INFORMED CONSENT TO PARTICIPATE IN RESEARCH

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

Principal Investigator(s): Megan M. Wenner, PhD, and Michael Stillabower, MD

You are being invited to participate in a research study. This consent form tells you about the study including its purpose, what you will be asked to do if you decide to take part, and the risks and benefits of being in the study. Please read the information below and ask us any questions you may have before you decide whether or not you want to participate.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to understand what causes blood vessels to constrict or get smaller in women with high blood pressure. Women with high blood pressure have an increased risk for cardiovascular disease. One early indicator of cardiovascular disease is decreased blood vessel function. The inner lining of the blood vessels (called the endothelium), releases substances to help control blood vessel function. One of these substances is endothelin-1 (ET-1). Certain hormones that our bodies produce can influence the release of ET-1 like the hormone angiotensin II (ANG II), which is a common therapeutic target for high blood pressure. In this study we will determine if blocking both ET-1 and ANG II are more effective in reducing constriction compared to single therapy or control conditions. To do this we will measure blood flow in the very small blood vessels in your skin on your forearm while blocking the ET-1 receptors using a technique called microdialysis. This technique involves placing tiny sterile plastic tubes (microdialysis fibers) in the surface layer of your skin. The largest part of the tubing is about 6 times the diameter of human hair. During the experiment we will add substances to the fluid in the tubing, such as endothelin-receptor-A blocker (BQ-123), endothelin-receptor-B blocker (BQ-788), a mixture of both endothelin-receptors A and B blocker, and sodium nitroprusside (SNP). This will be done at baseline and again after 14 days.

You will be one of approximately 30 participants in this study (compared to 30 participants with high blood pressure).

WHY ARE YOU BEING ASKED TO PARTICIPATE?

You are being asked to participate because you are a postmenopausal woman between 50-70 years of age without high blood pressure (self-report).

You will not be allowed to participate in the study if you:

- Have a history of heart disease, stroke, cancer, diabetes, kidney or liver disease, blood clots
- Use tobacco products (including cigarettes and chewing tobacco)
- Have a body mass index is greater than 35
- Use hormone replacement therapy, or are pregnant

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- All medications you are taking at the time of this study will be evaluated by the investigators on an individual basis, to confirm you are eligible to participate.

WHAT WILL YOU BE ASKED TO DO?

Full participation in this study will require 4 visits for a total of approximately 11 hours. These visits will be held at the Nurse Managed Primary Care Center (Visits 1&2) and the Physiology Core Lab (Visits 3&4). Both of these facilities are located at 540 S. College Avenue (STAR Health Sciences Complex) in Newark, DE.

Visit 1: Participant Screening (1 visit to the Nurse Managed Primary Care Center; 1 hour in length)

- This visit will take place in the morning (between 7:30 and 8:30am). You will be asked to not eat food, alcohol, and caffeine for 12 hours and to not exercise for 24 hours. This is a screening visit where:
  1). You will complete a medical history and menstrual history questionnaire
  2). Your height and weight will be measured
  3). Resting blood pressure and a resting electrocardiogram (ECG: a test to record electrical activity of the heart) will be measured.
  4). A blood sample will be collected by inserting a needle into an arm vein (approximately 3 tablespoons of blood will be removed). The blood sample will be used to measure liver and kidney function, electrolytes such as sodium, cholesterol, red blood cells, glucose (blood sugar), and C-reactive protein.

The results from the screening, as well as the medical history form, will be reviewed by a nurse practitioner to determine if you qualify for the study. The investigators will discuss the results of these tests with you, and will make copies of the results available to you. In the event that one of the test results is abnormal, you will be referred to your personal physician for a follow-up exam and will not be able to participate in the research study. The results of your screening visit will be provided to you.

At the end of this visit, you will be given a 24-hour blood pressure monitor, and review instructions on how to wear the monitor. You will wear the monitor during the 24 hours after visit 1. The monitor measures blood pressure every 20 minutes during the day and every 30 minutes at night. You will return the monitor to the Physiology Core Lab the next day, at which time we will take your blood pressure again (3 measurements) with a standard automated cuff on your upper arm. You will be seated for ~5-10 minutes. At the end of this visit we will give you a blood pressure monitor to take home. You will be asked to take your blood pressure 3 days each week for the following 2 weeks. On each day you take your blood pressure, you will do so at 3 separate times: morning, noon, and evening. You should be seated for the readings. The device is worn on the upper arm and will take 3 measures and provide a reading which is the average of the three readings. You will be asked to write this number down in the space provided on the record log sheet. We will give you instructions and show you how to use the monitor. You will return the monitor at your next visit along with the record log.

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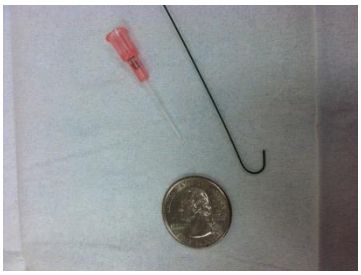
2-3 days before Visit 2, you will come to the lab to pick up a 24-hour blood pressure monitor, and review instructions on how to wear the monitor. You will wear the monitor during the 24 hours before visit 2.

Visit 2: Blood Vessel Function Assessment (Physiology Core Lab; 4.5 hours in length)

This visit will occur within 2 weeks of Visit 1. You will be asked not to drink alcohol and caffeine for 12 hours, food for at least 4 hours, and refrain from exercise for 24 hours prior to this visit. You will be asked to bring or wear a short sleeve shirt and shorts. During this visit the following will occur:

- You will have 3 self-adhesive electrodes placed on your chest to monitor heart rate, and a blood pressure cuff around the upper arm and finger to measure blood pressure.
- A catheter (i.e., a small needle encased in a flexible tube that is inserted into a vein) will be placed into a vein in your arm. The catheter in the vein will permit us to take a blood sample; approximately 6 tablespoons / 90 mL of blood will be drawn in total. The blood sample will be used to analyze hormones (estradiol, progesterone, follicle stimulating hormone, angiotensin II, aldosterone, renin, norepinephrine, and testosterone), a protein involved in regulating blood vessel function (endothelin-1), and cellular markers of blood vessel dysfunction. Blood samples will be stored in the freezer until the time of sample processing. Once processing is complete the samples will be destroyed.
- We will collect some cells from the wall of the vein. To do this, we will pass a very thin j-shaped wire through the intravenous catheter (see photo at right) and gently move it back and forth so it scrapes against the side of the vein wall. When this wire is moved against the vein wall, it will pick up some "endothelial" cells. We will then make measurements of different proteins in these cells that we collect.



- Next we will assess large blood vessel function using two separate non-invasive techniques. First we will place blood pressure cuffs around your arm and thigh and take measurements. Then we will record the pulse in your neck (carotid artery) by placing a pressure probe on the skin over the artery. The pressure probe looks like a pen. A cloth tape measure will be used to measure the distance between your neck and thigh. Second, a small blood pressure cuff will be placed on your forearm close to your elbow. We will use an ultrasound to take pictures of the blood vessel in your upper arm (brachial artery). The cuff will then be inflated for 5 minutes; the sensation felt will be similar to having fallen asleep on your arm (slight numbness). We will deflate the cuff and continue to take ultrasound pictures for 2 minutes.
- Next we will then prepare your arm for the microdialysis procedure. We will place a tight band around your upper arm to see your veins clearly. We will make 4 pairs of pen marks on your arm approximately 1 inch apart and away from veins to identify where the microdialysis tubing will be placed. We will then clean your arm with an orange-colored betadine fluid and alcohol. We will place

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an ice bag on your arm for 10 minutes to numb your skin. Then we will insert a thin needle into your skin at each entry mark. The needle's tip travels between the layers of skin for 2 cm and leaves your skin at the matching exit mark (See Figure 1). The microdialysis tubing is threaded through the needle, and then we will remove the needle leaving the tubing in your skin. You will have 4 sets of tubing in your skin. Any redness of your skin typically subsides in about 60 minutes.

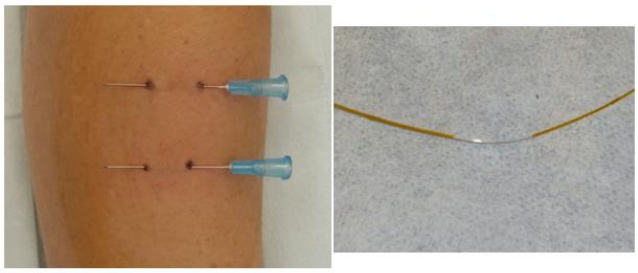

Figure 1. Procedure for placing microdialysis fibers (Left); Microdialysis fiber (Right).

- After the tubing is placed, we will tape a thin probe (Laser Doppler) and its holder over each area where the tubing is located. This probe uses a weak laser light to measure blood flowing in the small vessels in your skin. We can also control the temperature of the holders. The temperature will start at 91.4˚F. Then we will start a salt like solution (Lactated Ringer's solution) to flow (perfuse) through the tubing in your skin (60-90 min). Lactated Ringer's solution is a salt like solution containing sodium, chloride, potassium, calcium, and lactate.
- When the redness on your arm is gone, the experiment begins. We will make resting measures for ~20 minutes. Then we will add the test substances to the plain fluid running through the tubing for 45 minutes:
  - -Tubing site 1 will receive salt like solution (Lactated Ringer)
  - -Tubing site 2 will receive salt like solution + ET-A receptor blockade (BQ-123)
  - -Tubing site 3 will receive salt like solution + ET-B receptor blockade (BQ-788)
  - -Tubing site 4 will receive salt like solution + BQ-123 + BQ-788
- After 45 minutes we will increase the temperature of the local heaters to 104˚F and heat the small area of your skin. We maintain this temperature for about 40 minutes. When the skin blood flow becomes stable again, we will stop the flow of test substances through the tubing. Lastly, the salt like solution + SNP will flow through all of the tubing and we heat the skin to 108˚F for 30-45 minutes. This creates the greatest amount of blood flow possible.
- The trial then ends. We will remove the tubing from your arm and clean all the places on your arm where the tubing was with alcohol. We place a sterile bandage over the sites where the tubing was in your skin. We will check your heart rate and blood pressure again before you leave.
- At the completion of this visit, you will be given a 24-hour blood pressure monitor, and review instructions on how to wear the monitor. You will wear the monitor during the 24 hours preceding visit 3.

Visit 3: Blood Vessel Function Assessment (Physiology Core Lab; 4.5 hours in length)

This visit will occur 2 weeks after Visit 2, and will be approximately 4.5 hours in length. You will be asked not to drink alcohol and caffeine for 12 hours, food for at least 4 hours, and refrain from exercise for 24 hours prior to this visit. This visit will be identical to visit 2.

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Visit 4: Exercise Test (VO_2max test; Nurse Managed Primary Care Center, 1 hour in length)

- You will be asked to not eat food for 4 hours, alcohol or caffeine for 12 hours, and to not exercise for 24 hours prior to this visit. You will be asked to bring or wear comfortable clothing and athletic shoes (sneakers) to walk on the treadmill.
- We will place 10 electrodes on your torso to monitor your heart at rest and throughout the test. We will also measure your blood pressure at rest and throughout the test. If your resting BP is ≥200/110 mmHg we will not proceed with the test. You will wear a small mask over your nose and mouth so that we can measure the oxygen and carbon dioxide you breathe at rest and throughout the test.
- You will begin the test with a warm up by walking on the treadmill. Then, every 1.5 minutes the speed and grade (incline) of the treadmill will increase. We will ask you to rate how hard you are working by using a number scale (Borg scale) that we will explain to you before the start of the test. The test will last ~8-10 minutes.
- We may stop the test at any time because of signs of fatigue or changes in your heart rate, electrocardiogram, or blood pressure, or symptoms you may experience (for example, chest pain, dizziness). You may also stop the test because of feelings of fatigue or any other discomfort.
- We will continue to measure your heart rate and blood pressure for at least 5 minutes after the end of the test, while you will complete a 'cool down' period (walking slowing on the treadmill with no incline). We will continue to measure your heart rate and blood pressure for an additional 5-10 minutes after you are off the treadmill. The results of the test will be reviewed with the study physician. In addition, 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 your primary care doctor.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

- There are no known risks associated with obtaining your height, weight, resting electrocardiogram, or resting blood pressure. You may have pain and/or bruising at the site where blood is taken, and there is a small risk of infection (this will be minimized by using sterile, single-use catheters). Signs of infection include pain, swelling, and redness. If these signs occur, you should contact the study investigators. Fainting sometimes occurs during or shortly after blood is drawn. There are minimal risks associated with the assessment of blood vessel function. You may feel some pressure or minor discomfort from the probes. You may feel minor discomfort when the blood pressure cuff is inflated, and may feel numbness and tingling in your arm and hand (similar to if your arm fell asleep). The 24-hour blood pressure monitor may wake you up during your sleep.
- There may also be risks associated with obtaining endothelial cells from your vein. The risks associated with endothelial cell collection are similar to those of an intravenous catheter and include pain, inflammation of the vein, infection and blood clots, although the risks of blood clots and infection

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may be slightly greater (compared to intravenous placement alone) due to increased manipulation of the blood vessel. There is a low likelihood that the j-shaped wire could get stuck in the vein and/or be damaged; if this were to happen the nurse will assess the situation and call 911 if needed. If you require additional medical treatment, you will be responsible for the cost.

- Microdialysis fiber placement into the skin may produce some short-term pain or discomfort. Risks associated with insertion of the microdialysis fiber include irritation to the skin, and rarely, infection. You will probably experience some pain and bruising like that from a blood draw. However, we use ice to numb your skin during insertion of the tubing. Also, the small needle reduces pain during placement of the tubing. You will probably not have pain after the tubing is in place. You may feel a little pain when the tubing is removed from your skin. You may become lightheaded or faint. Mild pressure with sterile gauze stops any slight bleeding that may occur. Infection is possible. Sterile techniques and supplies like those used in a hospital keep the risk minimal. We apply a sterile bandage after the experiment and tell you how to take care of the site.
- The amount of drug that enters the skin is very small. However, there is a chance of you having a bad reaction to the substances. This reaction could produce redness, itching, rash, and/or swelling. Although unlikely, it is possible that a worse reaction could also cause fever, breathing problems, changes in heart rate, convulsions, and/or collapse. If a bad reaction should occur, medical help will be summoned right away. We have no knowledge of any adverse events associated with these substances utilized during this technique.
- Lactated Ringer's solution: The fluid is similar to natural fluids in your skin. This fluid contains salt, potassium, lactate, and chloride. The acid content is like that of your body's fluids. A bad reaction to this fluid is highly unlikely.
- ET-A receptor blockade (BQ-123), ET-B receptor blockade (BQ-788) and sodium nitroprusside (SNP): Only minute amounts of these substances enter the nickel-sized area of skin around the tubing. Other researchers have used these substances in human skin and there have been no reports of bad reactions.
- Laser Doppler: The laser Doppler will not damage the skin. 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 your skin under the holders. The skin will feel very warm but will not hurt. The heating will make the skin of your arm under the holders red like when you take a hot bath. The redness will not last more than several hours. Some people may be more sensitive to the heating than others. If your arm feels too hot, you will tell us, and we will reduce or stop the heating.
- 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 (10th ED, 2018), there are ~6 cardiac events per 10,000 tests.
- What steps will be taken to minimize risks?

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- 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. Discomfort due to microdialysis fiber placement is minimized by the application of ice to the area for 10 minutes prior to fiber insertion. 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.

WHAT IF YOU ARE INJURED DURING YOUR PARTICIPATION IN THE STUDY?

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

WHAT ARE THE POTENTIAL BENEFITS?

You may not benefit directly from taking part in this research. However, the knowledge gained from this study may contribute to our understanding of blood pressure control in adults (particularly women). The data collected at the screening session will be provided to you upon completion of the study.

NEW INFORMATION THAT COULD AFFECT YOUR PARTICIPATION:

During the course of this study, we may learn new information that could be important to you. This may include information that could cause you to change your mind about participating in the study. We will notify you as soon as possible if any new information becomes available.

HOW WILL CONFIDENTIALITY BE MAINTAINED? WHO MAY KNOW THAT YOU PARTICIPATED IN THIS RESEARCH?

- 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 and will be encrypted. The list will be kept for 5 years after study completion; the list will be deleted and digital media scrubbed. All de-identified data will be stored in a locked cabinet or encrypted 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. Information obtained from the medical history in participants who are not allowed to

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participate will be stored in a locked cabinet or encrypted password protected computer indefinitely (HIPAA compliant database at the Nurse Managed Health Center).

- In the event of any publication or presentation resulting from the research, no personally identifiable information will be revealed.
- The confidentiality of your records will be protected to the extent permitted by law. Your research records may be viewed by the University of Delaware Institutional Review Board, which is a committee formally designated to approve, monitor, and review biomedical and behavioral research involving humans. This research is being funded by the National Institutes of Health (NIH); thereby they have the right to view the research data.
- A description of this clinical trial will be made available on http://www.ClinicalTrials.gov as required by U.S.Law. This website will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site any time.

HIPAA AUTHORIZATION

State and federal privacy laws protect your PHI. These laws say that, in most cases, your health care provider can release your PHI for the purpose of conducting research only if you give permission by signing an Authorization.

If you agree to participate in the research study, the research team will need to collect and use your PHI. To allow your health care provider to share your PHI with the research team, your approval is required. Signing this Authorization is completely voluntary. However, if you do not sign this Authorization, then you may not participate in the research study.

Who May Disclose and Who may Use and/or Receive my PHI?

By signing this document, you are hereby permitting the Nurse Managed Primary Care Center at the University of Delaware to disclose the PHI described in this Authorization to the research team involved in this project; the study sponsor and its employees; the Institutional Review Board (IRB) and other regulatory agencies responsible for overseeing research.

Once your PHI is shared with these persons, you understand that the PHI may no longer be protected by federal or state privacy laws.

What PHI Will Be Disclosed and Used, and for What Purpose?

The following PHI may be disclosed to, collected by, used by and shared with those listed above for the following purpose to determine your eligibility for this study: Medical history/treatment; Laboratory/diagnostic tests; EKG/EEG report.

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This Authorization will expire at the conclusion of the research study. You may cancel this Authorization at any time before, during, or after your participation in this study by giving a written request with your signature on it to the Principal Investigator at ***mwenner@udel.edu***. If you cancel this Authorization, your PHI obtained before that date may still be used for this research study.

I hereby authorize the disclosure and use of ***my Personal Health Information***.

_____ _____

Signature of Patient or Authorized Representative Date

Printed Name of Person Signing: _____

Relationship to Patient: _____

WILL THERE BE ANY COSTS TO YOU FOR PARTICIPATING IN THIS RESEARCH?

- There are no costs associated with participating in the study.

WILL YOU RECEIVE ANY COMPENSATION FOR PARTICIPATION?

- You will receive $200.00 for completion of all aspects of the study. You will receive compensation at completion of the study in the form of a check. If you only complete 3 visits, you will receive $150.00.

DO YOU HAVE TO TAKE PART IN THIS STUDY?

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

WHO SHOULD YOU CALL IF YOU HAVE QUESTIONS OR CONCERNS?

If you have any questions about this study, please contact the Principal Investigator, Megan M. Wenner, PhD, Assistant Professor, Department of Kinesiology and Applied Physiology (302-831-7343).

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If you have any questions or concerns about your rights as a research participant, you may contact the University of Delaware Institutional Review Board at hsrb-research@udel.edu or (302) 831-2137.

Your signature on this form means that: 1) you are at least 18 years old; 2) you have read and understand the information given in this form; 3) you have asked any questions you have about the research and those questions have been answered to your satisfaction; 4) you accept the terms in the form and volunteer to participate in the study. You will be given a copy of this form to keep.

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

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

Do we have your permission to contact you regarding participation in future studies? Please write your initials next to your preferred choice.

_____ YES _____ NO

Participant's Initials _____

INFORMED CONSENT TO PARTICIPATE IN RESEARCH

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

Principal Investigator(s): Megan M. Wenner, PhD, and Michael Stillabower, MD

You are being invited to participate in a research study. This consent form tells you about the study including its purpose, what you will be asked to do if you decide to take part, and the risks and benefits of being in the study. Please read the information below and ask us any questions you may have before you decide whether or not you want to participate.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to understand what causes blood vessels to constrict or get smaller in women with high blood pressure. Women with high blood pressure have an increased risk for cardiovascular disease. One early indicator of cardiovascular disease is decreased blood vessel function. The inner lining of the blood vessels (called the endothelium), releases substances to help control blood vessel function. One of these substances is endothelin-1 (ET-1). Certain hormones that our bodies produce can influence the release of ET-1 like the hormone angiotensin II (ANG II), which is a common therapeutic target for high blood pressure. In this study we will determine if blocking both ET-1 and ANG II are more effective in reducing constriction compared to single therapy or control conditions. To do this we will measure blood flow in the very small blood vessels in your skin on your forearm while blocking the ET-1 receptors using a technique called microdialysis. This technique involves placing tiny sterile plastic tubes (microdialysis fibers) in the surface layer of your skin. The largest part of the tubing is about 6 times the diameter of human hair. During the experiment we will add substances to the fluid in the tubing, such as endothelin-receptor-A blocker (BQ-123), endothelin-receptor-B blocker (BQ-788), a mixture of both endothelin-receptors A and B blocker, and sodium nitroprusside (SNP). This will be done at baseline and again after 14 days of taking the blood pressure lowering drug called losartan.

You will be one of approximately 30 participants in this study (compared to 30 participants without high blood pressure).

WHY ARE YOU BEING ASKED TO PARTICIPATE?

You are being asked to participate because you are a postmenopausal woman between 50-70 years of age with high blood pressure (self report).

You will not be able to participate in the study if you:

- Have a history of heart disease, stroke, cancer, diabetes, kidney or liver disease, blood clots
- Use tobacco products (including cigarettes and chewing tobacco)
- Have a body mass index greater than 35

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- Use hormone replacement therapy, or are pregnant
- Are currently taking medication for high blood pressure
- If your resting blood pressure is greater than 180/100 mmHg or less than 100/60 mmHg, you will not be able to participate. All medications you are taking at the time of this study will be evaluated by the investigators on an individual basis, to confirm you are eligible to participate.

WHAT WILL YOU BE ASKED TO DO?

Full participation in this study will require 5 visits, for a total of approximately 12 hours. These visits will be held at the Nurse Managed Primary Care Center (Visits 1&2) and the Physiology Core Lab (Visits 3-5). Both of these facilities are located at 540 S. College Avenue (STAR Health Sciences Complex) in Newark, DE.

Visit 1: Participant Screening (1 visit to the Nurse Managed Primary Care Center; 1 hour in length)

- This visit will take place in the morning (between 7:30 and 8:30am). You will be asked to not eat food, alcohol, and caffeine for 12 hours and to not exercise for 24 hours. This is a screening visit where:
  1). You will complete a medical history and menstrual history questionnaire
  2). Your height and weight will be measured
  3). Resting blood pressure and a resting electrocardiogram (ECG: a test to record electrical activity of the heart) will be measured.
  4). A blood sample will be collected by inserting a needle into an arm vein (approximately 3 tablespoons of blood will be removed). The blood sample will be used to measure liver and kidney function, electrolytes such as sodium, cholesterol, red blood cells, glucose (blood sugar), and C-reactive protein.

The results from the screening, as well as the medical history form, will be reviewed by a nurse practitioner to determine if you qualify for the study. The investigators will discuss the results of these tests with you, and will make copies of the results available to you. In the event that one of the test results is abnormal, you will be referred to your personal physician for a follow-up exam and will not be able to participate in the research study. The results of your screening visit will be provided to you.

At the end of this visit, you will be given a 24-hour blood pressure monitor, and review instructions on how to wear the monitor. You will wear the monitor during the 24 hours after visit 1. The monitor measures blood pressure every 20 minutes during the day and every 30 minutes at night. You will return the monitor to the Physiology Core Lab the next day, at which time we will take your blood pressure again (3 measurements) with a standard automated cuff on your upper arm. You will be seated for ~5-10 minutes. At the end of this visit we will give you a blood pressure monitor to take home. You will be asked to take your blood pressure 3 days each week for the following 2 weeks. On each day you take your blood pressure, you will do so at 3 separate times: morning, noon, and evening. You should be seated for the readings. The device is worn on the upper arm and will take 3 measures and provide a reading which is the average of the three readings. You will be asked to write this number down in the space provided on the record log sheet.

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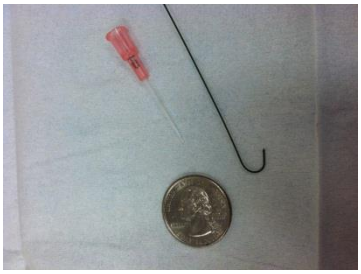
We will give you instructions and show you how to use the monitor. You will return the monitor at your next visit along with the record log.

2-3 days before Visit 2, you will come to the lab to pick up a 24-hour blood pressure monitor, and review instructions on how to wear the monitor. You will wear the monitor during the 24 hours before visit 2.

Visit 2: Blood Vessel Function Assessment (Physiology Core Lab; 4.5 hours in length)

This visit will occur within 2 weeks of Visit 1. You will be asked not to drink alcohol and caffeine for 12 hours, food for at least 4 hours, and refrain from exercise for 24 hours prior to this visit. You will be asked to bring or wear a short sleeve shirt and shorts. During this visit the following will occur:

- You will have 3 self-adhesive electrodes placed on your chest to monitor heart rate, and a blood pressure cuff around the upper arm and finger to measure blood pressure.
- A catheter (i.e., a small needle encased in a flexible tube that is inserted into a vein) will be placed into a vein in your arm. The catheter in the vein will permit us to take a blood sample; approximately 6 tablespoons / 90 mL of blood will be drawn in total. The blood sample will be used to analyze hormones (estradiol, progesterone, follicle stimulating hormone, angiotensin II, aldosterone, renin, norepinephrine, and testosterone), a protein involved in regulating blood vessel function (endothelin-1), and cellular markers of blood vessel dysfunction. Blood samples will be stored in the freezer until the time of sample processing. Once processing is complete the samples will be destroyed.
- We will collect some cells from the wall of the vein. To do this, we will pass a very thin j-shaped wire through the catheter (see photo at right) and gently move it back and forth so it scrapes against the side of the vein wall. When this wire is moved against the vein wall, it will pick up some "endothelial" cells. We will then make measurements of different proteins in these cells that we collect.



- Next we will assess large blood vessel function using two separate non-invasive techniques. First we will place blood pressure cuffs around your arm and thigh and take measurements. Then we will record the pulse in your neck (carotid artery) by placing a pressure probe on the skin over the artery. The pressure probe looks like a pen. A cloth tape measure will be used to measure the distance between your neck and thigh. Second, a small blood pressure cuff will be placed on your forearm close to your elbow. We will use an ultrasound to take pictures of the blood vessel in your upper arm (brachial artery). The cuff will then be inflated for 5 minutes; the sensation felt will be similar to having fallen asleep on your arm (slight numbness). We will deflate the cuff and continue to take ultrasound pictures for 2 minutes.
- Next we will then prepare your arm for the microdialysis procedure. We will place a tight band around your upper arm to see your veins clearly. We will make 4 pairs of pen marks on your arm approximately 1 inch apart and away from veins to identify where the microdialysis tubing will be placed. We will then clean your arm with an orange-colored betadine fluid and alcohol. We will place an ice bag on your arm for 10 minutes to numb your skin. Then we will insert a thin needle into your

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skin at each entry mark. The needle's tip travels between the layers of skin for 2 cm and leaves your skin at the matching exit mark (See Figure 1). The microdialysis tubing is threaded through the needle, and then we will remove the needle leaving the tubing in your skin. You will have 4 sets of tubing in your skin. Any redness of your skin typically subsides in about 60 minutes.

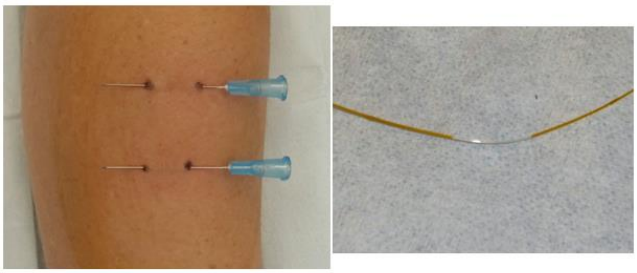

Figure 1. Procedure for placing microdialysis fibers (Left); Microdialysis fiber (Right).

- After the tubing is placed, we will tape a thin probe (Laser Doppler) and its holder over each area where the tubing is located. This probe uses a weak laser light to measure blood flowing in the small vessels in your skin. We can also control the temperature of the holders. The temperature will start at 91.4˚F. Then we will start a salt like solution (Lactated Ringer's solution) to flow (perfuse) through the tubing in your skin (60-90 min). Lactated Ringer's solution is a salt like solution containing sodium, chloride, potassium, calcium, and lactate.
- When the redness on your arm is gone, the experiment begins. We will make resting measures for ~20 minutes. Then we will add the test substances to the plain fluid running through the tubing for 45 minutes:
  -Tubing site 1 will receive salt like solution (Lactated Ringer)
  -Tubing site 2 will receive salt like solution + ET-A receptor blockade (BQ-123)
  -Tubing site 3 will receive salt like solution + ET-B receptor blockade (BQ-788)
  -Tubing site 4 will receive salt like solution + BQ-123 + BQ-788
- After 45 minutes we will increase the temperature of the local heaters to 104˚F and heat the small area of your skin. We maintain this temperature for about 40 minutes. When the skin blood flow becomes stable again, we will stop the flow of test substances through the tubing. Lastly, the salt like solution + SNP will flow through all of the tubing and we heat the skin to 108˚F for 30-45 minutes. This creates the greatest amount of blood flow possible.
- The trial then ends. We will remove the tubing from your arm and clean all the places on your arm where the tubing was with alcohol. We place a sterile bandage over the sites where the tubing was in your skin. We will check your heart rate and blood pressure again before you leave.
- At the end of the visit you will be given the study medication, losartan, which is a drug that blocks the receptor for angiotensin II. You will be given 7 days of the medication, and instructed to take 1 pill each day at nighttime (before bed). You should avoid alcohol while using losartan, and not take any potassium supplements or salt substitutes, or NSAIDs (advil, ibuprofen).

Visit 3: Blood draw to assess electrolytes and kidney function (Physiology Core Lab; 30 min in length)

This visit will occur on day 7 of taking losartan. You will be asked to report to the laboratory in the morning after an overnight fast for a blood draw and blood pressure recording. The blood draw will be used to assess your blood electrolytes and markers of kidney function. The study physician will review the results to determine if you can continue the study medication.

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- If your blood electrolyte levels or markers of kidney function are elevated, you may be instructed to stop the study medication, and may be excluded from the study. Rarely, if these markers are severely elevated, you may be instructed to go to the emergency room.
- If your blood pressure recording is less than 100/60 mmHg, you may be instructed to stop the study medication, and may be excluded from the study.
- You will be given another 7 days of the study medication, and instructed to take 1 pill each day at nighttime (before bed). At the completion of this visit, you will be given a 24-hour blood pressure monitor, and review instructions on how to wear the monitor. You will wear the monitor during the 24 hours preceding visit 4.

Visit 4: Blood Vessel Function Assessment (Physiology Core Lab; 4.5 hours in length)

This visit will occur 1week after Visit 3, or 2 weeks after starting losartan, and will be approximately 4.5 hours in length. You will be asked not to drink alcohol and caffeine for 12 hours, food for at least 4 hours, and refrain from exercise for 24 hours prior to this visit. This visit will be identical to visit 2.

Visit 5: Exercise Test (VO_2max test; Nurse Managed Primary Care Center, 1 hour in length)

- Your screening and health information will be reviewed by the study physician, Dr. Stillabower, for clearance to perform the exercise test. You will be asked to not eat food for 4 hours, alcohol or caffeine for 12 hours, and to not exercise for 24 hours prior to this visit. You will be asked to bring or wear comfortable clothing and athletic shoes (sneakers) to walk on the treadmill.
- We will place 10 electrodes on your torso to monitor your heart at rest and throughout the test. We will also measure your blood pressure at rest and throughout the test. If your resting BP is $\geq$200/110 mmHg we will not proceed with the test. You will wear a small mask over your nose and mouth so that we can measure the oxygen and carbon dioxide you breathe at rest and throughout the test.
- You will begin the test with a warm up by walking on the treadmill. Then, every 1.5 minutes the speed and grade (incline) of the treadmill will increase. We will ask you to rate how hard you are working by using a number scale (Borg scale) that we will explain to you before the start of the test. The test will last ~8-10 minutes.
- We may stop the test at any time because of signs of fatigue or changes in your heart rate, electrocardiogram, or blood pressure, or symptoms you may experience (for example, chest pain, dizziness). You may also stop the test because of feelings of fatigue or any other discomfort.
- We will continue to measure your heart rate and blood pressure for at least 5 minutes after the end of the test, while you will complete a 'cool down' period (walking slowing on the treadmill with no incline). We will continue to measure your heart rate and blood pressure for an additional 5-10 minutes after you are off the treadmill. The results of the test will be reviewed with the study physician. In addition, the NMPCC will send the test results to be read and interpreted by their

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cardiologist, Dr. Goldenberg. In the event of a positive test or if additional follow up is needed, the NMPCC will contact your primary care doctor.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

- There are no known risks associated with obtaining your height, weight, resting electrocardiogram, or resting blood pressure. You may have pain and/or bruising at the site where blood is taken, and there is a small risk of infection (this will be minimized by using sterile, single-use catheters). Signs of infection include pain, swelling, and redness. If these signs occur, you should contact the study investigators. Fainting sometimes occurs during or shortly after blood is drawn. There are minimal risks associated with the assessment of blood vessel function. You may feel some pressure or minor discomfort from the probes. You may feel minor discomfort when the blood pressure cuff is inflated, and may feel numbness and tingling in your arm and hand (similar to if your arm fell asleep). The 24-hour blood pressure monitor may wake you up during your sleep.
- There may also be risks associated with obtaining endothelial cells from your vein. The risks associated with endothelial cell collection are similar to those of an intravenous catheter and include pain, inflammation of the vein, infection and blood clots, although the risks of blood clots and infection may be slightly greater (compared to intravenous placement alone) due to increased manipulation of the blood vessel. There is a low likelihood that the j-shaped wire could get stuck in the vein and/or be damaged; if this were to happen the nurse will assess the situation and call 911 if needed. If you require additional medical treatment, you will be responsible for the cost.
- Microdialysis fiber placement into the skin may produce some short-term pain or discomfort. Risks associated with insertion of the microdialysis fiber include irritation to the skin, and rarely, infection. You will probably experience some pain and bruising like that from a blood draw. However, we use ice to numb your skin during insertion of the tubing. Also, the small needle reduces pain during placement of the tubing. You will probably not have pain after the tubing is in place. You may feel a little pain when the tubing is removed from your skin. You may become lightheaded or faint. Mild pressure with sterile gauze stops any slight bleeding that may occur. Infection is possible. Sterile techniques and supplies like those used in a hospital keep the risk minimal. We apply a sterile bandage after the experiment and tell you how to take care of the site.
- The amount of drug that enters the skin is very small. However, there is a chance of you having a bad reaction to the substances. This reaction could produce redness, itching, rash, and/or swelling. Although unlikely, it is possible that a worse reaction could also cause fever, breathing problems, changes in heart rate, convulsions, and/or collapse. If a bad reaction should occur, medical help will be summoned right away. We have no knowledge of any adverse events associated with these substances utilized during this technique.
- Lactated Ringer's solution: The fluid is similar to natural fluids in your skin. This fluid contains salt, potassium, lactate, and chloride. The acid content is like that of your body's fluids. A bad reaction to this fluid is highly unlikely.

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- ET-A receptor blockade (BQ-123), ET-B receptor blockade (BQ-788) and sodium nitroprusside (SNP): Only minute amounts of these substances enter the nickel-sized area of skin around the tubing. Other researchers have used these substances in human skin and there have been no reports of bad reactions.
- Laser Doppler: The laser Doppler will not damage the skin. 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 your skin under the holders. The skin will feel very warm but will not hurt. The heating will make the skin of your arm under the holders red like when you take a hot bath. The redness will not last more than several hours. Some people may be more sensitive to the heating than others. If your arm feels too hot, you will tell us, and we will reduce or stop the heating.
- Losartan is a medication that is commonly prescribed for 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, low blood pressure, dizziness or light-headed, high potassium levels, 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. You will be given a sheet with potential side effects and instructions to either call the study investigators (PI, Megan Wenner, 609-413-1418) or 911 if a severe allergic reaction should occur.
- 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 (10th ED, 2018), there are ~6 cardiac events per 10,000 tests. The study physician, Dr. Stillabower, will determine whether you are able to complete the exercise test based on your blood pressure and screening results.
- 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. 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. You will be instructed not to drink alcohol while taking losartan, and also to avoid potassium supplements or salt substitutes. We will assess your electrolytes and creatinine levels on day 7 of losartan administration. If serum potassium is >5.2 mmol/L or creatinine is >50% over baseline, the study physician will be contacted. He may advise you to discontinue the study medication and determine if you should discontinue the study. In rare cases, severe elevations in these blood markers may require you to go to the emergency room. You will keep in close contact with study investigators. A list of potential side effects of using losartan will be given to you, 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. You will be given contact information to reach the study investigator and Nurse Managed Primary Care Center if questions or

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concerns arise during the study protocol. The study investigator can reach Dr. Stillabower, who can then call you 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.

WHAT IF YOU ARE INJURED DURING YOUR PARTICIPATION IN THE STUDY?

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

WHAT ARE THE POTENTIAL BENEFITS?

You may benefit directly from taking part in this research. Using the drug losartan may lower your blood pressure; however there is no guarantee. Furthermore, the knowledge gained from this study may contribute to our understanding of blood pressure control in adults (particularly women) with high blood pressure. The data collected at the screening session will be provided to you upon completion of the study.

NEW INFORMATION THAT COULD AFFECT YOUR PARTICIPATION:

During the course of this study, we may learn new information that could be important to you. This may include information that could cause you to change your mind about participating in the study. We will notify you as soon as possible if any new information becomes available.

HOW WILL CONFIDENTIALITY BE MAINTAINED? WHO MAY KNOW THAT YOU PARTICIPATED IN THIS RESEARCH?

- 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 and will be encrypted. The list will be kept for 5 years after study completion; the list will be deleted and digital media scrubbed. All de-identified data will be stored in a locked cabinet or encrypted 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. Information obtained from the medical history in participants who are not allowed to participate will be stored in a locked cabinet or encrypted password protected computer indefinitely (HIPAA compliant database at the Nurse Managed Health Center).

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- In the event of any publication or presentation resulting from the research, no personally identifiable information will be revealed.
- The confidentiality of your records will be protected to the extent permitted by law. Your research records may be viewed by the University of Delaware Institutional Review Board, which is a committee formally designated to approve, monitor, and review biomedical and behavioral research involving humans. This research is being funded by the National Institutes of Health (NIH); thereby they have the right to view the research data.
- A description of this clinical trial will be made available on http://www.ClinicalTrials.gov as required by U.S.Law. This website will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site any time.

HIPAA AUTHORIZATION

State and federal privacy laws protect your Protected Health Information (PHI). These laws say that, in most cases, your health care provider can release your PHI for the purpose of conducting research only if you give permission by signing an Authorization.

If you agree to participate in the research study, the research team will need to collect and use your PHI. To allow your health care provider to share your PHI with the research team, your approval is required. Signing this Authorization is completely voluntary. However, if you do not sign this Authorization, then you may not participate in the research study.

Who May Disclose and Who may Use and/or Receive my PHI?

By signing this document, you are hereby permitting the Nurse Managed Primary Care Center at the University of Delaware to disclose the PHI described in this Authorization to the research team involved in this project; the study sponsor and its employees; the Institutional Review Board (IRB) and other regulatory agencies responsible for overseeing research.

Once your PHI is shared with these persons, you understand that the PHI may no longer be protected by federal or state privacy laws.

What PHI Will Be Disclosed and Used, and for What Purpose?

The following PHI may be disclosed to, collected by, used by and shared with those listed above for the following purpose to determine your eligibility for this study: Medical history/treatment; Laboratory/diagnostic tests; EKG/EEG report.

This Authorization will expire at the conclusion of the research study. You may cancel this Authorization at any time before, during, or after your participation in this study by giving a written request with your signature

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on it to the Principal Investigator at ***mwenner@udel.edu***. If you cancel this Authorization, your PHI obtained before that date may still be used for this research study.

I hereby authorize the disclosure and use of ***my Protected Health Information***.

_____ _____

Signature of Patient or Authorized Representative Date

Printed Name of Person Signing: _____

Relationship to Patient: _____

WILL THERE BE ANY COSTS TO YOU FOR PARTICIPATING IN THIS RESEARCH?

- There are no costs associated with participating in the study.

WILL YOU RECEIVE ANY COMPENSATION FOR PARTICIPATION?

- You will receive $275.00 for completion of all aspects of the study. You will receive compensation at completion of the study. If you drop out or are excluded after Visit 3 (Blood draw to check electrolytes and kidney function), you will receive $100.00. If you complete 4 visits (everything except the exercise test) you will receive $225.00.

DO YOU HAVE TO TAKE PART IN THIS STUDY?

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

WHO SHOULD YOU CALL IF YOU HAVE QUESTIONS OR CONCERNS?

If you have any questions about this study, please contact the Principal Investigator, Megan M. Wenner, PhD, Assistant Professor, Department of Kinesiology and Applied Physiology (302-831-7343), study

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physician, Michael Stillabower (302-463-5371), or Nurse Managed Primary Care Center (302-831-3195; during normal business hours).

If you have any questions or concerns about your rights as a research participant, you may contact the University of Delaware Institutional Review Board at hsrb-research@udel.edu or (302) 831-2137.

Your signature on this form means that: 1) you are at least 18 years old; 2) you have read and understand the information given in this form; 3) you have asked any questions you have about the research and those questions have been answered to your satisfaction; 4) you accept the terms in the form and volunteer to participate in the study. You will be given a copy of this form to keep.

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

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

Do we have your permission to contact you regarding participation in future studies? Please write your initials next to your preferred choice.

_____ YES _____ NO

Participant's Initials _____