by the questions. Reporting on stressful events may result in some sadness or anger or remind the participant of previous stressful experiences. Completing the "brain games" may cause some frustration.

  Mitigation: Participants may choose not to answer the questions and decline to be in the study. We do not use the tools to decide a recommendation for healthcare. We keep the data resulting from the completed assessments confidential and secure. Only approved staff may access the results.

- Cognitive Function Assessments: These tests help us to interpret the physiological data we record during the experiment. Completing the "brain games" may cause some frustration. Subjects may feel shy or uncomfortable by the tasks.

  Mitigation: They may choose not to answer the questions and decline to be in the study. We do not use the tools to decide a recommendation for healthcare. We keep the completed forms confidential and secure. Only approved staff may access the results.

- Surveys (Physical Activity Questionnaire, Life Events Checklist, etc.): These surveys help us learn of a subject's experiences that are useful for the purposes of this study. The surveys help us to interpret the physiological data we record during the experiments. Subjects may feel shy or disturbed by the questions. Subjects may be uncomfortable giving information about their lives. Reporting on stressful events may result in some sadness or anger or remind the participant of previous stressful experiences.

  Mitigation: They may choose not to answer the questions and decline to be in the study. We do not use the tools to decide a recommendation for healthcare. We keep the completed forms confidential and secure. Only approved staff may access the results.

Screening/Experimental Procedures

- Intradermal Microdialysis: Intradermal microdialysis commonly causes some pain and bruising similar to that experienced during venipuncture. There is usually no pain after the probe is in place. The participant may experience mild pain while the researchers remove probe. Minor bleeding may occur. As with routine venipuncture, a participant who is nervous about needles could have increased heart rate and blood pressure, become lightheaded or nauseated, or could faint.

  Mitigation: Only trained personnel perform this technique. Ice numbs the skin, and the small needle reduces pain during insertion. Subjects are asked about allergy to iodine and, for those who are, alcohol is used instead. The researchers stop any bleeding following probe insertion by applying mild pressure to the site with sterile gauze. In the unlikely event that the individual

has an allergic reaction, the researchers stop microdialysis immediately. If the reaction becomes severe, the researchers seek emergency medical assistance. As with venipuncture or any event that breaks the skin, infection is possible, but proper aseptic technique and sterile solutions/supplies keep this risk minimal. Additionally, no participants in any of the PI's experiments have reported infection (over 1,500 probes have been placed by Dr. Greaney). The researchers place a sterile bandage on the site after the experiment. Although rare, if the membrane should break in half during removal, they remove the remaining half by gently pulling the attached tubing. This presents no additional risk to the participant.

- Microdialysis Perfusates/Pharmacological Agents: Lactated Ringer's solution flows through the microdialysis probes. An allergic reaction to this physiological saline solution is highly unlikely. All substances (LNAME, Tempol, mitoTempol, sodium nitroprusside) added to the lactated Ringer's perfusing the microdialysis probes have been used previously clinically and/or in research in humans. Microdialysis delivers small amounts of the substances to a nickel-sized area of the skin. The small quantities used and the extremely localized administration during microdialysis does not produce systemic effects. To the researchers' knowledge, there are no reports of long or short-term side effects of these substances administered through microdialysis. The chance of adverse reactions to these substances is extremely small given the minute amount delivered to a very small area of skin, the lack of adverse reactions to similar amounts delivered via microdialysis in many other studies, and lack of adverse effects in human cell cultures. There is a slight chance of allergic reaction to these substances that could produce redness, itching, rash, and/or swelling. A severe reaction (anaphylactic shock) could also cause fever, difficulty in breathing, changes in pulse, convulsions, and/or loss of consciousness/lightheadedness. Although unlikely, in the case of a severe reaction to the perfusate, the researchers call 911.

  General Note: Intradermal Microdialysis is a safe and effective method to locally deliver pharmacological agents directly to the cutaneous vasculature. This technique is widely used to administer small quantities of investigational agents to the cutaneous vasculature, and the PI has extensive experience with this technique. Pharmacological agents delivered using microdialysis do not affect the systemic circulation. All investigational agents have been previously used in humans. It is common in microdialysis studies to obtain research grade formulations of these agents because of their purity.

  Mitigation: The perfusate is sterile. The researchers prepare the perfusate in their laboratory using sterile techniques and supplies commonly used for this purpose in research laboratories. The Ringer's solution is sterile as purchased. They add other solutions aseptically through 0.2 micron Gelman Sterile Acrodisc syringe filters. They mix the perfusate for same-day use and discard excess perfusate after the experiment. We use only USP grade when available or the purest form of the pharmacological perfusates possible (>98% purity). Although unlikely, in the case of a severe reaction to the perfusate, the researchers call 911.

- Flow-Mediated Dilation of the Brachial Artery: Placing the probe on the arm's skin may cause temporary minor redness. The temporary redness from the probe is unlikely to have lasting ill effects. The inflated cuffs may cause the participant's arms and feet to feel numb or tingly, and the skin's color to change slightly. The cuffs could cause mild bruising. The gel may feel cool or cold on the skin.

  Mitigation: Only trained personnel perform this technique. Measures will be taken efficiently. The cuffs inflate for a minimal amount of time. The investigator can manually deflate the cuff at any time during the procedure, if necessary. The gel is the same as that used with medical ultrasound tests. A bad reaction to the gel is highly unlikely.

- Pulse Wave Analysis and Pulse Wave Velocity: The proposed assessments of arterial stiffness are non-invasive. The inflated cuffs may cause the participant's arm and/or feet to feel numb or tingly.

  Mitigation: Only trained personnel perform this technique. Measures will be taken efficiently. The cuffs inflate for a minimal amount of time. The investigator can manually deflate the cuff at any time during the procedure, if necessary.

- Intravenous Catheterization: Insertion of an intravenous catheter can cause anxiety (with increased heart rate and blood pressure), mild pain, swelling, nausea, lightheadedness, fainting, or bleeding. There is a slight chance of infection or small blood clot.

  Mitigation: Subjects may decline the procedure. A competent and trained research nurse performs the technique using standard procedures and techniques that minimize the chance of infection. Sterile supplies are used. Participants will be supine for the procedure. Subjects will only be subjected to a maximum of two attempts.

- Venous Endothelial Cell Biopsy: The risks of venous endothelial cell biopsy are similar to those of intravenous catheterization, including some pain, redness, swelling, or bruising. Rare side effects include a slightly greater risk of blood clots and infection than that of an intravenous catheter due to increased manipulation of the blood vessel. There is a low likelihood that the vein is damaged.

  Mitigation: Subjects may decline the procedure. A competent and trained research nurse performs the technique using standard procedures and techniques that minimize the chance of infection. Sterile supplies are used. Participants will be supine for the procedure.

- Venipuncture: Blood draws can cause anxiety (with increased heart rate and blood pressure), mild pain, swelling, nausea, lightheadedness, fainting, or bleeding. There is a slight chance of infection or small blood clot.

  Mitigation: Subjects may decline the procedure. A competent and trained investigator or research nurse performs blood draws using standard venipuncture-procedure and techniques that minimize the chance of infection. Sterile supplies are used. Participants may recline for the procedure. Subjects will only be subjected to a maximum of two attempts per arm by one person.

- Blood Pressure (automated): The automated methods use a cuff that inflates on the upper arm. The cuff slowly deflates. The inflated cuff may make the arm feel tingly and numb, and the cuff may temporarily bruise the arm. Efficient and competent measurement technique minimizes the duration of cuff inflation.

  Mitigation: Efficient and competent measurement technique minimizes the duration of automated cuff inflation. This includes, but is not limited to, using the appropriate cuff size, correctly positioning the cuff, reminding the participant to minimize movement during measurements, etc. The cuffs inflate for a minimal amount of time. The investigator can manually deflate the cuff at any time during the procedure, if necessary.

- Accelerometry: There are no known risks with wearing an accelerometer. Minor skin irritation may occur.

Mitigation: Subjects may decline the procedure.

- Confidentiality: There is a risk of loss of confidentiality when completing study assessments and procedures.

  Mitigation: All data are stored on password-protected computers in a locked room. Only authorized members of the lab have access to these files. REDCap (Research Electronic Data Capture) is a secure online platform for building and managing basic and clinical research protocols and features built-in data monitoring, back-up, and security (including electronic signatures and form/record locking). Qualtrics and Metricwire are secure survey administration applications designed to support data capture for research studies. Data are deleted from cloud servers within 24 hours. The data files are downloaded and stored on an external password-protected hard-drive.

In your opinion, are risks listed above minimal* or more than minimal? If more than minimal, please justify why risks are reasonable in relation to anticipated direct or future benefits.

The risks listed above present more than minimal risk of harm to subjects. Although the risks are more than minimal, they are low. Data from this study will help to understand the mechanisms of vascular dysfunction in young-to-middle aged adults with MDD.

*(*Minimal risk means the probability and magnitude of harm or discomfort anticipated in the research are not greater than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests)*

What steps will be taken to minimize risks?

Strategies to minimize risks associated with specific techniques and procedures are described in detail in the preceding section.

- General Procedures: The research group's members are trained and competent in their duties. The group, led by Dr. Greaney, evaluates the effectiveness and safety of protocols and procedures in an ongoing fashion. They discuss the protocol with candidates, invite questions, and offer tours of the laboratory. Prior to medical screening, candidates read and sign informed consent forms detailing protocols, procedures, risks, sensations, compensation, etc. The researchers give candidates witnessed copies of the signed consent forms. Guided by the inclusion and exclusion criteria, the investigators and clinical staff screen subjects before participation to ensure that the subjects meet the study's requirements. After enrolling participants in the study, the researchers discuss and review the procedures and protocols with them generally and at each step throughout the project. They frequently remind participants of the option to withdraw from the study at any time. Restricting access to experiments, data, and coding to authorized personnel maintains confidentiality. Lists of emergency numbers remain by lab telephones. At least one cell phone is present at each experiment. A hospital and emergency medical services are within 6 miles of the lab. An AED hangs nearby in the hallway.

- Availability of Medical/Psychological Resources: We have developed strict safety guidelines for this study, including detailed crisis protocols that can be implemented at any time for acute psychiatric issues. Subjects will be free to contact the PI or study team regarding any concerns or emergency during the study. All participants will be given materials on local resources for psychiatric care and suicide hotline information. To ensure safe participation, subjects will be asked to identify an emergency contact person and provide their contact information, so that the study team may contact these individuals in case of an emergency.

- Stoppage Criteria: Although such events are extremely unlikely to occur, the investigators are prepared to immediately stop experiments and seek medical assistance if the subjects should experience the more serious reactions, such as signs and symptoms of an allergic reaction, anaphylactic shock, and fainting. In the case of a medical emergency, emergency services will be contacted. The investigators end the experiments if the subject's blood pressure is greater than 220/110 mmHg or less than 80/50 mmHg, if heart rate is >150 bpm or <40 bpm, or the presence of symptoms. The investigators will stop the experiments at any time should a subject wish it. Also, the investigators will exercise the discretion to end a subject's participation if the subject should engage in behavior that could jeopardize his/her own health and well-being or that of others.

- Previously Unknown Medical Conditions: It is possible that the investigators will discover a participant's previously unknown medical condition because of the screening. At the time of screening, the investigators inform subjects of any condition identified that might require further treatment and will suggest follow-up with a healthcare provider. The investigators make the results from laboratory studies available to the subject as soon as possible.

Describe any potential direct benefits to participants.

All study subjects will be informed that there may not be any potential benefit to them individually because of taking part in the study. All study subjects will receive (without cost) an extensive psychiatric and medical evaluation, which may be of potential benefit for the subjects. Participants may gain knowledge about the biological and psychological processes of depression, as well as the implications for cardiovascular disease risk, which they may find interesting and helpful in dealing with the illness. The subjects receive financial compensation. No other direct benefits result from study participation.

Describe any potential future benefits to this class of participants, others, or society.

Approximately 50% of adults in the US will manifest cardiovascular disease (CVD) during their lifetime, contributing substantially to the global burden of disease and disability. This health issue is pervasive and exacts emotional, physical, and financial costs. Cardiovascular disease is the leading cause of morbidity and mortality in modern societies. This risk appears to be further heightened in adults with MDD. The information to be gained is essential to the understanding increased CVD risk in adults with MDD. The results from this study will determine if the links between emotional responsivity to daily stress and *1)* proinflammatory cytokine production, *2)* mitochondrial redox balance, and 3) vascular dysfunction are sensitized in adults with MDD. These data will provide information that could be valuable in designing interventions to promote mental and physical health in adults with and without MDD.

If there is a Data Monitoring Committee (DMC) in place for this project, please describe when and how often it meets.

No

9. **COMPENSATION**

Will participants be compensated for participation?

Participants will be compensated at the completion of each testing cycle or after the final visit to the lab.

If so, please include details.

Participants will receive this payment in the form of a check.

For each 14-day ambulatory assessment portion of the study (including daily saliva collection), there is a base compensation of $200, with a $50 bonus for the completion of both morning/evening surveys on ≥12 days. For example, if a participant finishes the 14-day protocol and completes both morning/evening surveys on 12 days total morning/evening surveys (out of 14 total), he/she will be compensated $250. If a participant finishes the 14-day protocol and completes both morning/evening surveys on 10 days (out of 14 total), he/she will be compensated $200. For each experimental laboratory visit, participants will be compensated $100. If a participant completes all parts of a testing cycle, the maximum compensation is $350.

If a participant completes all parts of both testing cycles, he/she will be compensated $700 (as described above), plus an additional $150 bonus. The maximum compensation for completion of the entire study is $850. If, for example, a participant receives the maximum compensation, he/she will receive one check for $350 after the first testing cycle and a second, separate, check for $500 after the second testing cycle.

The Internal Revenue Service (IRS) considers all payments made to research subjects to be taxable income. Participant's personal information, including their name, address, and social security number, may be acquired provided to the University of Delaware's accounting office for the purpose of payment. If total payments for the year exceed $600.00, the University of Delaware will report this information to the IRS as income and participants will receive a Form 1099 at the end of the year. If participants receive less than $600.00 total for payments in a year, they are personally responsible for reporting the payments to the IRS.

10. **DATA**

Will subjects be anonymous to the researcher?

No

If subjects are identifiable, will their identities be kept confidential? (If yes, please specify how)

Yes

All files will be coded with a unique code for each subject. Documents allowing identification of participants do not leave the investigator's labs and are only available to authorized persons. The list linking participant names to their participant numbers (codebook) will be digitized and stored in REDCap, in a project separate from all de-identified data. Records across the REDCap projects are not linked, thus personal identifiable information is not associated with any data collected except for the linking codebook document. The data captured in Project 1 will contain personally identifiable information and include the IRB-approved consent, the pre-screening survey, subject contact information, and the compensation report form. Project 1 will also contain the list linking participant names to their participant numbers, which will be the only way to link the participant ID with their personal identifiable information. Project 2 will not contain personal identifiable information and will include all other coded data collected via survey, as well as all other coded data collected during the experimental visit. Any paper forms of data will be digitized and imported into REDCap. REDCap is a HIPAA compliant database. Only approved project staff will have access to the REDCap databases, which will only be accessed via secure connection (UD VPN).

Note: REDCap eConsent will be used to secure electronic signatures for adult participants,

parents, legal guardians, children, witnesses, and study personnel. The REDCap eConsent will contain identical information as a paper consent, and will have automatic archiving of signed consent documents with date and time stamps for audit purposes. PDF copies of the signed consent document can be generated to give to participants. Electronic consents will be stored in a file repository on the REDCap database system in a separate REDCap database not linked to any other REDCap databases or data.

Subjects may give permission to have their contact information retained in the investigator's secured files if they wish to be considered for participation in future studies. After the investigators complete the study, they remove all identifiers from the data and store the data indefinitely.

How will data be stored and kept secure (specify data storage plans for both paper and electronic files. For guidance see http://www.udel.edu/research/preparing/datastorage.html)

Hard (paper) copies of informed consents will be scanned and uploaded to REDCap; hard paper copies will shredded.

Any paper files will be stored in a locked cabinet. These files are coded and do not contain personal identifying information. Only approved project staff will have access to the data.

All electronic data will be encrypted. All electronic data are coded and do not contain personal identifying information. The data will be stored using UD-sanctioned storage tools and archived for potential use in other, future studies. In the event of any publication or presentation resulting from the research, no personally identifiable information will be revealed. Only approved project staff will have access to the data.

Some data for this project will be stored in CHRC's REDCap instance, a highly secure and robust web-based research data collection and management system. REDCap is managed by CHRC's Data Systems Analysts ensuring fidelity of database configuration and back-ups. User activities are logged to enable auditing of all data changes. Each project must have a UD full time faculty member with the User Rights privileges and they are responsible for ensuring appropriate access/role to study data via the User Rights Module. REDCap employs a robust multi-level security system that enables researchers to easily implement "minimum necessary" data access for their research staff, including specification of data fields that are identifiers. This feature includes "single click" ability to provide completely deidentified (removing all identified data fields and shifting dates) for analysis or other purposes. All users are given individual user IDs and passwords and their access is restricted on a role-specific basis. Each user must be sponsored by a UD full time faculty member. User activities are logged to enable auditing of all data access.

Qualtrics will be accessed via The Pennsylvania State University institutional contract; associated data will be reduced and exported by our Co-Investigator David Almeida. Qualtrics is a secure website and survey application designed to support data capture for research studies. All web traffic to and from the Qualtrics application website is done via a Secure Socket Layer (SSL) that encrypts the data in transmission. The surveys contain statements advising of the limitations of the technology and that there is no confidentiality guarantee. Data are deleted from Qualtrics' servers within 24 hours after the user deletes it from the website. The cognitive function assessments administered via Gorilla experiment builder will not contain any identifiers; the link to access the Gorilla experiment builder (in Qualtrics) will include the unique identifier connecting the participant's Qualtrics survey results to their Gorilla task results. Data are removed from Gorilla's backup servers within 14 days of deletion.

Laboratory-provided smartphones are password protected and will only be used to capture de-

identified data streams. There will be no other identifying types of data captured.

Metricwire was built to protect the most sensitive research data and comply with the Health Insurance Portability and Accountability Act (HIPAA) and 21 CFR Part 11 (Electronic Signatures in Clinical Research) in the United States and the General Data Protection Regulation (GDPR) in the EU. The Metricwire mobile app is also FDA compliant. Data streams collected via the Metricwire mobile app are linked to a variable code that contains no identifying information. No identifiable information will be (or can be) entered into the Metricwire smartphone app, nor does the app collect any identifiable information from the smartphone. Data collected via the Metricwire mobile app are immediately synced to Metricwire cloud servers in the US via WiFi connection and/or mobile network and removed from the device. All data are encrypted end-to-end during transmission using TLS 1.3 Protocol, an updated and more secure version of SSL. If there is no network connection, data are temporarily stored on the mobile device until an online connection is re-established using AES-256 encryption. Temporarily stored data cannot be accessed using application interfaces. Metricwire servers use an Encryption Token to verify that the data are coming from the correct source (authenticity) and that data have not been modified in-transit (integrity). Data are stored without identifiers within the Metricwire platform/server until exported directly from the Metricwire server dashboard by the study team. The server is accessed via an encrypted website portal that is password protected and only accessible to study personnel. No data on the server will include participant identifiers.

How long will data be stored?

Upon study closure, all data will be de-identified and stored indefinitely in a locked cabinet or password protected computer. All data will be encrypted.

Will data be destroyed? ☐ YES **X** NO (if yes, please specify how the data will be destroyed)

Screening data from subjects who are not accepted into the study are shredded immediately.

Will the data be shared with anyone outside of the research team? ☐ YES **X** NO (if yes, please list the person(s), organization(s) and/or institution(s) and specify plans for secure data transfer)

How will data be analyzed and reported?

To ensure robust and unbiased results, we will use defined protocols for including/excluding, enrolling, and testing subjects. Multiple experimental controls and orthogonal approaches will be used, and relevant psychobiological variables are factored into the research design and analyses. Data analysis will be blinded, when applicable and to the extent that this is possible, and performed by two independent investigators.

All statistical analyses will be performed with SAS. Descriptive statistics will be calculated for all data. Prior to all analyses, we will examine the distributions of continuous variables for departures from normality, separately and in combination, for possible violations of assumptions (e.g., multicollinearity). For continuous variables, the mean and standard deviation will be calculated. Medians and interquartile ranges will be calculated if a continuous variable is skewed. The 95% confidence interval (CI) will be provided as appropriate. Model fit diagnostics will be used to evaluate the appropriateness of linear modeling for primary hypotheses. If appropriate, log transformation may be performed. Statistical significance will be accepted at $p<0.05$. The analyses will be based on a variety of statistical techniques. Most will focus on the use of multilevel modeling and multilevel structural equation modeling which allows the appropriate analysis of data

that are nested (that is, observations come from the same individual over time).

11. **CONFIDENTIALITY**
Will participants be audiotaped, photographed or videotaped during this study?

No

How will subject identity be protected?

Information obtained from this study will be kept strictly confidential. All files will be coded with a unique code for each subject. Documents allowing identification of participants do not leave the investigator's labs and are only available to authorized persons. The list linking participant names to their participant numbers (codebook) will be digitized and stored in REDCap, in a project separate from all de-identified data. While the results of the research may be published, subjects' names and identities will not be revealed.

Is there a Certificate of Confidentiality in place for this project? (If so, please provide a copy).

Yes (NIH funded study)

12. **CONFLICT OF INTEREST**
(For information on disclosure reporting see: http://www.udel.edu/research/preparing/conflict.html)

Do you have a current conflict of interest disclosure form on file through UD Web forms?

Yes

Does this project involve a potential conflict of interest*?

No

* As defined in the University of Delaware's Policies and Procedures ,a potential conflict of interest (COI) occurs when there is a divergence between an individual's private interests and his or her professional obligations, such that an independent observer might reasonably question whether the individual's professional judgment, commitment, actions, or decisions could be influenced by considerations of personal gain, financial or otherwise.

If yes, please describe the nature of the interest:

13. **CONSENT and ASSENT**

X Consent forms will be used and are attached for review (see Consent Template under Forms and Templates in IRBNet)

____ Additionally, child assent forms will be used and are attached.

____ Waiver of Documentation of Consent (attach a consent script/information sheet with the signature block removed).

____ Waiver of Consent (Justify request for waiver)

14. **Other IRB Approval**
Has this protocol been submitted to any other IRBs?

no

If so, please list along with protocol title, number, and expiration date.

15. **Supporting Documentation**
Please list all additional documents uploaded to IRBNet in support of this application.

2056784-12.protocol.svcMDD.20240701
2056784-11.IC.svcMDD.20240503

Advertisements

- Study Information.svcMDD.20240118
- CHS ad
- gen.recruit.ad.bookmark
- gen.recruit.ad.buisinesscard
- recruit general.unMDD_1
- recruit general.unMDD_2
- CPP Recruitment EDDM Postcard
- svcMDD.research match.20240701

Surveys

- Pre-Screening Survey.svcMDD.20240105
- CPP.Contact Information
- CPP.Medical History Form.svcMDD
- CPP.Females.Gyno+Meno.20240118
- CPP.Males.AMS.20231020
- MINI
- C-SSRS.triage
- Daily Stress Script.20240118
- Daily Cognitive Tasks
- PHQ-9
- GAD-7
- Big Five Inventory
- Life Events Checklist
- PSQI
- IPAQ
- PROMIS Cognitive Function
- CPP.Saliva Collection Sheet

Other

- C-SSRS.greaney completion certificate
- CPP.Crisis Protocol
- CPP.Mental Health Resources
- CPP.microdialysis discharge form
- CPP.Saliva Collection Instructions
- Jha.IMM Mtg1 pre-enroll.20240522
- IMM agenda.pre-enrollment.20240522.notes
- CPP.Metricwire Instructions

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Title of Study: Accelerated vascular aging in midlife as a mechanism linking daily stress to cognitive decline

Principal Investigator(s): Jody Greaney, PhD

This study is funded by the National Institute of Health.

KEY INFORMATION

Important aspects of the study you should know about first:

- **Purpose:** The purpose of the study is to better understand how day-to-day stress affects cardiovascular health and brain function in people with depression.
- **Procedures**: You will complete two 15-day "testing cycles" separated by ~3-6 months. During each cycle, you will complete two daily assessments of stress and your brain's thinking ability using a mobile survey tool for 14 days. You will also wear a wristwatch to track activity and sleep patterns. You will also collect saliva samples during 4 of these days. On the last day of each cycle, you will attend a laboratory visit, at which we will assess your mood, personality, health behaviors, and how your blood vessels work. We also collect a blood sample. Some techniques that we use to assess blood vessel function involve needles. Details are below.
- **Duration:** This study will take about 22 hours in total. An initial screening visit (like a physical at your doctor's office) takes about 1.5 hours. Then, each testing cycle takes about 2-3 hours for the 14-day remote assessment portion, about 1 hour for the 4-day saliva sample collection, and about 6 hours for the in-person portion. Up to two brief (~5-min) additional in-person visits may be required to pick-up study materials.
- **Risks**: The main risk from this study is pain/discomfort or bruising at the sites where needles are placed for blood sampling and the techniques to measure blood vessel health. Some survey and interview questions are personal and may cause sadness or anger.
- **Benefits:** There is no direct benefit to participating, but you may learn about the physical and mental processes that are associated with depression and cardiovascular health.
- **Alternatives:** There are no known alternatives available to you other than not taking part.
- **Costs and Compensation:** You could receive up to $850 for completing the study.
- **Participation:** Participating in this study is your decision. You can change your mind at any time.

Please carefully read the entire document. You can ask any questions you may have before deciding If you want to participate.

You are being invited to participate in a research study. This consent form tells you about the study including its purpose, what you will be asked to do if you decide to take part, and the risks and benefits of being in the study. Please read the information below and ask us any questions you may have before you decide whether you want to participate.

PURPOSE OF THE STUDY

The purpose of this study is to better understand how day-to-day stress affects cardiovascular and brain health in people with depression. We have previously shown that blood vessels do not work as well in people with depression, but we do not know why this happens. One potential reason is too much inflammation. Another possibility is an imbalance in the production of certain molecules from the mitochondria (the powerhouse of the cell). Stress can also cause both inflammation and mitochondrial distress. However, whether and how stress in our day-to-day lives influences inflammation and mitochondrial distress, as well as how this influences how well blood vessels work, in people with depression is not known. We also do not know how stress influences brain health in people with depression. This study will determine whether the complex links between daily stress and both inflammation and mitochondrial distress are stronger in people with depression compared to healthy people without depression. It will also determine how this impacts blood vessel and brain health.

WHO IS BEING ASKED TO PARTICIPATE?

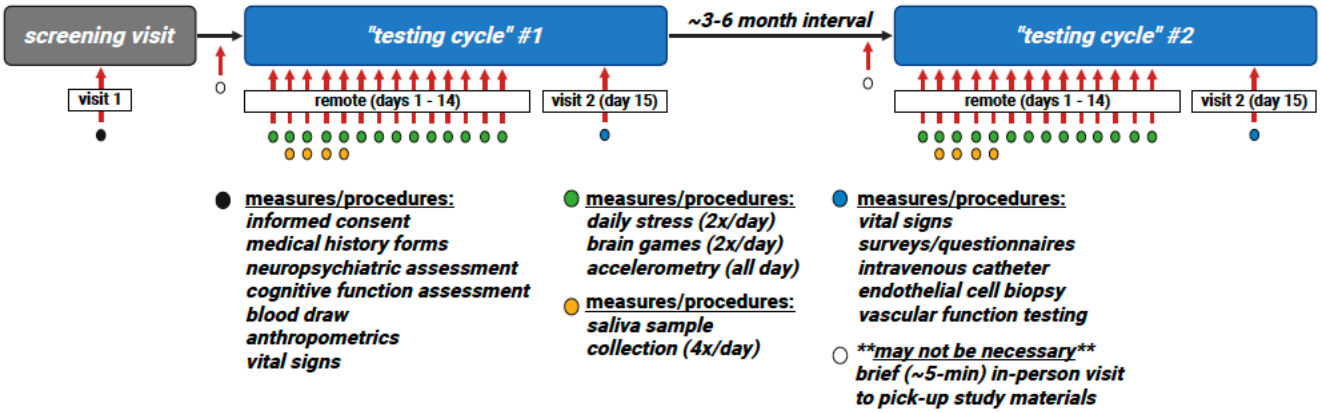
You will be one of approximately 150 participants in this study.

You are being asked to participate because you either 1) suspect that you may currently have depression, 2) suspect that you have had depression in the past, 3) you have been diagnosed with depression, or 4) you do not any history or evidence of depression. This will be confirmed by a diagnostic interview conducted by the researchers. To be included in this study, you must be between the ages of 18 and 55. During the screening visit, we will check to ensure that you meet these requirements.

You will not be allowed to participate in the study if: 1) you have a mental illness aside from depression; 2) if you are treated with a medication for depression other than a SSRI like Wellbutrin, Cymbalta, or Effexor (common SSRIs include Lexapro, Celexa, Zoloft, and Prozac); 3) if you are at high risk of suicide; 4) if you have active drug or alcohol dependence; 5) if you are treated with a medication that could alter how your brain or cardiovascular system functions; 6) if you make any changes or alterations in your medication status; 7) if you have a chronic disease aside from depression (e.g., heart disease, diabetes, kidney disease, psoriasis, etc.); 8) if you have a body mass index of <18.5 or >35 kg/m^2; 9) if you use tobacco or nicotine products; 10) if you are pregnant or breastfeeding; 11) if you do not have a reliable way to access and complete an online survey; or 12) if you have an allergy to study drugs. The research team will determine if you meet any of these criteria at the screening visit (described below). If you meet any of these criteria, we will document the reason why you are ineligible to participate, but all of your information will be de-identified and destroyed.

PROCEDURES: WHAT WILL YOU BE ASKED TO DO?

Time Commitment: Your participation in this study is expected to last for about 3-6 months and consists of an initial screening visit at the laboratory, followed by two 15-day "testing cycles". Each cycle consists of a 14-day remote portion and an in-person experimental visit at the laboratory. The laboratory visits will take place at the Tower at STAR on the University of Delaware campus. If you complete the entire study, your total time commitment will be about 22 hours. Of this total commitment, about 14 hours will take place in the laboratory (about 1.5 hours for the initial screening visit and about 6 hours for each experimental visit), and the remaining 8 hours will take place remotely (about 2-3 hours in total for the remote assessments of stress and brain health and about 1 hour for the daily saliva sample collection). An illustration is provided below (Fig. 1). You should avoid donating blood for 8 weeks before and after your participation in this study.



Research Procedures: This study involves at least three visits to the laboratory. Please read the descriptions of the measurements and procedures below.

Screening Laboratory Visit (about 1.5 hours):

1. The research staff measures your height, weight, waist circumference, blood pressure, heart rate, and temperature.
2. You will be asked to provide emergency contact information and complete a health history form. Women will also complete a gynecological and menstrual history form, whereas men will also complete a reproductive aging form. The research staff reviews your medical history.
3. If you are a woman who is not post-menopausal, you will have a urine pregnancy test.
4. You will be administered the Mini-International Neuropsychiatric Interview (MINI). The MINI has 16 sections. This interview helps the researchers look for signs of depression and other mental health illnesses.
5. You will be administered the Columbia-Suicide Severity Rating Scale (C-SSRS). The C-SSRS helps the researchers look for signs of active thoughts or plans of suicide.
6. You will be asked to complete an assessment of your brain's thinking ability on a tablet. You will take several different tests that assess memory, language, decision-making, and processing speed. Many of these tests are like "games." For example, in some tasks, you will be asked match patterns and in others you will be asked to match a word with a picture of its meaning. You will also be asked to complete a self-report assessment of your brain health. These assessments take about 30 minutes.
7. Blood Draw: The research team draws 100 ml (6.7 tablespoons) of blood from a vein in your arm. We send the blood to a lab to see if the proteins, blood cells, electrolytes, etc. are within normal levels. The researchers will provide you with a copy of these results. Some of the blood will be stored in a freezer and tested for substances of interest (inflammatory cytokines, indices of mitochondrial distress, peripheral blood

mononuclear cells, stress hormones, estradiol, progesterone, follicle stimulating hormone, luteinizing hormone, and testosterone). The researchers do not perform genetic analyses on the blood or look for presence of disease (e.g., HIV). You will be asked to avoid eating any food except water (fasting) for 12 hours before the blood draw. Depending on your schedule, this task may be completed at either the screening visit or the experimental visit.

8. You will be familiarized with the organization and flow of the daily morning and evening surveys and brain games and will practice completing these. You may be provided with a laboratory iPhone preloaded with the Metricwire mobile app for the completion of the daily morning and evening surveys and brain games. You will receive a wristwatch and will be instructed in how to use it. You will also be instructed in how to collect saliva samples and will receive a collection kit and instructions. Finally, you will also be familiarized with the techniques that will be performed at the experimental visit so that you know what to expect. Depending on the scheduling of "testing cycle #1," you may be asked to return to the laboratory to pick up this equipment prior to beginning Day 1 of the remote assessments. If necessary, this visit will take <5 minutes and will be scheduled at your convenience.

Remote Assessment of Daily Stress and Brain Health (about 2-3 hours in total; Days 1-14):

1. You will complete a morning assessment and an evening assessment each day for 14 consecutive days (Days 1-14) using either an online survey tool called Qualtrics (administered and analyzed by our collaborating investigators at The Pennsylvania State University and accessed on your personal mobile device) or the Metricwire mobile app on a laboratory-provided smartphone.
2. Beginning on Day 1, you will be notified by pre-scheduled alert notifications via email/text message twice per day (about 9am and about 7pm) to complete a short morning survey (about 2-3 minutes) and a slightly longer evening survey (about 5-7 minutes). These notifications are preprogrammed by the research team and include direct access to the associated survey. The surveys administered using Qualtrics can be completed on any personal device that has internet connection capabilities (smartphone, tablet, etc.). The surveys administered using Metricwire will be completed on the laboratory-provided smartphone.
3. The morning survey asks questions about your prior night's sleep and your outlook on the day ahead. The evening survey will ask you about your daily experiences and your mood. The investigators do not monitor your answers to these surveys on a daily basis; instead, these data are compiled at study completion.
4. For these surveys, some questions will ask you to select a single option, some questions will ask you to check the boxes of as many options as are relevant to their answer, some questions involve drop down menus, and some questions involve a slider scale.
5. After each survey, you will also complete three short "brain games." If you complete the surveys using Qualtrics, a link will be provided automatically taking you to a new browser window, where you these brain games using a tool called Gorilla. If you complete the surveys using Metricwire, you will also complete the brain games within this app. For both options, directions are provided on the screen before each game. In the first game, you will have to remember pairs of words and numbers. In the second game, you will see several numbers and have to remember the order in which they appeared. In the third game, you will see four squares that change color and will have to click a button on the screen when the same square changes color twice in a row.
6. During these same 14 days, you will also wear a wristwatch on your non-dominant wrist during all waking and sleeping hours to measure sleep patterns and activity/sedentary behaviors using accelerometry.
7. On Day 15, you will return to the laboratory for the experimental visit. You will return the wristwatch at this visit.

Remote Assessment of Indices of Stress in Saliva (about 1 hour):

1. For 4 consecutive days (Days 3-6 of the daily assessments described above), you will be asked to provide saliva samples. We will measure indices of stress, like the hormone cortisol and mitochondrial DNA, in these samples.
2. You will collect your saliva 4 times per day (once before you get out of bed, once 30 minutes after you get out of bed, once at lunch, and once in the evening). This is a total of 16 samples. You will document the time of day of each collection. Each sample collection takes <5 minutes.
3. To collect saliva, you will use a salivette, which includes a cotton swab that you will put in your mouth for 2 minutes.
4. After the 2 minutes is up, you will place the cotton swab back in the salivette tube.
5. You will be provided with a small cooler and will store these samples in the refrigerator. You will bring the samples with you to the experimental laboratory visit (Day 15).

Experimental Laboratory Visit (about 6 hours):

1. Vital Signs: When you arrive to the lab, the research team will measure your blood pressure, heart rate, and temperature. Women who are not post-menopausal will submit a urine sample for a pregnancy test.
2. Self-Report Assessments: You will be asked to complete several different surveys, listed below. Your answers to these assessments will help the research team understand things that may impact stress in your day-to-day life.
    a. *Patient Health Questionnaire-9 (PHQ-9)* to assess symptoms of depression (for example, if you have a poor appetite)
    b. *Generalized Anxiety Disorder-7 (GAD-7)* to assess symptoms of anxiety (for example, if you have trouble relaxing)
    c. *Big Five Inventory* to assess personality characteristics (for example, if you are a reliable worker or have an active imagination)
    d. *Life Events Checklist* to assess any major events in your life and how you responded (for example, getting married)
    e. *Pittsburgh Sleep Quality Index (PSQI)* to assess your sleep (for example, how often you need to get up during the night to use the bathroom)
    f. *International Physical Activity Questionnaire (IPAQ)* to assess how active you typically are (for example, how much physical activity you do as a part of your job or when taking care of your house)
3. Intravenous (IV) Catheterization: A research nurse will insert a tube into the vein of your dominant arm at the inner elbow using a small needle. This tube will allow the research nurse to draw 100 ml (6.7 tablespoons) of blood. The blood sample will be stored in a freezer and tested for substances of interest (inflammatory cytokines, indices of mitochondrial distress, peripheral blood mononuclear cells, stress hormones, estradiol, progesterone, follicle stimulating hormone, luteinizing hormone, and testosterone). The researchers do not perform genetic analyses on the blood or look for presence of disease (e.g., HIV).

4. Endothelial Cell Biopsy: A research nurse will also use the IV catheter to collect some cells from the wall of the vein. To do this, we pass a very thin, flexible j-shaped wire through the tube and gently move it back-and-forth (see photo in Fig. 2). When this wire is moved against the vein wall, it will pick up some endothelial cells. The cells will be fixed to slides, and we will examine them under a microscope to assess proteins that regulate vascular function.

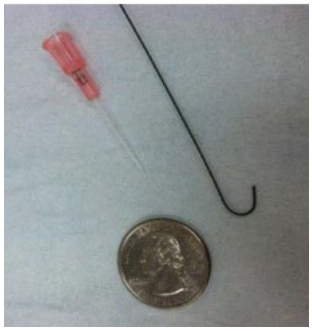


Figure 2. Intravenous (IV) catheter (left) and j-wire (right) depicted in relation to the size of a coin.

5. Flow-Mediated Dilation: We tape 3-5 ECG leads to your chest and abdomen to measure heart rate. We place a blood pressure cuff around the upper arm and on one of your fingers to measure blood pressure repeatedly during the experiment. We place a small blood pressure cuff around your forearm. We place gel on your upper arm just above the elbow. We place a Doppler ultrasound probe on the gel. The ultrasound makes sound waves to measure the size of blood vessels and the speed of the blood. We make a "resting" measurement before we inflate the blood pressure cuff. Then we inflate the cuff for 5 minutes to stop blood flow to and from the forearm (this is called arterial occlusion). We deflate the cuff and continue measuring for 3 minutes. We may repeat this measurement several times. You will be asked to avoid eating any food (fasting) for 6 hours before this procedure. Depending on your schedule, this task may be completed at either the screening visit or the experimental visit.
6. Applanation Tonometry: A pen-like probe will be placed over your skin to detect a pulse from the artery. At the same time, a blood pressure cuff on your upper leg will inflate and then deflate. These pulses will be used to assess the speed of the pulse in the artery. Depending on your schedule, this task may be completed at either the screening visit or the experimental visit.
7. Intradermal Microdialysis and Laser Doppler Flowmetry:
    a. First, we insert intradermal microdialysis probes in your non-dominant forearm:
        i. We place a tight band around the forearm so we can see the veins.
        ii. For each site, we make pairs of pen marks on the arm ~2.5 cm (1 inch) apart and away from veins. The marks serve as entry and exit points for the microdialysis tubing. We then remove the tight band.
        iii. We then clean that area of the arm and place an ice bag on the skin for 5 minutes to numb the area.
        iv. We insert a thin needle into the skin near each entry mark. The needle's tip travels between the layers of skin for 2.5 cm (1 inch). The needle exits the skin near the matching exit mark.
        v. We thread the microdialysis tubing (~1/100th of an inch in diameter; the size of 6 human hairs) through the needle and then withdraw the needle, leaving the tubing in the skin. The tubing in the skin has microscopic holes, which allows the investigator to slowly deliver solutions through the tubing assembly to your blood vessels in the immediate area surrounding the tubing.
        vi. We prepare up to 3 microdialysis sites. During this time, lactated Ringers solution and the test substances flow through the microdialysis tubing. Lactated Ringers solution is a saline fluid like that found throughout your body. This study involves the use of some test substances or drugs that are not approved by the FDA to treat disease. All of the test substances or drugs have been used in humans by us or others in the past.
            - lactated Ringers
            - lactated Ringers + MitoTempol
            - lactated Ringers + Tempol
    b. Next, we tape thin fiber optic laser Doppler flowmeter probes and their holders to your forearm at each microdialysis site. The thin probe measures skin blood flow with a weak laser light. We also tape 3-5 ECG leads to your chest and abdomen to measure heart rate. We place a blood pressure cuff around the

upper arm (opposite arm of the one with microdialysis probes) to measure blood pressure repeatedly during the experiment.

c. At first, we collect baseline data. Then, we slowly increase the temperature of probe holders to 42°C (107.6°F) until skin blood flow stabilizes. Then, we switch the fluids at all sites to L-NAME until skin blood flow stabilizes a second time. Next, we switch the fluids at all sites to sodium nitroprusside. At the same time, we warm the probe's holder to 43°C (108°F). This causes the blood vessels to dilate as much as they can. In total, this procedure takes about 4 hours.
d. At the end of the experiment, we remove the microdialysis tubing from your skin. We place sterile bandages over the sites. If you desire, we place a bag of ice on the sites for 10 minutes to reduce the chance of bruising.

At the end of the experiment, we remove any equipment. We measure blood pressure and heart rate before you leave the lab.

You will be asked to return to the laboratory to pick up all necessary equipment prior to beginning Day 1 of the remote assessments as a part of "testing cycle #2." This visit will take <5 minutes and will be scheduled at your convenience.

WHAT ARE POSSIBLE RISKS AND DISCOMFORTS?

Individually, each procedure should not cause you much discomfort. However, it is possible that you may experience discomfort due to the additive effect of all of these procedures. The protocol is designed to reduce the likelihood of this occurring by minimizing the duration of each procedure. Nevertheless, if you experience a degree of discomfort that is greater than your expectations, we will stop the procedure(s) at your request. All tests that are to be performed have safely been used in both healthy and depressed individuals. Throughout the tests you will be closely monitored. Any new information developed during the study that may affect your willingness to continue participation will be communicated to you.

Although such events are extremely unlikely, the investigators are prepared to immediately stop experiments and seek medical assistance if you should experience a medical emergency. In this case, emergency services will be contacted.

Neuropsychiatric Interview and Depressive Symptom Severity/Suicidality Assessments: These tools help us to sort people into the groups for this study. Answering questions related to your mental health and past experiences may be stressful or uncomfortable, and you may feel shy or disturbed by some questions. You may choose not to answer the questions and decline to be in the study. You may feel that these tools lead us to put you into the wrong group. We do not use these tools to diagnose depression. We do not use the tools to decide a recommendation for healthcare. The tools help us to find people who may have certain experiences useful for the purposes of this study. The tools help us to find those who have forms of psychological distress that are useful for this study. If you indicate a high risk of suicide, either the UD Helpline or the State of DE Crisis Helpline will be called for an external evaluation by a mental health professional. We keep the completed forms confidential and secure. Only approved staff may access the results. We treat all groups in the same way throughout the study. No one other than you and the approved staff know the group to which you have been assigned. If you have concerns about the results, we suggest that you seek follow-up with a healthcare or mental healthcare provider. If you do not have access to one, the researchers will provide you with resources and help connect you with providers if needed.

Self-Report Assessments (Interviews/Surveys): These surveys help us learn of your experiences that are useful for the purposes of this study. The surveys help us to interpret the data we produce in the experiments. You may feel shy or disturbed by the questions. Reporting on stressful events may result in some sadness or anger or remind the participant of previous stressful experiences. Completing the "brain games" may cause some frustration. You may choose not to answer the questions and decline to be in the study. We do not use the tools to decide a recommendation for healthcare. We keep the completed forms confidential and secure. Only approved staff may access the results.

Brain Function Assessment: This assessment helps us to interpret the data we produce in the experiments. Completing the "brain games" may cause some frustration, and you may feel uncomfortable or embarrassed. You may choose not to answer the questions and decline to be in the study. We do not use the tools to decide a recommendation for healthcare. We keep the completed forms confidential and secure. Only approved staff may access the results.

Intravenous (IV) Catheterization and Endothelial Cell Biopsy: IV catheterization may cause mild pain, bruising, swelling, or bleeding. There is also a slight chance of infection or small clot. If you are nervous about needles, blood pressure and heart rate may increase for a little while and you may feel anxious or stressed. You may also feel lightheaded, sick to your stomach, or may faint. The risks associated with endothelial cell biopsy are similar to those of IV catheterization. The risk of blood clots and infection may be slightly greater due to increased manipulation of the blood vessel. Using the same techniques used in hospitals keeps the chance of infection minimal. You will be laying down for these procedures.

Blood Draw: Blood draws often cause mild pain, bruising, swelling, or bleeding. There is also a slight chance of infection or a small clot. If you are nervous about needles, blood pressure and heart rate may increase for a little while and you may feel anxious or stressed. You may also feel lightheaded, sick to your stomach, or may faint. Using the same techniques used in hospitals keeps the chance of infection minimal.

Intradermal Microdialysis: The risks are less than that for a blood draw because microdialysis uses only a small, local area of skin. In contrast, a blood draw involves not only skin, but also larger blood vessels and blood. You are likely to have some pain and bruising like that from a blood draw. However, we use ice to numb your arm when we insert the tubing. Also, the use of such a small needle reduces pain when we insert the tubing. You are not likely to have pain after the tubing is in place. You may feel a little pain when we remove the tubing from your skin. Needles make some people feel sick to their stomach, lightheaded, or may cause them to faint. Although rare, the tubing could break as we remove it from the skin. Then we remove the tubing still in your skin by pulling on the other end of it. This presents no added risk for you. We stop any mild bleeding with mild pressure and sterile gauze. Infection is possible. We keep the risk of infection very small by using sterile techniques and supplies like those used with blood draws. We apply a sterile bandage to the site after the experiment. We tell you how to take care of the site.

Fluid flowing through the tubing (perfusate): The substances flowing through the tubing only go to a 2.5 cm^2 (0.4 $inch^2$) area of skin at each tubing site. The amount that enters the skin is very small. However, there is a chance of having a bad reaction to the substances. This reaction could produce redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse, convulsions, and/or fainting/lightheadedness. We and other researchers have used these substances with microdialysis in skin. There have been no reports that these substances caused bad reactions. If a bad reaction should occur, we summon medical help.

Lactated Ringers solution: This fluid is similar to the natural fluids in your skin. The fluids contain salt, potassium, lactate (Ringer's only), and chloride. The acid content is like that your body's natural fluids. A bad reaction to these fluids is highly unlikely.

L-NAME/MitoTempol/Tempol/sodium nitroprusside: These substances mimic the action of your body's natural chemicals upon the blood vessels in the skin. A small amount of these substances enters the skin around the tubing. This only affects the blood flow in the vessels in that nickel-sized area of skin. The effect of these substances is gone within an hour after the experiment.

Laser Doppler Flowmetry and Local Heating: Weak lasers can hurt your eye if you stare into the light for a long time. We do not turn on the laser until the probes are taped to the surface of your skin. The tape may irritate your skin. We measure the temperature of the skin under the holders. During heating, the skin feels very warm but does not hurt. The heating makes the skin under the holder red, like when you take a hot bath. The redness disappears within a few hours. Some people may be more sensitive to heating. If your arm feels too hot, tell the investigators, and we will reduce or stop the heating.

Flow-Mediated Dilation and Arterial Occlusion: These are common research techniques. There is a small chance the probe could irritate the skin. Minor redness may occur where the researchers place the probe against the arm. This is temporary. The gel is the same as that used with medical ultrasound tests. The gel may feel cool or cold on the skin. A bad reaction to the gel is highly unlikely. During arterial occlusion, a blood pressure cuff will be inflated very high so that there is no blood flow to the limb below where the cuff is placed. This will be maintained for 5 minutes. You will feel temporary discomfort such as numbness and/or tingling of the distal digits. If you become too uncomfortable you may ask for the cuff to be deflated. Symptoms subside within 30 seconds following cuff deflation.

Applanation Tonometry: This is a common research technique. During the short time we inflate the cuff, your arm or leg may feel numb or tingly. The cuff could cause mild bruising. We can deflate the cuff at any time if it is too uncomfortable.

Fasting: You may feel lightheaded, dizzy, or faint. You will be provided a small snack at the completion of the techniques that require you to avoid eating.

Medical Screening: You may feel shy about giving health information. The staff collects the information in a private and professional manner. You may feel shy about being measured. You may request someone of the same sex to conduct parts of the screening.

Blood Pressure: We measure blood pressure with the method used in a doctor's office. A cuff inflates on the upper arm and then slowly deflates. During the short time we inflate the cuff, your arm may feel numb or tingly. The cuff could cause mild bruising. We can deflate the cuff at any time if it is too uncomfortable.

Accelerometry: Minor skin irritation may occur from wearing the wristwatch.

Povidone Iodine: Researchers and hospitals use this orange-colored fluid to clean the skin. You could have a bad reaction to the fluid if you are allergic to iodine. You inform us if you have this allergy. In this case, we use only alcohol instead. A bad reaction could cause redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse, convulsions, and/or fainting.

Confidentiality: There is a risk of loss of confidentiality if your information or your identity is obtained by someone other than the investigators, but precautions will be taken to prevent this from happening. The confidentiality of your electronic data created by you or by the researchers will be maintained to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed.

WHAT ARE POTENTIAL BENEFITS FROM THE STUDY?

We cannot promise any benefits to you or others from your taking part in this research. The main possible direct benefit to you from participating in this research study is learning about the physical and mental processes that are associated with depression and how they impact cardiovascular and brain health. You could gain knowledge about how your body works, and you might personally gain insights concerning your behaviors. Other possible benefits include a screening that informs you about your health such as your current blood pressure and blood cholesterol levels. There will be no charge for any tests required for the study. You may also enjoy the knowledge that you are contributing to information that may be valuable in designing new programs to improve overall health in people with depression.

NEW FINDINGS THAT COULD AFFECT YOUR PARTICIPATION

During this study, we may learn new important information. This may include information that could affect your decision about participating in the study. If any new important information becomes available while you are a participant, we will let you know.

CONFIDENTIALITY: WHO MAY KNOW THAT YOU PARTICIPATED IN THIS RESEARCH?

Your study data will be handled as confidentially as possible. The findings of this research may be presented or published. If this happens, no information that gives your name or other details will be shared. In the event that the investigators have to reach out to your emergency contact, the confidentiality of your participation cannot be guaranteed.

To minimize the risks to confidentiality, we will code all files with a unique code for each person. We keep the list that matches your name with your code number in REDCap, which is a secure online platform for managing research studies. Qualtrics and Metricwire are secure survey administration applications designed to support data capture for research studies. Data are deleted from the cloud servers within 24 hours.

We label your research records with your code number and keep them in a locked file or on a password-protected computer in a room that is locked when unoccupied. Some research records contain your name for a brief period until they are coded or destroyed. We also label any samples that we collect with your code number. We keep the samples in a dedicated freezer until analysis. All research specimens sent to outside labs for analysis are identified only by code number. Your name and other information that can directly identify you will be stored securely and separately from the research information we collected from you.

We will keep your study data confidential and only those with permission in the research team will have access to information that identifies you. We may have to report certain information for legal or ethical reasons, such as child abuse, or intent to hurt yourself or others. If required, your records may be inspected by authorized personnel in the following groups and agencies: the University of Delaware Institutional Review Board.

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. With this Certificate, researchers cannot be forced to disclose information that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings.

Exceptions: A Certificate of Confidentiality does not prevent researchers from voluntarily disclosing certain information about you for legal or ethical reasons. For example, we will report information about child abuse, or intent to hurt yourself or others. If an insurer, employer, or other person obtains your written consent to receive research information, we cannot use the Certificate to withhold that information. In addition, the Certificate may not be used to withhold information from the federal government needed for auditing or evaluating federally funded projects or information needed by the FDA, e.g., for quality assurance or data analysis.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

USE OF DATA COLLECTED FROM YOU IN FUTURE RESEARCH:

Identifiers about you might be removed from the identifiable private information and after such removal, the information could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from you or your legally authorized representative.

COSTS AND COMPENSATION

There are no costs associated with participating.

If you agree to take part in this research study, we will pay you for your time and effort. You will be compensated when you complete the study or choose to quit the study. You will receive this payment in the form of a check.

For each 14-day remote assessment portion (including daily saliva collection), you will be compensated $200. If you complete both the morning and evening surveys on ≥12 days, you will receive a bonus of $50. The maximum compensation for this portion of the study is $250. For each in-person experimental laboratory visit, you will be compensated $100. If you complete all parts of a testing cycle, the maximum compensation is $350. If you complete all parts of both testing cycle, you will be compensated $700, plus an additional $150 bonus. These payments will be made separately following each cycle. The maximum compensation for completion of the entire study is $850. If you receive the maximum compensation, you will receive one check for $350 after the first testing cycle and a second, separate, check for $500 after the second testing cycle.

The Internal Revenue Service (IRS) considers all payments made to research subjects to be taxable income. Your personal information, including your name, address, and social security number, may be acquired from you and provided to the University of Delaware's accounting office for the purpose of payment. If your total payments for the year exceed $600.00, the University of Delaware will report this information to the IRS as income and you will receive a Form 1099 at the end of the year. If you receive less than $600.00 total for payments in a year, you are personally responsible for reporting the payments to the IRS.

WHAT IF YOU ARE INJURED DURING PARTICIPATION IN THE STUDY?

If you are injured during your participation in the study, you will be offered first aid at no cost to you. If you need additional medical treatment, the cost of this treatment will be your responsibility or that of a third-party payer (for example, your health insurance). By signing this document, you are not waiving any rights that you may have if injury was the result of negligence of the university or its investigators.

DO YOU HAVE TO TAKE PART IN THIS STUDY?

Taking part in this research study is your decision. You do not have to participate in this research. If you choose to take part, you have the right to stop at any time. If you decide later not to participate, or if you decide to stop taking part in the research, there will be no penalty or loss of benefits to which you are otherwise entitled. Your decision to stop participation, or not to participate, will not influence current or future relationships with the University of Delaware.

As a student, if you decide not to take part in this research, your choice will have no effect on your academic status or your grade in any class.

INSTITUTIONAL REVIEW BOARD

This research study has been reviewed and approved by the University of Delaware Institutional Review Board (UD IRB), which is a committee formally designated to approve, monitor, and review biomedical and behavioral research involving humans. If you have any questions or concerns about your rights as a research participant, you may contact the UD IRB at hsrb-research@udel.edu or (302) 831-2137.

CONTACT INFORMATION

If you have any questions about the purpose, procedures, or any other issues related to this research study you may contact the Principal Investigator, Jody Greaney, PhD at (302) 831-2193 or jgreaney@udel.edu

CONSENT TO PARTICIPATE IN THE RESEARCH STUDY:

I have read and understood the information in this form and I agree to participate in the study. I am 18 years of age or older. I have been given the opportunity to ask any questions I had and those questions have been answered to my satisfaction. I understand that I will be given a copy of this form for my records.

_____	_____	_____
Printed Name of Participant (PRINTED NAME)	Signature of Participant (SIGNATURE)	Date
_____	_____	_____
Person Obtaining Consent (PRINTED NAME)	Person Obtaining Consent (SIGNATURE)	Date

<u>OPTIONAL</u> CONSENT TO BE CONTACTED FOR FUTURE STUDIES:

Do we have your permission to contact you regarding participation in future studies? If you agree to being contacted in the future, we will keep your contact information. Please write your initials next to your preferred choice.

_____ YES _____ NO