Sex Differences in the Dilatory Response of Compound 21

NCT05576155

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Sex Differences in the Dilatory Response of Compound 21

PI: Anna Reid-Stanhewicz
IRB ID #: 202206519

Project Details

I. Project Introduction

I.1 ***Project to be reviewed by:***
IRB-01

I.2 ***Project Title:***
Sex Differences in the Dilatory Response of Compound 21

I.3 ***Short Title (optional):***

I.4 ***Provide a short summary of the purpose and procedures of the study proposed in this IRB application.***

- ***DO NOT include information on studies not proposed in this application.***
- ***Use LAY terminology only. This must be easily understandable by IRB community members and nonscientists.***
- ***DO NOT cut and paste technical abstracts from funding applications that may not be understood by a general audience.***

When blood pressure decreases, your body receptor, Angiotensin II type II receptors (AT2R), cause blood vessels to get bigger in diameter (i.e., vasodilation). These two responds by naturally producing and releasing a chemical called Angiotensin II into your bloodstream. This substance is widely known to act on blood vessels causing them to become smaller in diameter (i.e., vasoconstriction) through Angiotensin II type I receptors (AT1R). However, a lesser-known receptors normally maintain blood pressure and blood volume in the body. Excess Angiotensin II released in the blood stream may reduce sensitivity of AT2Rs, leading to excessive activation of AT1Rs. This results in increased constriction which plays a major role in diseases such as high blood pressure, hardening of the arteries, and heart failure. In the body, Angiotensin II production is reduced in the presence of estrogen, as seen in pre-menopausal women. Pre-menopausal women have a greater protection against cardiovascular diseases compared to age-matched males, likely due to the protective effects of estrogen. However, the extent that estrogen may impact the sensitivity of Angiotensin II receptors in pre-menopausal is unknown.

The purpose of this study is to examine the microvascular differences in young men and women after activation of AT2Rs in the skin. This will help us better understand the mechanisms of Angiotensin II receptors in men and women and how Compound 21 may restore their balance.

In this study, we use the blood vessels in the skin as a representative vascular bed for examining mechanisms of microvascular dysfunction in humans. Using a minimally invasive technique (intradermal microdialysis for the local delivery of pharmaceutical agents) we examine the blood vessels in a dime-sized area of the skin in healthy young women and men. As a compliment to these measurements, we also draw blood from the subjects and measure circulating factors that may contribute to cardiovascular health.

I.5 ***Specify your research question(s), study aims or hypotheses (do not indicate "see protocol")***
The aim of this proposal is to define the mechanistic role of activation of AT2Rs in young men and young women. To accomplish this aim, we will test the following hypotheses:

Hypothesis 1: AT2R activation (C21) will have greater microvascular dilation in young women vs young men.
Hypothesis 2: The dilatory response to C21 will be enhanced in both young women and men with AT1R inhibition (losartan) vs control.

I.6 ***Background and significance and/or Preliminary studies related to this project.***
(do not indicate "see protocol")
Pre-menopausal women have a significantly lower incidence of cardiovascular disease (CVD) than age-matched men due to the protective benefits of estrogen. Estrogen decreases levels of Angiotensin II, allowing less Angiotensin II to act on its receptors: AT1Rs (cause constriction) and AT2Rs (cause dilation). Excess Angiotensin II disrupts the balance between these two receptors in the body acts mainly through AT1Rs, leading to higher risk of CVD and related diseases. Because estrogen downregulates Angiotensin II, there is a balance between activation of AT1Rs and AT2Rs. In young males, this balance can be dysregulated when excess Angiotensin II is produced. A new pharmacological agent, known as Compound 21 (C21), activates AT2Rs to cause vasodilation. Besides vasodilation, activation of AT2Rs with C21 also elicits anti-inflammatory and anti-fibrotic effects in animal models. In humans, this compound has been shown to cause dilation in the lungs and the peripheral vessels (e.g., skin) when given orally. Thus, the purpose of this study is to determine the extent that dilation with AT2R activation differs in young men and women. As young women have more protection from heart diseases than age-matched young males, we propose that activation of AT2Rs will be more sensitive in young women than in young men. This will help us better understand why women have enhanced cardio-protection prior to menopause.

I.7 ***Literature cited / references (if attaching a grant or protocol enter N/A).***
N/A

II. Research Team

II.1 ***Principal Investigator***

Name	E-mail	College
Anna Reid-Stanhewicz	anna-stanhewicz@uiowa.edu	College Lib Arts and Sciences

II.2 ***Team Members***
UI Team Members

Name	E-mail	College	Contact	Key Prsn	UI COI	VAMC COI	Consent Process Involvement	Deactivated
[redacted]		College Lib Arts and Sciences	Yes	Yes	No		Yes	No
		College Lib Arts and Sciences	No	No	No		Yes	No
		Carver College of Medicine	No	Yes	No		No	No
		Graduate College	No	No	No		Yes	No
		College of Liberal Arts and Sciences	No	No	No		No	No
		Carver College of Medicine	No	No	No		No	No
		Graduate College	No	No	No		Yes	No
		College of Liberal Arts and Sciences	No	No	No		No	No

Non-UI Team Members

Name Institution Location FWA Role DHHS Contact Key Prsn UI COI VAMC COI Consent Process Involvement Email

Nothing found to display.

II.3 ***The Principal Investigator of this study is:***

Faculty

II.6 ***Identify the key personnel. The system will automatically designate the PI and all faculty members on the project as "key personnel." For information about other team members who should be designated as "key personnel" please click on the help information.***

Name	Is Key Personnel
[redacted]	

II.5 ***Select research team member who is the primary contact for study participants.***

Kelsey Schwartz

III. Funding/Other Support

III.1 ***Funding Sources***

Source Entered as Text	DSP Link	Type	Source	Grant Title	Nar
Source is entered as text no		Federal Agency	US Department of Health & Human Services, National Institutes of Health	Role of Angiotensin II and Chronic Inflammation in Persistent Microvascular Dysfunction Following Preeclamptic Pregnancy	Ani Stai

* new source name

III.2 ***What type of funding agreement would be completed?***

Federal/State/Local Agency/Non-Profit Funded/Other

III.3 ***Does any member of the research team have a financial conflict of interest related to this project according to the Conflict of Interest in Research policy? If yes, please indicate which members below.***

Name	Has Conflict of Interest
[redacted]	No
	No
	No
	No
	No
	No
	No
	No

III.5 *What is the current status of this funding source?*

Source	Status	Other Status Description
US Department of Health & Human Services, National Institutes of Health	Awarded	

IV. Project Type

IV.1 *Do you want the IRB to give this project*
Regular (expedited or full board) review

IV.2 *Enter the date you will be ready to begin screening subjects/collecting data for this project. (If you do not have a specified date, add "upon IRB approval")*
upon IRB approval

IV.3 *Are you requesting a waiver of informed consent/authorization (subjects will not be given any oral or written information about the study)?*
No

V. Other Committee Review

V.1 *Does this project involve any substance ingested, injected, or applied to the body?*

- *Do not answer yes, if the involvement includes a device, wire, or instrument*

Yes

V.1.a *What is/are the substance(s):*
Povidone iodine (SOC)

*Note: All of the below substances have been approved by the FDA for use with microdialysis under IND #124,294
Sodium Nitroprusside (SNP), Powder
Lactated Ringer's, Liquid
Losartan, Powder
Compound 21 (C21), Powder

V.1.b *Are any of these substances defined as a Schedule I - V Controlled Substance?*
No

V.2 *Are any contrast agents used for any purpose in this study?*
No

V.4 *Are all drugs or substances in this study being used within the FDA approved population (i.e., children, adults)?*
Yes

V.5 *Are all drugs or substances in this study being used within the FDA approved indication (i.e., disease, condition)?*
No

V.6 *Are all drugs or substances in this study being used within the FDA approved dose?*
No

V.7 *Are all drugs or substances in this study being used within the FDA approved route of administration?*
No

V.8 *Drugs used in study that are not FDA approved for the population, indication, dose, or route of administration*

Lactated Ringer's solution ()

Name of Sponsor	Anna Stanhewicz
Investigator's Brochure Version	NA
Investigator's Brochure Date	NA
Who is supplying the drug	VWR
Who is dispensing the drug	PI/Research Team
Where will the drug be stored	118 PSRB
IND#	124,294
Dose	NA
Route of administration	intradermal microdialysis

Sodium nitroprusside ()

Name of Sponsor	Anna Stanhewicz
Investigator's Brochure Version	NA
Investigator's Brochure Date	NA
Who is supplying the drug	USP
Who is dispensing the drug	PI/Research Team

Where will the drug be stored	118 PSRB
IND#	124,294
Dose	<0.1 mg
Route of administration	intradermal microdialysis

Losartan ()

Name of Sponsor	Anna Stanhewicz
Investigator's Brochure Version	NA
Investigator's Brochure Date	NA
Who is supplying the drug	USP
Who is dispensing the drug	PI/Research Team
Where will the drug be stored	118 PSRB
IND#	124,294
Dose	<0.01 mg
Route of administration	intradermal microdialysis

Compound 21 ()

Name of Sponsor	Anna Stanhewicz
Investigator's Brochure Version	NA
Investigator's Brochure Date	NA
Who is supplying the drug	Sigma-Aldrich
Who is dispensing the drug	PI/Research Team
Where will the drug be stored	118 PSRB
IND#	124,294
Dose	<0.1 mg
Route of administration	intradermal microdialysis

V.9 ***Will any subject be asked to undergo a diagnostic radiation procedure (including radiographic, nuclear medicine, DEXA)?***
No

V.14 ***Will any subject be asked to undergo a radiation therapy procedure (including external beam therapy, brachytherapy, or nuclear medicine therapy)?***
No

V.20 ***Does this project involve the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into one or more human research participant?***
No

V.21 ***Will any portion of this project be conducted in the CRU, or does it use any CRU resources?***
No

V.22 ***Will this project use:***

- ***any resource/patients of the Holden Comprehensive Cancer Center***
- ***involve treatment, detection, supportive care, or prevention of cancer***

No

V.25.a ***Will the study involve <u>any</u> of the following activity at UI Health Care, even if subjects or their insurance will not be billed for the item or service, and regardless of the study funding source (including studies with departmental or no funding)?***

- ***Procedures, tests, examinations, hospitalizations, use of Pathology services, use of clinic facilities or clinical equipment, or any patient care services, including services conducted in the Clinical Research Unit; or***
- ***Physician services or services provided by non-physicians who are credentialed to bill (ARNPs, Physician Assistants, etc.)***

No

V.26 ***The study involves Department of Nursing Services and Patient Care nursing, nursing resources or evaluates nursing practices at UI Health Care.***
No

V.27 ***Will the study involve the use of the I-CTMS (OnCore) for clinical trial data management? Select yes if any or all of the following apply:***

- ***Any study required to register subjects in EPIC are encouraged to use the I-CTMS***
- ***Best practice is to use the I-CTMS for any new study that involves subject tracking or sponsor invoicing***

Note: This question is for non-oncology studies only. For oncology studies use existing HCCC OnCore processes by selecting V.22
Yes

V.28 ***Identify the role of each research team member.***

Name	Role
[redacted]	

Name	Role
[redacted]	

VI. Subjects

VI.1 ***How many adult subjects do you expect to consent or enroll for this project?***
44

VI.2 ***What is the age of the youngest adult subject?***
18.0

VI.3 ***What is the age of the oldest adult subject?***
35.0

VI.4 ***What is the percentage of adult male subjects?***
50

VI.5 ***What is the percentage of adult female subjects?***
50

VI.6 ***How many minor subjects do you expect to consent or enroll for this project?***
0

VI.13 ***Describe EACH of your subject populations***

- ***Include description of any control group(s)***
- ***Specify the Inclusion/Exclusion criteria for EACH group***

In this study we are recruiting 2 subject groups. This will include 1) young women and, 2) young men. Participants must be 18-35 years old with a body mass index between 18 and 30 kg/m2, a systolic blood pressure <140, and diastolic blood pressure <90 mmHg.

Subjects will be excluded for any of the following reasons: 1) known skin allergies, skin disorders, or skin diseases such as Raynaud's phenomenon or other history of cold intolerance, 2) diagnosed or suggested history of metabolic or cardiovascular disease, 3) taking medications that could alter vascular function, including antidepressants, anxiety medications, or cholesterol or blood pressure lowering drugs, 4) oligo- or amenorrhea, 5) women that are pregnant or nursing, 6) currently tobacco use, 7) a body mass index <18 kg/m2 or >30 kg/m2, 8) allergy to materials used during the experiment (e.g. latex).

Because sex hormones may influence skin blood flow mechanisms, all young women will be eumenorrheic, and will be tested during the low hormone phase of the menstrual cycle (within 7 days of cessation of menses), or taking
oral contraceptives.

VI.14 ***Provide an estimate of the total number of subjects that would be eligible for inclusion in each of your study populations (include your control population if applicable)***
We will recruit subjects from Johnson and surrounding counties in Iowa. The University of Iowa has a student population of ~25,000 undergraduates, a majority of whom would qualify for the study based on age.

VI.15 ***Describe how you will have access to each of your study populations in sufficient number to meet your recruitment goals.***
We will advertise for participants by posting fliers in the community where permission has been granted (see attached flier). We will also post this flier in digital spaces such as on facebook and twitter. The flier will also be made into table tents which will be displayed on tables in areas the researchers are granted permission (e.g. around campus, bars, restaurants, etc.). Participants will be recruited from the University of Iowa and surrounding area, by mass email (see attached mass email text) and the Noon News (see attached Noon news posting) a newsletter available on campus at UIHC. We will also display digital signage around the university's campus (see attached digital sign). All individuals interested in participating will be directed to contact research staff by phone or email. All individuals interested in the study will be invited to complete the RedCap eligibility survey sent via email. If an individual appears to be eligible for the study based on their RedCap Eligibility survey responses, a member of the research team will contact them via phone or email to tell the individual more about the study, answer any questions, and perform a phone screening to determine eligibility.

VI.16 ***Do you plan to recruit/enroll non-English speaking people?***
No

VI.18 ***Do you propose to enroll any of the following in this study as subjects?***

- ***Employee of the PI or employee of a research team member***
- ***Individual supervised by PI or supervised by member of research team***
- ***Individual subordinate to the PI or subordinate to any member of the research team***
- ***Student or trainee under the direction of the PI or under the direction of a member of the research team***

No

VI.20 ***Will subjects provide any information about their relatives?***

No

VI.23 ***Will anyone (other than the subject) provide you with information about the subject (e.g. proxy interviews)?***
No

VI.26 ***Is this project about pregnant women?***
No

VI.27 ***Will this project involve fetuses?***
No

VI.28 ***Does this project involve adult subjects who may be incompetent or have limited decision-making capacity on initial enrollment into the study?***
No

VI.32 ***Does this project involve subjects whose capacity to consent may change over the course of the study?***
No

VI.37 ***Does this project involve prisoners as subjects?***
No

VII.A. Project Description (A)

VII.A.1 ***Where will project procedures take place (check all that apply)?***
- Other UI campus site - 118 PSRB

VII.A.2 ***Is this project also being conducted by other researchers at their own sites (e.g. a multi-site collaborative project)?***
No

VII.B. Project Description (B)

VII.B.1. ***Does this project involve any of the following (Check all that apply):***

- ☑ **Interventional –** Includes **Clinical (or Treatment) trial**, **Physiology intervention/study**, **Behavioral intervention/study**, **Diagnostic Trial**.
  - ☐ **Clinical (or Treatment) trial –** A prospective biomedical or behavioral research study of new treatments, new drug or combinations of drugs, new devices, or new approaches to surgery or radiation therapy. (NIH and ClinicalTrials.gov & FDA)
  - ☑ **Physiology intervention/study –** A pharmacologic or measurement study aimed at understanding basic mechanisms of disease and/or of normal human physiology, often without any therapeutic intent (though a clinical trial could include such components, often labeled as "translational" or "basic science" aims.) Measurements in such studies could include, but are not limited to, a blood draw, EKG, EEG, MRI, auditory or sensory testing, checking vital signs, DEXA scans, eye tracking, specimen collection, exercise, fasting, special diets, etc.
  - ☐ **Behavioral intervention/study –** May be used to refer to studies of individual or group behavior. This option does not include drugs, biologics, or devices but could include psychotherapy, lifestyle counseling, behavior modification, etc.
  - ☐ **Diagnostic trial –** Protocol designed to evaluate one or more interventions aimed at identifying a disease or health condition (ClinicalTrials.gov & FDA)
- ☐ **Observational**
- ☐ **Expanded Access –** A process regulated by the Food and Drug Administration (FDA) that allows manufacturers to provide investigational new drugs to patients with serious diseases or conditions who cannot participate in a clinical trial. Examples of expanded access include non-protocol access to experimental treatments, including protocol exception, single-patient IND, treatment IND, compassionate use, emergency use, continued access to investigational drug, and parallel track (ClinicalTrials.gov & FDA).
- ☐ **Registry –** The collection and maintenance of data (not including biologic samples) in which: (1) the individuals in the registry have a common or related condition(s), and/or (2) the individuals in the registry are interested in being contacted for future studies by investigators other than those listed in Section II of this project.(UI Guide)
- ☑ **Repository –** The collection, storage, and distribution of human biologic samples and/or data materials for research purposes. Repository activities involve three components: (i) the collection of data and/or specimens such as blood, tissue, saliva, etc.; (ii) the storage of data or specimens, and data management function; and (iii) the sharing of data/specimens with recipient investigators other than the original investigators. (paraphrased from OHRP)
- ☐ **Other**

VII.B.2 ***Does this project involve a drug washout (asking subject to stop taking any drugs s/he is currently taking)?***
No

VII.B.11 ***Is there a separate, written protocol that will be submitted in addition to this IRB New Project form? (Note: a grant application is not considered to be a protocol)***
Yes

VII.B.12 ***Who initiated/provided the protocol?***
UI Investigator

VII.B.12.c ***Would you like to use information from this IRB application to have a ClinicalTrials.gov record started on your behalf?***
No

VII.B.14 ***Protocol#:***
202206519

VII.B.15 ***Protocol Version#:***
1

VII.B.16 ***Protocol Date:***
7/13/2022

VII.B.17 ***Amendments submitted with this application:***

Amend. #	Amend. Date

Nothing found to display.

VII.B.18 ***Does this project involve the evaluation, or testing, of the safety and/or efficacy of a medical device?***
No

VII.C. Project Description (C)

VII.C.1 ***Does this project involve any research on genes or genetic testing/research?***
No

VII.D. Project Description (D)

VII.D.1 ***Check all materials/methods that will be used in recruiting subjects (you will need to attach copies of all materials at the end of the application):***

- Advertisements -
- Posters -
- E-mail -
- Website - UIHC Clinical Trials and Research Website https://microvascularphys.lab.uiowa.edu/research-studies

VII.D.1.a ***Will any of the materials/methods below be used by researchers (or their colleagues) to recruit subjects into this study?***

- ***the potential subject is a patient OR***
- ***use of any information considered to be Protected Health Information (PHI) OR***
- ***review of patient/clinic records be used in recruiting subjects***

No

VII.D.8 ***Will a member of the research team discuss the study with the subject in person prior to the subject agreeing to participate?***
Yes

VII.D.9 ***Describe the physical location where the consent process will take place:***
The research staff will discuss the study with potential subjects in the research lab space in 118 PSRB.

VII.D.10 ***Will a member of the research team discuss the study with the subject by phone prior to the subject agreeing to participate?***
Yes

VII.D.11 ***Describe:***
Research staff will discuss the project over the phone with individuals who are interested in participating in the study. (see pre-consent phone script). This will take place in the research lab in 118 PSRB. During the call, the research team member will answer any questions and describe the study to the individual as needed. If the subject is still interested in participating they will be given access to the RedCap pre-consent screening survey. If the individual continues to meet eligibility after completing this survey, they will be given information about the study, but will not be consented over the phone. If the subject agrees, they will come to the Field House and be consented and proceed with Visit 1.

VII.D.12 ***Who will be involved in the consent process (including review of consent document, answering subjects' questions)?***

Name	Consent Process Involvement
[redacted]	

Name	Consent Process Involvement
[redacted]	

VII.D.15 ***Check all materials that will be used to obtain/document informed consent:***

- Consent Document
- Consent Summary (or Key Information Sheet)

VII.D.16 ***Are you requesting a waiver of documentation of consent (either no subject signature or no written document)?***
No

VII.D.19 ***Before the subject gives consent to participate are there any screening questions that you need to directly ask the potential subject to determine eligibility for the study?***
Yes

VII.D.20 ***List any screening questions you will directly ask the potential subject to determine eligibility.***
Eligibility Survey: (see attached pre-consent online survey) This survey will be administered via REDCap link delivered by email. Participants will be invited to complete a survey to determine their eligibility.

If subjects' responses to the online screening survey deem them eligible, they will be contacted by a member of the research team via phone to confirm their answers to the pre-consent online survey, answer additional questions, and schedule a study visit.

VII.D.21 ***Will you keep a screening log or other record that would include information on people who do not enroll in the study?***
Yes

VII.D.22 ***Describe the information being collected and the purpose for keeping this information.***
The following information will be collected in a screening log:
1. Subject's name
2. Age
3. Sex
4. How they heard about study
5. Date that they contacted the study
6. How they contacted us (phone/email)
7. Date of phone screening
8. Phone number
9. Email address
10. Pass online screening; yes or no
11. If did not pass online screening, reason?
12. Pass phone screen? Yes or No
13. If did not pass phone screen, reason?
14. If passed phone screening, date of consent
15. Signed informed consent

VII.D.23 ***Will this information be shared with anyone outside the UI research team members?***
No

VII.D.25 ***After the subject agrees to participate (signs consent), are there any screening procedures, tests, or studies that need to be done to determine if the subject is eligible to continue participating?***
Yes

VII.D.26 ***List and describe screening***
Complete the participant info survey (see attached RedCap document "participant info survey") for study records
Medical history and physical exam
Resting blood pressure and heart rate
Height and weight measurements
Women must complete a urine pregnancy test

VII.D.27 ***Discuss how much time a potential subject will have to agree to consider participation and whether or not they will be able to discuss the study with family/friends before deciding on participation.***
There is no time limit for the subject to agree to consider to be in the study as long as the study is actively recruiting subjects and they are still eligible. Subjects are allowed to discuss the study with family/friends before deciding on participation.

VII.D.28 ***How long after the subject agrees to participate do study procedures begin?***
The procedures in visit 1 (screening visit) can occur on the same day as consent. The experimental visits will begin within 2 weeks or less of visit 1.

VII.D.29 ***Provide a description of the enrollment and consent process for adult subjects***

- ***Describe each study population separately including control population***
- ***Include when recruitment and consent materials are used***
- ***Use 3rd person active voice "The Principal Investigator will identify subjects. For example, the principal investigator will identify potential subjects, the study coordinator will discuss the study with subjects over the telephone and schedule the first study visit, etc..."***
- ***Describe the steps that will be taken by the research team to minimize the possibility of coercion or undue influence during the consent process***

The subjects will consist of young, healthy men and women between the ages of 18-35 years of age. The PI and research staff will recruit subjects from Johnson county and surrounding areas via flyers, table tents, digital signage, emails, The UI mass-email system, newspaper and newswire ads, and posting on the UIHC website for research volunteers. The UIHC Clinical Trials and Research Website will have this research study available as

a listed research study for potential research subjects to find out more information and express interest in participating. Subjects will be asked to contact the research staff via phone or email. Based on how the subject reached out, a study team member will then either 1) contact the potential participant by phone (see pre-consent phone script) or 2) email the participant back with study information (see email correspondence to recruit subjects). If the potential subject is still interested, they will be sent a link to the pre-consent screening survey (see pre-screening survey). If the potential subject DOES NOT qualify based on the pre-screening survey, researchers will notify them and list a reason for ineligibility. If the subject DOES qualify, researchers will reach out by phone or email to schedule visit 1. Subjects will be sent the consent summary by e-mail before visit 1. Visit 1 will include a detailed description of the study and review of the informed consent, including risks. If subjects are not able to understand the protocol and instructions for any reason, written or verbal, they are not included in the study. Subjects are informed throughout the consenting, screening, and conduction of the study that they may discontinue their participation at any time with no penalty to them. If the subject signs the informed consent, they are given a copy of the signed document to take home with them.

VII.D.37 ***Does the study include any form of deception (e.g., providing participants with false information, misleading information, or withholding information about certain study procedures)?***

Examples:

- ***Procedure includes a cover story that provides a plausible but inaccurate account of the purposes of the research.***
- ***Participants will be provided with false information regarding the particular behaviors of interest in the research.***
- ***Procedures include a confederate pretending to be another participant in the study.***
- ***Participants will be told that the research includes completion of a particular task, when in fact, that task will not be administered.***
- ***Study is designed to introduce a new procedure (or task) that participants are not initially told about.***
- ***If yes, a waiver of informed consent must be requested under question IV.3.***

No

VII.E. Project Description (E)

VII.E.1 ***Will subjects be randomized?***
No

VII.E.3 ***Will any questionnaires, surveys, or written assessments be used to obtain data directly from subjects in this study?***
Yes

VII.E.4 ***List all questionnaires, surveys, written assessments and ATTACH each one to the application. (NOTE: You are NOT prohibited from attaching copyrighted materials to this application)***
Participant information survey
Health history questionnaire

VII.E.5 ***Does this project involve creating any audiotapes, videotapes, or photographs?***
No

VII.E.6 ***Provide a detailed description in sequential order of the study procedures following the consent process - DO NOT cut and paste from the Consent Document.***

Describe study populations separately if they will be participating in different procedures - include CONTROL population if applicable.

DESCRIBE:

- ***What subjects will be asked to do/what happens in the study (in sequential order)***
- ***The time period over which procedures will occur***
- ***The time commitment for the subject for individual visits/procedures***
- ***Long-term followup and how it occurs***

Visit 1 - Consent and Screening (approximately 1 hour)
The PI or research staff explain the study and go over the informed consent with the participant. After the participant signs the informed consent, the PI or research staff performs the physical screening that includes urine pregnancy test for women, heart rate (HR), blood pressure (BP), height, and weight measurements. The participant fills out the health history questionnaire and participant info survey.

Visit 2- Experimental Visit (approximately 4 hours)
Note: In the event of an experimental failure (e.g. equipment stops working, building power outage during study visit, etc) subjects may be asked to repeat a trial.

Experimental procedures
Regularly menstruating women and women taking hormonal contraceptives will be tested during the early follicular/placebo phase to minimize variability due to circulating sex-steroid hormones. Participants will be asked to fast 3 hours prior, avoid alcohol and forms of heavy exercise 24 hours prior, avoid caffeine 12 hours prior, and avoid supplements and over-the-counter medications the morning of the experimental visit. Participants should NOT stop taking medications as prescribed by their physician.

Subjects will come to the Pharmaceutical Sciences Research Building (PSRB) and be escorted by a member of the research team to 118 PSRB for the blood draw and experiment (intradermal microdialysis). A blood draw involves a research nurse or trained lab personnel drawing blood (15 ml, ~1 Tablespoon) for substances of interest (e.g. reactive oxygen species) and blood chemistry.

Microdialysis Probe Insertion: (NOTE: The intradermal microdialysis insertion procedure used here is identical to the one approved in IRB #201909818, #202006148, #202009357, #202203433, and #202104188) Please see the attached "intradermal microdialysis" document for general information about intradermal microdialysis, schematic representation of the procedure, and references. The researchers place a tight band around the forearm so they can visualize veins. For each MD site, they make pairs of pen-marks on the arm 2.5 cm (1 inch) apart and away from veins. They remove the tight band. The MD tubing enters and exits the skin at the marks. The researchers clean the arm with povidone iodine and alcohol, and place an ice bag on the site for 5 minutes to numb the skin. Then they 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. They thread the microdialysis tubing through the

needle and then withdraw the needle leaving the tubing in the skin. Any hyperemia related to the insertion subsides in about 60 minutes. When the hyperemia induced by inserting the tubing in the skin subsides, the experiment begins. During this time Lactated Ringer's perfuses the tubing. The researchers tape a fiber optic laser Doppler flowmeter probe and its holder over each microdialysis site. The researchers control the temperature of the holders. The holders start at 33°C (91.4°F). During the experiment, the computerized data acquisition system records heart rate, skin blood flow, and skin temperatures continuously. The researcher and/or an automated critical care monitor measures blood pressure at 5-7 minute intervals.

Local Heating Protocol: The intradermal microdialysis probes are randomly numbered 1 or 2 and assigned to receive doses of Lactated Ringer's (probe 1) or Losartan (probe 2).

Probe 1. Lactated Ringer's only
Probe 2. Lactated Ringer's + Losartan

Initially, Lactated Ringer's flows through all probes. Then the investigational agents are added to the Lactated Ringer's flowing through MD probe 2 for 60 minutes. When the skin blood flow is stable, we perform a 20-minute baseline and set of measurements, including placement of 3 ECG electrodes for the collection of heart rate. After we have collected baseline data, we begin the dose-response protocol. We add identical concentrations of C21 to the perfusate in probes 1 and 2. At 10-minute intervals, we increase the concentration of C21 in the perfusates in identical fashion in probes 1 and 2. The participant receives 10 different concentrations of C21. After perfusing the last concentrations, all MD sites are then warmed to 43°C (108°F) to perform Maximal Skin Blood Flow. At the same time, the researchers switch the fluids at all sites to Ringer's + SNP for about 30 minutes. This causes the blood vessels at the MD sites to dilate or enlarge as much as they can. The researchers then de-instrument the subject, remove the microdialysis fibers, and apply sterile dressings. The subject is given verbal and written instructions for how to care for the microdialysis sites. The researcher measures final vitals before the subject departs.

VII.E.7 ***Will you attempt to recontact subjects who are lost to follow-up?***
No - followup is not required in this study

VII.E.9 ***Will subjects be provided any compensation for participating in this study?***
Yes

VII.E.10 ***Cash***
No

VII.E.11 ***Gift Card***
No

VII.E.12 ***Check***
Yes

VII.E.13 ***Who will be providing the research compensation check to the subject?***
Accounting Services directly via the e-Voucher system

VII.E.16 ***Other***
Yes

VII.E.17 ***Describe:***
Parking vouchers will be provided during study visits.

VII.E.18 ***If you plan to compensate subjects using cash, checks or cash equivalent does your unit have a Cash Handling Procedure in place that has been approved by Accounting Services?***
Yes

VII.E.19 ***Describe the compensation plan including***

- ***Compensation amount and type per visit***
- ***Total compensation***
- ***Pro-rating for early withdrawal from study***

Experimental visits:
$15/microdialysis probe
$7.50/hour for completing the experiment

Total for completing the study: $60 (one completed study visit)
Parking pass for time involved at study and screening visits.

Pro-rating: Subjects can receive payment for pre-treatment and experiments not completed. The researchers pay an amount of money equal to the part completed. For instance, if a subject completes half of Experiment 1, the subject receives $15.00 for each probe inserted plus $7.50 for each hour they completed. The researchers may ask subjects to repeat a trial. If subjects agree to repeat a trial, they receive payment for the repeated trial as stated above.

VIII. Risks

VIII.1 ***What are the risks to subjects including***
- emotional or psychological
- financial
- legal or social
- physical?
Physical Risks:
Microdialysis: Intradermal microdialysis is a specialized research technique. Dr. Stanhewicz is highly skilled in this technique and has been using the procedures in this protocol (including fiber placement, perfusate preparation, subject monitoring, and fiber removal) for over 10 years with no

adverse events and have been reviewed and approved by Dr. Diana Jalal at the University of Iowa.

Cutaneous 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. Participants may feel faint with probe placement or removal. As with any event that breaks the skin, infection is possible. However, no participants in any of the researchers' prior experiments have reported infection. In the unlikely event in which the membrane breaks during removal leaving an isolated piece of membrane under the skin, they treat the piece of membrane in a manner similar to that for a splinter in the skin. In this case, participants will be referred to UIHC for care where a small incision may be made to remove the piece of membrane in the forearm. Such an event has never occurred in any projects overseen by Dr. Stanhewicz.

Microdialysis delivers small amounts of pharmacological substances to a nickel-sized area of the skin. Therefore there are no individual risks associated with the drugs perfused through the fibers (Losartan, C21, SNP, lactated Ringer's) at the concentrations applied in the microdialysis procedure. The small quantities used and the 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 small given the small amount delivered to a very small area of skin, the lack of adverse reactions to similar amounts delivered via MD 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.

Local Heating: The local heating control unit (Moor Instruments) precisely controls and monitors the temperature of the heated probe holders used with the Laser Doppler Flowmeter. The system has programmed maximum temperature limits. To determine the maximal SkBF, the researchers increase the temperature of the heating units slowly (about 0.1°C every 1 second). The skin feels very warm but not painful. Local heating causes temporary redness of the skin that subsides within several hours. This technique is very unlikely to produce long-term ill effects.

Povidone Iodine: Hospitals and researchers use povidone iodine to clean and sterilize the skin. Participants could be allergic to iodine. An allergic reaction could cause redness, itching, rash, and/or swelling. Staff use only alcohol on participants with iodine allergy as identified during screening.

Tape and adhesive disks: Participants could be sensitive to the adhesive of the tape, ECG electrodes, and double-sided adhesive disks used in the study causing redness, rash, tenderness, and/or itching. The researchers remove these items carefully.

Blood draws: Potential risks associated with obtaining blood samples are minimal but include slight bruising, pain, a temporary feeling of faintness, and/or a small risk of infection. All blood draws will be performed by a research nurse or team member trained in drawing blood.

Emotional or Psychological risks: There are no foreseeable psychological risks with this study.

Social Risks: There are no foreseeable social risks with this study

Legal Risks: There are no foreseeable legal risks with this study.

Loss of confidentiality is possible.

VIII.2 ***What have you done to minimize the risks?***

- ***If applicable to this study ALSO include:***
  - ***How you (members of your research team at Iowa) will monitor the safety of individual subjects.***
  - ***Include a description of the availability of medical or psychological resources that subjects might require as a consequence of participating in this research and how referral will occur if necessary (e.g. availability of emergency medical care, psychological counseling, etc.)***

Risks Associated with Intradermal Microdialysis: Research techniques are only performed by personnel who are trained and approved by Dr. Stanhewicz to complete these procedures. Dr. Stanhewicz has 10 years of experience utilizing intradermal microdialysis without unanticipated adverse events. All laboratory personnel are trained in CPR and basic first aid. In the event of a life threatening emergency, lab personnel call 911. The PSRB is equipped with AED and lab personnel are trained in how to use them if necessary. Prior to placing the microdialysis fibers, the researcher puts on sterile gloves and cleans the skin with iodine and alcohol to reduce the risk of infection. The researchers apply ice to the skin for 5 minutes before placing the microdialysis fibers to reduce any pain associated with placement. Once the fibers are in place, research personnel constantly monitor subjects for adverse reactions (e.g. pain, itching, redness, swelling) to the microdialysis fibers or the perfusates. Participants are never left unsupervised with the fibers in place. An automated monitor measures blood pressure every 5 minutes and constantly records heart rate. The researchers clip the ends of the fibers and clean them with an alcohol swab before removing them from the skin to reduce the risk of infection. They stop any bleeding by applying mild pressure to the sites with a sterile gauze pad. The researchers apply a clean, sterile bandage to the area before the participant leaves. Participants are given verbal and written instructions on how to care for the sites and to call Dr. Stanhewicz or the research nurse if they have any questions or concerns about the sites after they have left the study visit.

Risks associate with blood draw: Only appropriate trained laboratory personnel perform the blood draw procedure. A sterile gauze pad and pressure wrap (latex free coban) are applied to the site after the blood draw to stop any bleeding and minimize bruising.

Risk of loss of confidentiality: The investigators collect the minimum amount of confidential data in order to complete the aims. All subjects are assigned a code and data and specimens are collected using the code only. The only time subject names and codes appear together is on their file which is kept in a locked cabinet in the PI's office. Only IRB approved personnel have access to these files. All data files are stored on password protected computers and servers and only IRB approved personnel have access. Extensive details on how the risk of loss of confidentiality is minimized are provided in section X. Privacy and Confidentiality.

Laboratory Space: The Microvascular Physiology Lab (118 PSRB) is a designated laboratory space on the University of Iowa campus. It is fully compliant with Environmental Health and Safety standards and undergoes annual inspection. The lab space is equipped with state of the art equipment to perform the proposed research procedures. All equipment is certified for human use and checked and calibrated before each experiment. The lab is equipped with a first aid kit and all lab personnel are trained in CPR and basic life support. An AED is located in the hallway just outside the lab space.

VIII.3 ***Does this study have a plan to have an individual or committee review combined data from all subjects on a periodic basis (such as summary or aggregate safety and/or efficacy data)?***
No

IX. Benefits

IX.1 ***What are the direct benefits to the subject (do not include compensation or hypothesized results)?***
The study procedures and/or findings do not provide direct benefits to the subjects participating in the study. Subjects receive a medical screening that could inform them about their health, including resting heart rate and blood pressure measurements. We can provide subjects with their heart rate and blood pressure values if they ask, but we will not advise them on any unexpected findings.

IX.2 ***What are the potential benefits to society in terms of knowledge to be gained as a result of this project?***
Pre-menopausal women have significantly lower incidence of cardiovascular disease (CVD) than age-matched men due to the protective benefits of estrogen. Estrogen decreases levels of Angiotensin II, allowing less Angiotensin II to act on its receptors: AT1Rs (cause constriction) and AT2Rs (cause dilation). Excess Angiotensin II disrupts the balance between these two receptors in the body acts mainly through AT1Rs, leading to higher risk of CVD and related diseases. Because estrogen downregulates Angiotensin II, there is a balance between activation of AT1Rs and AT2Rs. In young males, this balance can be dysregulated when excess Angiotensin II is produced. A new pharmacological agent, known as Compound 21, activates AT2Rs to cause vasodilation. Besides vasodilation, activation of AT2Rs with Compound 21 also elicits anti-inflammatory and anti-fibrotic effects in animal models. In humans, this compound has been shown to cause dilation in the lungs and the peripheral vessels (e.g., skin) when given orally. Thus, the purpose of this study is to determine the extent that dilation with AT2R activation differs in young men and women. As young women have more protection from heart diseases than age-matched young males, we propose that activation of AT2Rs will be more sensitive in young women than in young men. This will help us better understand why women have enhanced cardioprotection prior to menopause.

X. Privacy & Confidentiality

X.1 ***What are you doing to protect the privacy interests of the subjects?***
The minimum amount of data necessary to complete the aims will be collected during the study. Data are collected about the subject's normal daily activities (physical activity, nutrition, etc.) as these factors all can influence a person's vascular function. Differences in our data may be explained by these variables, which we can then confirm by looking back at their health history questionnaire when discrepancies arise. The informed consent process will be conducted in a private exam room in the private lab (118 PSRB) with the door closed. All screening and experimental procedures will be conducted in the laboratory in 118 PSRB with the door closed and locked. Only personnel directly involved in the study will be allowed in the rooms.

X.2 ***Are you collecting the Social Security Number of any subjects for any purpose?***
No

X.4 ***How will information/data be collected and stored for this study (check all that apply):***

- Electronic records (computer files, electronic databases, etc.) - Data will be entered using subject ID code into the ICTS REDCap web-based database application that is password protected. REDCap questionnaires will be filled out on the participants electronic device (e.g. phone, tablet, personal computer) or a printed paper copy if they prefer. No personal identifiable data will be entered. Only research staff on the IRB approved study will be allowed access this database. The ICTS REDCap staff are responsible for maintaining security of the data. Some data using subject ID code will also be entered into a Microsoft Excel and SPSS datasheets that will be kept in a shared server for CLAS that is password protected. Only research staff on the IRB approved study will have access to the folder the study on the server.
  - Name - [redacted]
  - Title - IT Support Consultant
  - University Job Classification - Faculty/Staff
- Biologic samples (blood draws, check swabs, saliva samples, tissue samples, etc.) - Blood samples will be sent to the UIHC pathology lab for analyses. Remaining biological specimens, such as blood, will be labeled with subject code, date collected and IRB protocol number and transported to the PIs laboratory (118 PSRB) in a secure unbreakable biohazard container. Samples will be stored in the PIs laboratory in a -80C freezer in 111 PSRB. All samples will be labeled with date collected and subject ID code only. No personal identifiable information will be labeled on the sample. Only the PI and her research staff will have access to the samples.
  - Name - Anna Reid-Stanhewicz
  - Title - Assistant Professor
  - University Job Classification - Faculty/Staff
- Paper/hard copy records (hard copy surveys, questionnaires, case report forms, pictures, etc.) - Most of the data are coded and do not contain personal identifying information. Some data will temporarily contain the subject's name, address, and/or telephone number (Phone Interview Form). Documents allowing identification of participants do not leave our labs and are only available to authorized persons. Only authorized personnel may access the lab computer. Data forms containing identifiable information are shredded when no longer needed (within 5 years after publication of results). We keep data in the laboratory in locked cabinets, the password-protected folder on the secured University of Iowa RDSS server, and on password-protected computers maintained in locked laboratory rooms. Only authorized personnel have access. Coded data shared with unauthorized persons cannot be traced to individuals. The list linking code numbers to participants is not shared with unauthorized persons and destroyed when the project is completed and within 5 years of publication of the data. Screening data from subjects who are not accepted into the study are shredded when the project ends. 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 we complete the study, we remove all identifiers from the data and store the data indefinitely. Individual data may be used without identifying the subject to illustrate representative responses. Any hard paper copy of subject lists or data will be kept in a locked cabinet in the locked office of the PI. Subjects will be assigned a code for the study. Files will be labeled with this code for confidentiality. Study data including laser-Doppler flowmetry data will be stored on password protected computer hard-drives and password protected server folders which are only accessible to research team members via password. Subject confidentiality will be maintained in all presentations and publications and information/records pertaining to subject participation in the research project will not be released without prior authorization by the subjects.

X.5 ***Do the confidentiality protections indicated above allow only members of the research team to access the data/specimens?***
Yes

X.7 ***Does your study meet the NIH criteria for a Certificate of Confidentiality or will you be applying for Certificate of Confidentiality?***
No

XI. Data Analysis

XI.1 ***Describe the analysis methods you will use, including, if applicable, the variables you will analyze***

The primary outcome variable will be cutaneous vascular conductance, which is calculated from laser-Doppler flux/mean arterial pressure. Three-way repeated measures ANOVA (group, MD site, dose) will be used to examine group differences and local microdialysis treatment differences across the doses of pharmacological stimuli. Appropriate post-hoc analyses with corrections for multiple comparisons will be performed when main effects are identified, including potential confounding variables as covariates. Paired t-tests will be used to examine group differences in subject characteristics.

XI.2 ***Provide the rationale or power analysis to support the number of subjects proposed to complete this study.***
Using previously published data that determined differences in cutaneous vascular conductance (CVC), an index of skin blood vessel dilation, the results of a power analysis indicated that 24 young men and women (12 subjects x 2 groups) will be sufficient to detect a meaningful physiological difference (power=0.80, a=0.05) of a ~10% change in CVC from baseline between microdialysis treatment sites and sex differences (men and women). Based on the available literature and on prior experience with microdialysis studies, the standard deviation of the difference is ~10%. After accounting for attrition, withdrawals, and screen failures, we conservatively estimate that 44 people will need to be consented and screened in order to arrive at this target number of 24.

XII. Future Research

XII.1 ***Do you wish to keep any information about subjects involved with this research project so that members of the current research team may contact them in the future for your own research projects?***
Yes

XII.2 ***Do you wish to keep any information about subjects involved with this research project so that other researchers may contact them for future research?***
No

XII.3 ***List the data or information you will keep:***
Name
Race/ethnicity
Birthdate
Sex
Contact information (mailing address, e-mail address, phone number)

XII.4 ***Does this project involve storing any data, tissues or specimens for future research?***
Yes – contribution for future use is optional

XII.5 ***Describe how you will keep track of those who consent to future use and those who do not and how you will prevent future use for those who do not consent.***
We will keep track of who consents to future use on our enrollment log.