Vascular Dysfunction in Hypertensive Postmenopausal Women

Registered Clinical Trial # NCT03371823

IRB Protocol Approved February 13, 2019

HUMAN SUBJECTS PROTOCOL
University of Delaware

Protocol Title: Losartan and ET-1 mediated constriction in postmenopausal women with high blood pressure

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Other Investigators: Michael Stillabower, MD.

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: COBRE (NIH)

2. Research Site(s)

X University of Delaware

☐ Other (please list external study sites)

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

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

No

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

Hypertension is major health problem for postmenopausal women. Hypertension is more likely to be uncontrolled in women, despite medication use. Medications to block the angiotensin II pathway are commonly used to treat hypertension. However, recent data in animals suggest that multiple pathways are involved in blood pressure control in females. Specifically, blocking the angiotensin II pathway along with the endothelin-1 pathway resulted in a larger drop in blood pressure in these animals compared to single drug therapy. These blood pressure lowering effects observed in the female animals may be related to the interactions between angiotensin II and endothelin-1. Both angiotensin II and endothelin-1 are potent vasoconstrictors that can cause an increase in blood pressure, and these substances interact to cause additive effects on constricting blood vessels. The aims of this study are to examine how angiotensin II and endothelin-1 interact to regulate blood vessel function in hypertensive postmenopausal women. To do this, we will recruit women with elevated blood pressure not taking blood pressure medications, and test their blood vessel function on 2 separate visits: 1) baseline, and 2) after 14-days of losartan (an angiotensin II receptor blocker). Blood vessel function will be assessed using a technique called microdialysis, which involves placing tiny sterile plastic tubes (microdialysis probes) in the surface layer of the skin on the forearm, and blood flow will be measured using a small disk with a laser. We will also recruit normotensive women to complete 2 visits to assess blood vessel function pre/post 14 days as a control (no losartan administration).

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

We will recruit postmenopausal women with high BP ($\geq$130 and/or 80 mmHg) and normotensive postmenopausal women (ages 50-70 years). Women with high BP will complete 5 visits (screening/orientation, VO_2max test, Blood Vessel Assessment 1, Blood draw to check electrolytes and kidney function, and Blood Vessel Assessment 2), and normotensive women will complete 4 visits (screening/orientation, VO_2max test, Blood Vessel Assessment 1, and Blood Vessel Assessment 2).

<u>Procedures in Women with high BP (5 visits):</u>

Visit 1: Screening/orientation: The first visit is about 1 hour long and includes

1). Completion of informed consent, medical history, and menstrual cycle history
2). Measures of height and weight,
3). Measures of resting blood pressure and 12-lead electrocardiogram
4). Blood Draw

Participants will be fasted from food, caffeine, and alcohol (12 hours) and exercise (24 hours). Informed consent will be obtained by Megan Wenner, study PI, Joshua Hobson, Lab/Study Coordinator, or doctoral student trained by Dr. Wenner. Subjects will also be familiarized with the laboratory and experimental procedures. The blood draw and electrocardiogram will be done by a trained healthcare professional at the Nurse Managed Primary Care Center (NMPCC). Blood will be analyzed for cholesterol, electrolytes, kidney and liver function, c-reactive protein, and blood glucose (sugar). All screening results will be reviewed by a Nurse Practitioner at the NMPCC. In the event that the test results are abnormal, the NMPCC will refer the subject to their physician for follow-up and they will not be able to participate in the research study. Participants will be provided a HIPAA authorization form that includes permission for sharing of patient protected health information with the research team. Women will also be given a 24 hour ambulatory blood pressure monitor, and instruction on how to wear and operate the monitor. They will don the monitor at the completion of Visit 1. The blood pressure monitor measures BP every 20 minutes during the day, and every 30 minutes at night. Women will return the monitor the next day. When they return the monitor, we will ask that they be seated for 5-10 minutes, and we will take 3 resting blood pressure measures using an automated brachial blood pressure cuff. At the end of this visit we will give participants a standard blood pressure monitor to take home (Omron). This device is often used for adults to monitor blood pressure at home. The device takes 3 blood pressures and reports the average of these readings on the device. Women will be instructed to take seated resting blood pressures at home 3 times each week for 2 weeks. Blood pressures should be taken in the morning, at lunchtime, and again in the evening. Instructions will be provided along with a log to record the readings and time of day. Participants will return the BP monitor and log when they return to the lab prior to Visit 2.

Two to three days prior to Visit 2, women will be given a 24 hour ambulatory blood pressure monitor, and instruction on how to wear and operate the monitor. They will don the monitor the day before Visit 2. The blood pressure monitor measures BP every 20 minutes during the day, and every 30 minutes at night. Women will return the monitor the third visit.

Visit 2: Blood Vessel Assessment 1: The second visit lasts approximately 5 hours. Measurements will be conducted in the Cardiovascular Core Lab at STAR Health Sciences Complex. Subjects will be asked not to drink alcohol and caffeine for 12 hours, food for 4 hours, and refrain from exercise for 24 hours prior to this visit. Subjects will be asked to wear a short-sleeved shirt. If they do not bring one, we will provide one for them.

Endothelial Function: An 18 gauge catheter will be placed in an antecubital vein by a registered nurse or nurse practitioner. We will collect blood samples for the measurement of electrolytes, serum creatinine, estradiol, progesterone, testosterone, follicle stimulating hormone, angiotensin II, aldosterone, renin, norepinephrine, and endothelin-1 (90 mL). The blood samples will be stored in a freezer in the Physiology Core Lab until the time of analysis. After the samples are analyzed they will be destroyed at UD. The tubes will be labeled with subject numbers; no names will appear on the tubes. In addition we will collect some cells from the wall of the vein. Two sterile J-wires are inserted through the IV catheter and gently moved back and forth inside the vessel. One wire is used at a time. The wires are then transferred to a 50mL conical tube with endothelial dissociation buffer. The cells are rinsed and recovered by centrifugation. The cells are then fixed to coverslips, and stored in the freezer to be stained and analyzed for protein expression of ET-1 and ET-B receptors in batches at a later date. Large blood vessel function will then be assessed using two non-invasive techniques. First, a pressure probe is placed over the radial artery to record pressure waveforms. Then, the pressure probe will be placed over the carotid and femoral arteries to record pressure waveforms. A cloth tape will measure the distance between the carotid and femoral sites. Second, an ultrasound will be used to image the brachial artery. A small blood pressure cuff is placed just distal to the olecranon process. This cuff will be inflated for 5 minutes. Images of the brachial artery are collected continuously before, during, and for 3 minutes after cuff deflation. Changes in brachial artery diameter are used as an index of endothelial function.

Microdialysis placement: Four microdialysis fibers (Bioanalytical Systems) with a membrane length of 10mm and a 20-kDa cutoff will be inserted on the dorsal side of the forearm. Microdialysis fibers will be placed by Megan Wenner, PhD or doctoral student trained by Dr. Wenner. Under sterile conditions the fibers are placed in the intradermal space with a 23-gauge needle used as a guide cannula. Ice is used on the site locally to numb the skin, reduce blood flow, and minimize release of vasoactive substances. The entrance and exit site on the skin are separated by at least 2 cm. The guide cannula is inserted horizontally in the dermis and the microdialysis fiber is fed through the guide cannula. The needle is removed, and the fiber left in place at a depth of ≈ 2-5 mm. After placement, the fiber is perfused with lactated Ringers at a rate of 2 µl/min using a syringe pump (Bioanalytical Systems, Inc.) for 90 minutes. This recovery period (after fiber insertion) is required to allow local skin blood flow (SkBF) to return to baseline before data measurements can be made. During the recovery period, the subject will be instrumented for the measurement of heart rate (ECG) and blood pressure.

ET-1 receptor antagonists: ET-A and ET-B receptor antagonists (BQ-123 and BQ-788, respectively, Sigma) will be perfused via intradermal microdialysis fibers while measuring skin blood flow (SkBF) at rest and during local heating. The antagonists will be dissolved in lactated Ringers solution and filtered using syringe microfilters (Whatman Puradisc 13 mm Syringe Filters, Florham Park, NJ). Following recovery from the microdialysis fiber placement, we will measure resting SkBF using laser Doppler flowmetry (LDF) for 15 min at all sites with the local heating devices off. Laser Doppler probes (MoorLAB, Temperature Monitor SH02, Moor Instruments, Devon, UK) which both measure and control local skin temperature, will be placed on the surface of the skin on the dorsal forearm over the microdialysis site. The laser Doppler probes will be maintained at 33°C during baseline and ET receptor antagonist infusion. Microdialysis fibers will then be perfused at 5.0 µl/min for 45 minutes with the following drugs: the first fiber will be perfused with lactated Ringers; the second fiber will be perfused with 500 nM of the ET-A receptor antagonist (BQ-123) [1]; the third fiber will be perfused with 300 nM of ET-B receptor antagonist (BQ-788) [1]; the fourth fiber will be perfused with combined ET-A and ET-B receptor subtype antagonists (i.e. BQ-123 and BQ-788; 500 and 300 nM respectively). After perfusing the blocking agents, all four local heating devices will be raised until the skin temperature under the LDF probes reaches a stable temperature of 42°C [2]. The SkBF response will be measured at each microdialysis site until a stable plateau is reached after heating. The microdialysis perfusions of antagonists will continue throughout the local heating response to maintain ET-A and ET-B receptor inhibition. Each series of perfusions will be followed by a perfusion of sodium nitroprusside (SNP, 28 mM) through the microdialysis fiber combined with local heating to 43°C to determine maximal SkBF. Cutaneous vascular conductance (CVC) will be calculated as mean SkBF/mean arterial pressure. We will collect blood samples for the measurement of estradiol, progesterone, testosterone, and endothelin-1at the end of the recovery period just prior to beginning the perfusions, and again prior to maximal heating.

Perfusion of the ET-A and ET-B receptor antagonists, BQ-123 and BQ-788, either in arteries or skin vessels in humans, is an acceptable and safe experimental technique. Included is a list of the published research studies utilizing this technique:

- [1]Wenner MM et al. Endothelin B receptor contribution to peripheral microvascular function in women with polycystic ovary syndrome. JPHYS 2011.
- Kellogg DL et al. Gender differences in the endothelin-B receptor contribution to basal cutaneous vascular tone in humans. J Appl Phys 2001.
- Stauffer BL et al. Sex differences in endothelin-1-mediated vasoconstrictor tone in middle-aged and older adults. Am J Physiol Regul Integr Comp Physiol. 2010.

Losartan administration: At the completion of visit 3, women with high BP will be given 7 days of losartan (50mg), and be instructed to take the study medication once per day (at nighttime before bed). Losartan is a medication given to treat hypertension. Losartan administration will be overseen by Dr. Michael Stillabower.

Visit 3: Blood draw. This visit will occur one week after starting losartan. This visit will last ~20 minutes. Participants will report to the Physiology Core Laboratory at the STAR Health Sciences complex. Participants will be seated, and 10mL of blood will be drawn from a vein. Serum electrolytes and creatinine will be assessed to ensure using losartan in not having adverse effects on kidney function or electrolytes. If serum potassium is >5.2 mmol/L or creatinine is >50% over baseline values, the PI will contact the study physician Dr. Stillabower, and he will determine if the medication will be discontinued and the participant be removed from the study. Elevated levels

typically resolve on their own upon cessation of drug therapy. Rarely, acute renal injury or cardiac arrhythmia's may develop. Additional medical follow-up may be instructed by Dr. Stillabower, determined on a case-by-case basis (depending on individual responses to the medication), either to the emergency room or to the PCP. They will also be weighed. Women will be given another 7 days of losartan (50mg) and instructed to take the medication once per day (at nighttime before bed). Women will also be given a 24 hour ambulatory blood pressure monitor, and instruction on how to wear and operate the monitor. They will don the monitor the day before Visit 4 The blood pressure monitor measures BP every 20 minutes during the day, and every 30 minutes at night. Women will return the monitor the fourth visit.

Visit 4: Blood Vessel Assessment 2: The fifth visit is will occur 14 days after beginning losartan. This visit is approximately 5 hours long, and is identical to Visit 2 (Blood Vessel Assessment 1).

Visit 5: VO_2max Testing. This visit will last ~1 hour. Participants will be fasted from alcohol and caffeine for 12 hours, food for 4 hours, and exercise for 24 hours prior to testing. Exercise tests will be conducted in the stress testing laboratory in the NMPCC and overseen by a Certified Exercise Physiologist and Nurse Practicioner after clearance from the study physician, Dr. Stillabower. A resting 12-lead ECG and blood pressure will be taken in the supine and standing position. If resting BP is $\geq$200/110 mmHg, the test will not be performed. A standard ramp treadmill protocol will be used, consisting of 1.5-minute stages where the speed and grade is increased incrementally. Expired gases will be collected and analyzed using the Parvo Medics Metabolic Cart. Exercise BP, HR, and ECG will be taken throughout, along with ratings of perceived exertion. This is a symptom-limited metabolic stress test; test termination criteria are based on ACSM guidelines (RER $\geq$1.10, plateau in HR or VO2 despite increased workload), and absolute indications for test termination such as chest pain, ECG abnormalities or arrhythmias, BP >250/115 mmHg, or participants request to stop. Dr. Stillabower will review the exercise test results. The NMPCC will send the test results to be read and interpreted by their cardiologist, Dr. Goldenberg. In the event of a positive test or if additional follow up is needed, the NMPCC will contact the participant's primary care doctor.

Procedures in Normotensive Women (4 visits):

Visit 1: Screening/orientation: The first visit is about 1 hour long and includes

1). Completion of informed consent, medical history, and menstrual cycle history
2). Measures of height and weight,
3). Measures of resting blood pressure and 12-lead electrocardiogram
4). Blood Draw

Participants will be fasted from food, caffeine, and alcohol (12 hours) and exercise (24 hours). Informed consent will be obtained by Megan Wenner, study PI, or doctoral student trained by Dr. Wenner. Subjects will also be familiarized with the laboratory and experimental procedures. The blood draw and electrocardiogram will be done by a trained healthcare professional at the Nurse Managed Primary Care Center (NMPCC). Blood will be analyzed for cholesterol, c-reactive protein, electrolytes, kidney and liver function, and blood glucose (sugar). All screening results will be reviewed by a Nurse Practitioner at the NMPCC. In the event that the test results are abnormal, the NMPCC will refer the subject to their physician for follow-up and they will not be able to participate in the research study. Participants will be provided a HIPAA authorization form that includes permission for sharing of patient protected health information with the research team. Women will also be given a 24 hour ambulatory blood pressure monitor, and instruction on how to wear and operate the monitor. They will don the monitor at the completion of Visit 1. The blood pressure monitor measures BP every 20 minutes during the day, and every 30 minutes at night. Women will return the monitor the next day. When they return the monitor, we will ask that they be seated for 5-10 minutes, and we will take 3 resting blood pressure measures using an automated brachial blood pressure cuff. At the end of this visit we will give participants a standard blood pressure monitor to take home (Omron). This device is often used for adults to monitor blood pressure at home. The device takes 3 blood pressures and reports the average of these readings on the device. Women will be instructed to take seated resting blood pressures at home 3 times each week for 2 weeks. Blood pressures should be taken in the morning, at lunchtime, and again in the evening. Instructions will be provided along with a log to record the readings and time of day. Participants will return the BP monitor and log when they return to the lab prior to Visit 2.

Two to three days prior to Visit 2, women will be given a 24 hour ambulatory blood pressure monitor, and instruction on how to wear and operate the monitor. They will don the monitor the day before Visit 2. The blood pressure monitor measures BP every 20 minutes during the day, and every 30 minutes at night. Women will return the monitor the third visit.

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Endothelial Function: An 18 gauge catheter will be placed in an antecubital vein by a registered nurse or nurse practitioner. We will collect blood samples for the measurement of electrolytes, serum creatinine, estradiol, progesterone, testosterone, follicle stimulating hormone, angiotensin II, aldosterone, renin, norepinephrine, and endothelin-1 (90 mL). The blood samples will be stored in a freezer in the Physiology Core Lab until the time of analysis. After the samples are analyzed they will be destroyed at UD. The tubes will be labeled with subject numbers; no names will appear on the tubes. In addition we will collect some cells from the wall of the vein. Two sterile J-wires are inserted through the IV catheter and gently moved back and forth inside the vessel. One wire is used at a time. The wires are then transferred to a 50mL conical tube with endothelial dissociation buffer. The cells are rinsed and recovered by centrifugation. The cells are then fixed to coverslips, and stored in the freezer to be stained and analyzed for protein expression of ET-1 and ET-B receptors in batches at a later date. Large blood vessel function will then be assessed using two non-invasive techniques. First, a pressure probe is placed over the radial artery to record pressure waveforms. Then, the pressure probe will be placed over the carotid and femoral arteries to record pressure waveforms. A cloth tape will measure the distance between the carotid and femoral sites. Second, an ultrasound will be used to image the brachial artery. A small blood pressure cuff is placed just distal to the olecranon process. This cuff will be inflated for 5 minutes. Images of the brachial artery are collected continuously before, during, and for 3 minutes after cuff deflation. Changes in brachial artery diameter are used as an index of endothelial function.

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microdialysis perfusions of antagonists will continue throughout the local heating response to maintain ET-A and ET-B receptor inhibition. Each series of perfusions will be followed by a perfusion of sodium nitroprusside (SNP, 28 mM) through the microdialysis fiber combined with local heating to 43°C to determine maximal SkBF. Cutaneous vascular conductance (CVC) will be calculated as mean SkBF/mean arterial pressure. We will collect blood samples for the measurement of estradiol, progesterone, testosterone, and endothelin-1at the end of the recovery period just prior to beginning the perfusions, and again prior to maximal heating.

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At the completion of Visit 2, women will be given a 24 hour ambulatory blood pressure monitor, and instruction on how to wear and operate the monitor. They will don the monitor the day before Visit 3. The blood pressure monitor measures BP every 20 minutes during the day, and every 30 minutes at night. Women will return the monitor the third visit.

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Visit 4: VO_2max Testing. This visit will last ~1 hour. Participants will be fasted from alcohol and caffeine for 12 hours, food for 4 hours, and exercise for 24 hours prior to testing. Exercise tests will be conducted in the stress testing laboratory in the NMPCC and overseen by a Certified Exercise Physiologist and Nurse Practitioner. A resting 12-lead ECG and blood pressure will be taken in the supine and standing position. If resting BP is $\geq$200/110 mmHg, the test will not be performed. A standard ramp treadmill protocol will be used, consisting of 1.5-minute stages where the speed and grade is increased incrementally. Expired gases will be collected and analyzed using the Parvo Medics Metabolic Cart. Exercise BP, HR, and ECG will be taken throughout, along with ratings of perceived exertion. This is a symptom-limited metabolic stress test; test termination criteria are based on ACSM guidelines (RER $\geq$1.10, plateau in HR or VO2 despite increased workload), and absolute indications for test termination such as chest pain, ECG abnormalities or arrhythmias, BP >250/115 mmHg, or participants request to stop. The NMPCC will send the test results to be read and interpreted by their cardiologist, Dr. Goldenberg. In the event of a positive test or if additional follow up is needed, the NMPCC will contact the participant's primary care doctor.

7. **STUDY POPULATION AND RECRUITMENT**

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

Normotensive and postmenopausal women and women with high BP (ages 50-70 years) will be recruited. A total of 60 women will be recruited (30 normotensive and 30 high BP). Subjects will be recruited from campus and local community- posting flyers and advertisement in local newspapers, in physicians' offices, community centers or events, through professional societies, or online (UD classifieds, website). In addition, we will conduct blood pressure screenings in the community.

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

Recruitment flyers are attached.

Describe what exclusionary criteria, if any will be applied.

Women will be excluded from the study if they have a history of any heart disease, kidney or liver disease, history of blood clots, pulmonary embolism, deep vein thrombosis, stroke, cancer, diabetes, or any other chronic disease. Women will also be excluded from the study if they use tobacco products (including cigarettes and chewing tobacco), are using hormone therapy, are pregnant, or if their body mass index is greater than 35. Women will be excluded if resting blood pressure is greater than 180/100 mmHg, or less than 100/60 mmHg at the initial screening.

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

We are assessing serum electrolytes, creatinine and blood pressure at baseline and after 1 week of losartan administration. If serum electrolytes (potassium >5.2 mmol/L), creatinine levels (>50% over baseline values) rise too high, or blood pressure (less than 100/60 mmHg) drops too low, the study physician Dr. Stillabower will be contacted to determine if the subject will be instructed to stop taking the medication and will be excluded from the study.

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).

There are no known risks associated with obtaining height, weight, resting electrocardiogram, and resting blood pressure. The 24-hour blood pressure monitor may interrupt sleep in some participants.

Microdialysis: Subjects will probably experience some pain and bruising like that from a blood draw. However, we will use ice to numb the skin during insertion of the fibers. Also, the small needle size (23 gauge) reduces pain during placement of the fibers. Subjects will probably not have pain after the fiber is in place but may feel a little pain when the fiber is removed from the skin. Similar to a blood draw subjects may become lightheaded or faint. Mild pressure with sterile gauze would be applied to stop any slight bleeding that may occur. Infection is possible. Sterile techniques and supplies are used to minimize risk. We will apply a sterile bandage after the experiment.

Substances infused through microdialysis fibers: The amount of substance that enters the skin is very small. However, there is a chance of having an adverse reaction to the substances. This reaction could produce redness, itching, rash, and/or swelling. Although unlikely, it is possible that a more serious reaction could also cause fever, breathing problems, changes in heart rate, convulsions, and/or collapse. If a serious reaction should occur, emergency medical help will be summoned right away.

Lactated Ringer's solution: This fluid contains salt, potassium, lactate, and chloride. The acid content is similar to the body's fluids. A bad reaction to this fluid is highly unlikely.

BQ-123, BQ-788, and SNP: Only minute amounts of these substances enter the nickel-sized area of skin around the tubing. Dr. Wenner has previously used BQ-123, BQ-788, and SNP infusions in the skin without adverse reactions[1,2]. Additionally, these drugs have been used in the skin by other researchers without adverse reactions[3].

Laser Doppler: Lasers can damage the eye if pointed into the eye for a prolonged period of time. We will not turn the laser on until it is taped to the skin surface and will turn it off before removing it.

Local Heating: We measure the temperature of the skin under the holders. The skin will feel very warm but will not hurt. The heating will make the skin under the holders red similar to taking a hot bath. The redness will not last more than several hours. Some people may be more sensitive to the heating than others. If a subject's arm feels too hot, we will reduce or stop the heating.

Losartan: Losartan is a drug commonly used to treat high blood pressure. Common side effects may include cold or flu symptoms (stuffy/runny nose, sneezing, sore throat, fever), dry cough, muscle cramps, pain in the legs or back, stomach pain, nausea, or diarrhea, headache, hypotension, dizziness or light-headed, hyperkalemia, tired feeling, trouble concentrating, or sleep problems (insomnia). Other side effects may include pain or burning when urinating, wheezing or chest pain, rapid heart rate, increased thirst, loss of appetite, swelling, weight gain, urinating less than usual or not at all, confusion, or mood changes. Women will be given a list of potential side effects with instructions to either call 911 (severe allergic reaction) or call the study investigators.

Exercise Test: The risks of performing an exercise test are low. As summarized in the American College of Sports Medicine Guidelines for Exercise Testing and Prescription (10^{th} ED, 2018)[4], there are ~6 cardiac events per 10,000 tests. All participants (normotensives and hypertensives) are initially screened by the NMPCC to ensure they are otherwise healthy and not high risk (i.e., no known disease, no signs or symptoms of disease). The study physician, Dr. Stillabower, will determine whether hypertensive participants are able to complete the exercise test based on their blood pressure and screening results since hypertension is a CVD risk factor.

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.

*(*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)*

More than minimal, however the risks associated with the study are low. Data collected from this study will help to understand the mechanisms contributing to hypertension in women.

What steps will be taken to minimize risks?

The risk of infection during blood draws, endothelial cell collection, and microdialysis fiber placement is minimized by using sterile materials and technique throughout the protocol. Subject discomfort due to microdialysis fiber placement is minimized by the application of ice to the area for 10 minutes prior to fiber insertion. Losartan is a drug commonly used to treat patients with high blood pressure. Using losartan may cause an electrolyte imbalance, and rarely kidney failure. Women will be instructed not to drink alcohol while taking losartan, and also to avoid potassium supplements or salt substitutes, and NSAIDs. We will assess electrolytes and creatinine levels in all women taking losartan on day 7 of administration. If serum potassium is >5.2 mmol/L or creatinine is >50% over baseline, the study physician will be contacted to advise the participant to discontinue the study medication and determine whether they should discontinue the study. The study physician will determine if additional medical follow-up is needed (ER, PCP). Women will keep in close contact with study investigators. A list of potential side effects of using losartan will be given to women, with instructions to either call 911 (if signs of an allergic reaction occur such as hives, difficulty breathing, swelling of the face, lips, tongue, or throat), or to call the study investigators. The participant will be given contact information to reach the study investigator and Nurse Managed Primary Care Center if questions or concerns arise during the study protocol. The study investigator can reach Dr. Stillabower, who can then call the participant if needed.
A clinical exercise physiologist and nurse practitioner will directly supervise the exercise test, and follow set guidelines published by the American College of Sports Medicine for stopping the test. The NMPCC will send the test results to be read and interpreted by their cardiologist, Dr. Goldenberg. In the event of a positive test or if additional follow up is needed, the NMPCC will contact the participant's primary care doctor.

Describe any potential direct benefits to participants.

There may be no direct benefit to subjects for participating in this research study however they will be provided the results of screening blood work and VO2max test when they complete the study. The results will be interpreted and provided to the participants PCP if additional follow-up is needed.

Describe any potential future benefits to this class of participants, others, or society.
This study may provide new information regarding the mechanisms contributing to hypertension in women.

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

N/A

9. **COMPENSATION**
Will participants be compensated for participation?
Yes

If so, please include details.
Women with high BP: Subjects completing all aspects of the study will receive $275.00. If a participant is excluded due to elevated blood levels (visit 3), they will receive $100.00. Participants that do not complete visit 5 (exercise test) will receive $225.00. They will receive the compensation at the end of the study in the form of a check.
Normotensive women – hormone intervention: Subjects completing all aspects of the study will receive $200.00. They will receive the compensation at the end of the study in the form of a check. Participants that do not complete visit 4 (exercise test) will receive $150.00.

10. **DATA**
Will subjects be anonymous to the researcher?
Information obtained from this study will be kept strictly confidential. Participants are not anonymous to the researcher, and will not be individually identified except by a participant number known only to the investigators.

If subjects are identifiable, will their identities be kept confidential? (If yes, please specify how)
Informed consents and medical history questionnaires will be kept in a separate locked filing cabinet from any data with participant numbers. A list of names and participant numbers will be encrypted and kept on a password protected computer. The list will be kept for 5 years after study completion; the list will be deleted and digital media scrubbed. Information obtained in the medical history form is used only by the Nurse Managed Health Center at the University of Delaware, and electronic records are stored in a secure HIPAA compliant database.

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)
Information obtained from this study will be kept strictly confidential. Subjects will not be individually identified, except by subject number, known only to the investigators. All data stored as paper files or digitally will be kept indefinitely. The paper files are stored in a locked cabinet. A list of names and participant numbers will be kept on a password-protected computer. All data will be stored in a locked cabinet or password protected computer indefinitely. All data will be encrypted. The data will be stored and archived for potential use in other, future studies. Information obtained in the medical history form is used only by the Nurse Managed Health Center at the University of Delaware, and electronic records are stored in a secure HIPAA compliant database. In the event of any publication or presentation resulting from the research, no personally identifiable information will be revealed.

How long will data be stored?
All data will be stored in a locked cabinet or password protected computer indefinitely. All data will be encrypted.

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

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?
Cutaneous vascular conductance (CVC) will be calculated as mean SkBF/mean arterial pressure.
While the results of this research will be published, neither participant name nor identity will be revealed.

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. Subjects will not be individually identified, except by subject number, known only to the investigators. All data stored as paper files or digitally will be kept indefinitely. The paper files are stored in a locked cabinet. 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).
No

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.

REFERENCES:

1. Wenner MM, Taylor HS, Stachenfeld NS. Endothelin B receptor contribution to peripheral microvascular function in women with polycystic ovary syndrome. *J Physiol.* Oct 1 2011;589(Pt 19):4671-4679.

2. Wenner MM, Taylor HS, Stachenfeld NS. Progesterone enhances adrenergic control of skin blood flow in women with high but not low orthostatic tolerance. *J Physiol.* Dec 20 2011;589(4):975-986.

3. Kellogg DL, Jr., Liu Y, Pergola PE. Selected contribution: Gender differences in the endothelin-B receptor contribution to basal cutaneous vascular tone in humans. *J Appl Physiol.* Nov 2001;91(5):2407-2411; discussion 2389-2490.

4. ACSM's Guidelines for Exercise Testing and Prescription, 10th Ed. *2018*. Philadelphia, PA: Wolters Kluwer.

Statistical Plan

Sample size estimates were calculated for our main outcome variables in Specific Aim 1 based on previously published data in normotensive and pre-hypertensive adults utilizing arterial infusions of the ET-1 receptor antagonist. A three level 2x2 General Linear Mixed Model will be used to compare normotensive and hypertensive PMW on the outcomes. The hierarchical structure is needed as sites are nested within measurements, which are nested within individuals. The model assumptions of linearity and normality, will be evaluated using Box-Cox and Shapiro-Wilk tests, respectively. Outliers and influential cases will be screened for and removed. The covariance structure will be chosen using nested model comparisons. Violations of the assumptions will be remedied using transformations suggested by the Box-Cox test. *Post-hoc* comparisons will have the Bonferroni adjustment applied. The alpha is set at 0.05 for each analysis, and will be adjusted by the number of primary dependent variables to control for multiple comparisons. In order to find a large effect (${}_p\eta^2 = 0.50$), a sample of size of 38 women is required to achieve a power greater than 0.8. In order to account for screen failures, subject attrition and potential microdialysis fiber failure, we will recruit 60 women (30 normotensive and 30 hypertensive).

A Mixed Design ANOVA will be used to evaluate if losartan administration alters endothelial ET-1 protein expression ETB receptor expression on endothelial cells, along with ETA and ETB receptor expression on vascular smooth muscle cells in hypertensive PMW compared to that of normotensive PMW. Since values are expressed as ratios, if the assumptions of the Mixed Design ANOVA are not satisfied a Generalized Linear Mixed Model will be used. In order to find a moderately small effect (${}_p\eta^2 = .09$), a sample of size of 38 is required to achieve a power greater than 0.8. The model also assumes of linearity, sphericity, and normality, which will be evaluated using Box-Cox, Mauchly's, and Shapiro-Wilk tests, respectively. Outliers and influential cases will be screened for and removed. *Post-hoc* comparisons will have the Bonferroni adjustment applied. The alpha is set at 0.05 for each analysis, and will be adjusted by the number of primary dependent variables to control for multiple comparisons. Although we are planning on matching our groups based on age, body composition, VO_2max, and time since menopause, these may be potential covariates will be compared between groups using t-tests for continuous data and tests for χ^2 tests for categorical data. If a covariate is significantly different between groups it will be included in the analysis.