CONSENT FORM TO PARTICIPATE IN AMILORIDE/PLACEBO RESEARCH STUDY

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Project # 2012990

STUDY TITLE: ESTROGEN RECEPTOR ALPHA SIGNALING IN ENDOTHELIAL CELLS EXACERBATES ARTERIAL STIFFENING VIA UPREGULATION OF ENAC IN INSULIN RESISTANT FEMALES

INTRODUCTION

This consent may contain words that you do not understand. Please ask the investigator or the study staff to explain any words or information that you do not clearly understand. The Principal Investigator (also called the study doctor) is Dr. Camila Manrique. The people working with Dr. Manrique on this study are called the study team.

This study is being sponsored by the National Institutes of Health. In order to participate in this study, it will be necessary to give your written consent.

WHAT SHOULD I KNOW BEFORE I DECIDE WHETHER TO TAKE PART IN THIS STUDY

- Research studies help us to learn new things and test new ideas about treating certain conditions/diseases.
- Taking part in a research study is voluntary. You decide if you want to take part, and you can stop taking part at any time. Your regular medical care at the University of Missouri Hospitals and Clinics will not be affected now or in the future if you decide you do not want to be in this study.
- We are doing this study because we want to learn if taking the drug amiloride will improve blood vessel stiffness in overweight and obese subjects when compared to placebo.
- You are being asked to take part in this study because you may have characteristics of increased vascular disease risk.
- About 159 people will take part in this study at the University of Missouri.
- If you take part in this study, you will come to the Clinical Translational Science Unit (CTSU) for all scheduled in person visits. The CTSU, located on the 5th floor of the University Hospital and has clinic rooms specially designed for research. You will have vital signs, blood tests, DEXA scans, vascular measurements, questionnaires and physical exams completed during this study. We will explain these procedures in this form.
- If you join this study, you will not have to stop any current medical treatment for as long as you are in the study.
- The total amount of time you could be in this study is about 6 months.
- Taking part in this study may or may not make your health better. We hope that the information we learn from this study will help in the future treatment of people with increased vascular risk. **There is no guarantee that taking part in this research will result in any improvement in your condition.**
- As with any research study, there are risks that we know about and there may be some we don't know about. We will explain these risks in this form.
- We will only include you in this study if you give us your permission first by signing this consent form.

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PURPOSE OF THE RESEARCH

In this study, we want to find out more about a drug, called amiloride (also called the study drug in this form), for people with risks factors for blood vessel stiffness. We want to find out if amiloride reduces blood vessel stiffness.

Amiloride is approved by the U.S. Food and Drug Administration (FDA) for use but not specifically for patients with risks factors for blood vessel stiffness.

We are testing amiloride to see what effect it has on patients with risk factors for blood vessel stiffness. We don't know if this study will help you. Your condition may get better, but it could stay the same or even get worse. We hope the information we learn from this study will help us to develop a better treatment for blood vessel stiffness in the future.

WHY IS THIS STUDY BEING DONE AND HOW MANY PEOPLE WILL TAKE PART?

The goal of this study is to determine if a drug, amiloride, that blocks a channel called the epithelial sodium channel will improve blood vessel stiffness in overweight and obese subjects when compared to placebo. Our plan is to administer this medication for a period of 6 months by having you take the study medication and assess changes in the stiffness of your blood vessels. The tests performed are not part of standard medical care. About 400 people will be screened and 159 people will complete this study at the University of Missouri.

WHAT IS INVOLVED IN THE STUDY?

In general, this research is measuring how amiloride may impact cardiovascular health in the setting of overweight, obesity and insulin resistance. Based on your medical history, you are eligible to participate. If after obtaining the results of your blood tests you are found to be eligible, you will be assigned to the amiloride or to placebo group, which means you will be randomized to an active treatment (amiloride) or to a placebo treatment. If you chose to participate, you will be enrolled into a research study. As described below, this research includes today's consenting visit followed by 8 scheduled study visits over a 6-month period.

STUDY PLAN

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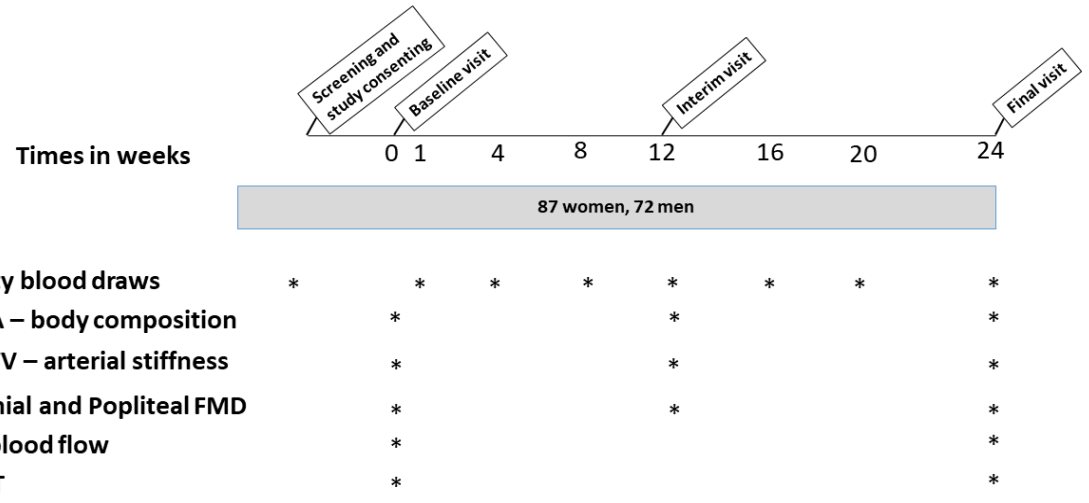
Figure 1



The following paragraphs describe the schedule and details of the procedures, study visits, and blood draws you will undergo while participating in this research study (**Figure 1 and 2**).

CONSENTING/SCREENING VISIT

Research staff will meet with you to review the study procedures, answer questions, sign this consent form, review your medical history, collect your date of birth (DOB). This visit will be located at the Clinical Translational Science Unit (CTSU) and requires you to fast for 12 hours (no food or drink, except water). The CTSU has clinic rooms specially designed for research. On testing days, you will arrive at your scheduled time after an overnight fast (no food or drinks, except water), having taken no morning medications and not exercised for 24 hours. The consenting visit will take up to 1 hour. Your waist circumference, height, weight, and vital signs will also be measured during this visit and blood will be drawn to check your general health (total amount about 2 tablespoons).

Within a week after the screening visit, we will receive the results of all screening tests and someone on the study team will go over the results with you. Since this is not a treatment study, these lab tests are not being used for standard medical care. Upon your request, you will be given a copy of the results which you can share with your primary physician.

If your screening results qualify you to be in the study, and if you are interested in proceeding with the study, you will be scheduled for a baseline visit. Again, you will come to the CTSU where parking is provided.

The timing of the study visits will occur at times that are agreeable to you and to the research staff (**Figure 1**). This consent form will explain your responsibilities during the study and the details of the procedures you will undergo.

Whether you receive Amiloride (5 mg) or placebo will be determined by chance. A placebo looks like the study drug but has no active medication. Randomization means that you are put into a group by chance. It is like flipping a coin. Neither you nor the researcher team will choose what group you will be in. You will have a one in two chance of being placed in any group

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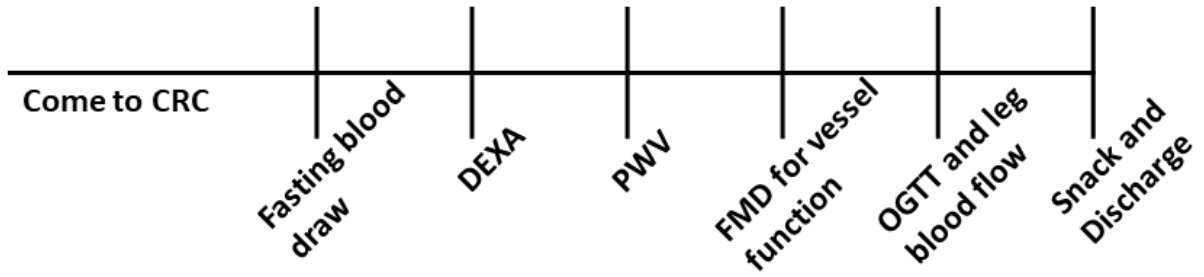
PROCEDURES

The following paragraphs describe the schedule for the study visits (**Figure 1 and Figure 2**).

Figure 2



BASELINE VISIT:

For 12 hours before the visit, do not use caffeine, alcohol, and vitamin supplements. You will come in after fasting overnight for 12 hours - no food or drink, except water. Please do not exercise the morning of this visit. This visit will take up to 6 hours. You will have measurements made of your weight, heart rate and blood pressure, and you will undergo several procedures.

During the visit you will have a DEXA scan to assess your body composition. Female subjects will have a pregnancy test prior to this scan (positive test is a contraindication to continue the study). Prior to and during the oral glucose tolerance test (OGTT) you will have vascular function measurements done.

During this visit you will also undergo an oral glucose tolerance test (OGTT) with measurements of leg blood flow. A venous catheter will be placed in the forearm for blood sampling and a baseline sample will be taken. Thereafter, you will consume a sugary drink which has 75 g of glucose. You will remain in reclined position during the following 2 hours. Blood draws will be taken every 15-30 min for measures of glucose and insulin. Total amount of blood drawn during this procedure will be about 3.5 tablespoons.

We will measure the blood flow to your leg by placing an ultrasound probe over the femoral artery during the OGTT. The probe will provide a measure of the speed at which your blood is traveling through your artery. OGTT will be completed when the sugary drink (glutol) is available. If not available, this portion of the study will not be done.

After the procedures are completed you will be offered a snack.

If all baseline procedures are successful, you will begin your 6-month drug treatment, overseen by the study physician, Dr. Camila Manrique. You will receive a pill bottle of drug and Dr. Manrique will instruct you how many pills to take daily.

Details on procedures:

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Body composition:

At baseline, interim and final visits, you will have a DEXA (Dual Energy X-ray Absorptiometry), which is a procedure to measure your body composition - how much fat and muscle your body has. It is a type of x-ray machine with a moving arm. This scan will expose you to a small amount of radiation. The amount of radiation received during the DEXA scan is less than that of an airline flight from California to New York and back.

Vascular function measures:

A blood pressure cuff will be placed around the forearm. This cuff will be inflated up to 250 mmHg, as is done when your blood pressure is being measured, but instead of deflating the cuff immediately it will remain inflated for 5 minutes. We will measure the blood flow to your arm and leg by placing an ultrasound probe over the brachial artery (upper arm artery) and popliteal artery (back of the leg) before, during and after inflating the cuff. The probe will provide a measure of the speed at which your blood is traveling through your artery and the extent to which your artery dilates (i.e., expands). A pressure sensor (tonometer, the size of a pencil) will be placed over the skin of the neck region to obtain the pressure wave form in the carotid artery. We will also measure the blood flow to your leg during the OGTT.

DOSE MONITORING VISITS:

After your baseline visit, you will be seen at 1 week, 4 weeks, 8 weeks, 16 weeks and 20 weeks after starting the medication to check your vital signs, safety questionnaire for side effects, and have your blood drawn (about 2 teaspoons) to monitor the kidney function and the potassium levels in your blood.If the prior safety visit labs have been within the study protocol ranges, visits at week 8, 16 and 20 may be completed as a phone call visit to collect safety questionnaire for side effects and any changes to your health. Vital signs, safety questionnaire for side effects and safety blood draws of up to 3 teaspoons may be taken at unscheduled safety visits. These visits will take place at the CTSU. Because you do not have to fast for this blood draw, these visits can be scheduled any time at your convenience. Dr. Manrique will review your blood test results and contact you by phone with instructions. Dr. Manrique will discuss with safety officer of this study if there are any safety concerns. Depending on your blood work and the responses to the safety questionnaire, the study medication can be put on hold on certain occasions, then laboratory values will be rechecked and a lower dose of the study medication can be restarted. An unscheduled visit to repeat safety labs may also occur if there are any issues with the processing of the labs or if there are any results with questionable accuracy.

STUDY MEDICATION

It is very important that you bring your study medication to the site at each visit – this will allow your doctor to confirm that the correct number of tablets has been taken.

INTERIM VISIT:

At 3 months, this visit will occur at the CTSU. You will come in fasting overnight for 12 hours; no food or drinks besides water, no caffeine, alcohol, or vitamin supplements.
Please do not exercise the morning of this visit. This visit will include blood drawing (about 2 tablespoons**)**, vital signs, weight, safety questionnaire for side effects, vascular function measurements and DEXA. Female subjects will have a pregnancy test prior to the DEXA scan (positive test is a contraindication to continue the study). You will be given a new bottle of medication and will continue for another 3 months. This visit will take up to 3 hours.

FINAL VISIT:

As you did with the baseline visit 1, you will come in fasting overnight for 12 hours; no food or drinks

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besides water, no caffeine, alcohol, or vitamin supplements. Please do not exercise the morning of this visit. This visit will be the exact same as the baseline visit, with all of the procedures being repeated plus additional blood drawn for safety labs, waist circumference, and safety questionnaire for side effects. This visit will take up to 6 hours and you will have about 4.5 tablespoons of blood drawn. The OGTT will be done when the sugary drink (glutol) is available in a similar volume as used in the baseline visit. If not available then this portion of the study will not be done. Following this visit, your participation in the study is completed.

WHAT ARE THE PROCEDURES AND RISKS OF BEING IN THIS STUDY?

BLOOD DRAWING AND ITS RISKS

During this study you will have blood drawn through a needle. Risks: Drawing blood from your arm can cause minimal discomfort and/or bruising. Infection, excess bleeding, clotting, and/or fainting also are possible, although unlikely. As a result of your participation in this study you will have given blood. If you wish to perform other research after you finish this project, you should let the investigator know that you have donated 8 oz. Your blood volume will be checked during screening to make sure that your volume is in safe limits.

INSERTION OF VENOUS CATHETERS

The potential risks of venous catheterization include infection, swelling and discomfort at the catheter insertion sites. Some bleeding may occur during the insertion of the catheters as well after the catheters have been removed. There is also the possibility of fainting, dizziness, and possible pain and bruising as a result of catheter insertion. These risks will be greatly minimized by using sterile procedures and having an experienced registered nurse placing the venous catheters.

ORAL GLUCOSE TOLERANCE TEST

This procedure could cause possible nausea from the sugary glucose drink.

BLOOD PRESSURE CUFF INFLATION AND VASCULAR FUNCTION MEASURES

The blood pressure cuff will squeeze your arm or leg tightly; however, any discomfort will be alleviated as soon as the pressure in the cuff is released.

MEASUREMENT OF BODY COMPOSITION BY DEXA

This procedure involves lying on a table for 15 minutes while the DEXA machine passes over your body. Although you will need to remain very still and quiet, you will feel nothing and should have no discomfort.

Risks: If you have participated in any other research study involving ionizing radiation exposure in the past 12 months, discuss this with the Investigator to determine if you are eligible to participate in this study. You will be exposed to a small radiation dose which is about 2% of the average radiation dose from all sources (natural background radiation, consumer appliances, radon gas, medical tests, etc.) that a person receives in the United States receives each year. However, radiation effects are cumulative. You

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should always inform future doctors of your participation in this study. For female participant, a urine pregnancy test will be performed before the scan.

STUDY DRUG: AMILORIDE

Amiloride is a common prescription drug given to patients with high blood pressure or heart disease. You will take amiloride as prescribed by the study physician, and the dose you are instructed to take will not change throughout the study. Amiloride is an FDA approved drug that is being used in an off label manner for the study.

Side effects: dizziness, fatigue, headache, high potassium. Your kidney function will also be closely monitored during the study to make sure levels are stable. After each safety visit, Dr. Manrique will review blood pressure and heart rate readings, as well as results of renal function and electrolyte levels (potassium, sodium and calcium). Dr. Manrique will reach out to the safety officer of this study in case of any concern and you will be informed regarding need for follow up blood work and potential need for holding the medication and/or starting a lower dose.

PLACEBO: PILL CAPSULE

There is a potential risk of an allergic reaction to the gelatin capsule.

BEING IN THE CONTROL/PLACEBO GROUP

As you are overweight or obese, you have increased risk for arterial stiffening. Currently, there is no clear proof that treating arterial stiffening impacts your overall risk of heart disease. For this research to be successful, it is important for us to monitor your vascular health over the next 6 months to determine if any changes occur naturally with aging.

CONSTANT DIET AND ACTIVITY

You will receive a drug or a placebo. For the researchers to determine the effect of that drug, we ask that you avoid making major changes to your diet and exercise regimens.

WHAT WILL BE MY RESPONSIBILITIES DURING THE STUDY AND ARE THERE BENEFITS?

While you are part of this study, the researchers will follow you closely to determine whether there are problems. It is your responsibility to do the following:

- Ask questions about anything you do not understand.
- Keep your appointments.
- Follow the researchers' instructions.
- Let the researchers know if your telephone number or address changes.
- Tell the researchers before you take any new medication, even if it is prescribed by another doctor for a different medical problem, or purchased over the counter.
- Report to the researchers any injuries or illnesses while you are on the study, even if you do not think they are related.

If you agree to take part in this study, there may not be direct medical benefit to you. You may also

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expect to benefit from taking part in this research to the extent that you are contributing to medical knowledge. We hope the information learned will benefit people at risk of heart disease in the future.

WILL YOU SHARE WITH ME ANY RESULTS OR HEALTH PROBLEMS/ISSUES THAT YOU LEARN ABOUT ME WHILE IN THE STUDY?

If we find any clinically relevant research results that include results about you, we will inform you as soon as possible. If the investigators identify potential health problems or issues as part of the research, you will be provided with this information and, with your permission, the investigators will share this information with your primary care physician or other healthcare provider of your choice. It is possible that you may need testing or treatment for a new or existing health condition. You and/or your health plan/insurance are responsible for the costs of this testing or treatment.

WHAT ARE THE COSTS?

You will not be charged for any procedures that are part of this research study. Parking will be provided but there is no compensation for travel to our facilities or for childcare during this study.

WILL I BE PAID FOR PARTICIPATING IN THE STUDY?

You will be compensated a total of $1005 for completing this study: $30 for screening visit, $100 will be given for the baseline visit, $50 for week 1, $100 for week 4, $75 week 8, $150 for week 12 (interim), $75 for week 16, $75 for week 20, and $350 for the final visit (week 24). Additional, unscheduled safety visits will be compensated at $50/ visit. Checks are sent to you through the mail and usually take 1-2 weeks to arrive.

We will need your Social Security Number in order to pay you. Any payment may need to be reported as income on your tax return. If you are not a resident/citizen (non-resident alien) of the United States, you will need to work with the MU Nonresident Tax Specialist at 573-882-5509.

WHAT OTHER OPTIONS ARE THERE?

You do not have to participate in this study

WHAT ABOUT CONFIDENTIALITY?

Information produced by this study will be stored in the investigator's file and identified by a code number only. The code key connecting your name to specific information about you will be kept in a separate, secure location. Information contained in your records may not be given to anyone unaffiliated with the study in a form that could identify you without your written consent, except as required by law. It is possible that your medical and/or research record, including identifying information, may be inspected and/or copied by the study sponsor (and/or its agent), the Food and Drug Administration (FDA), federal or state government agencies, MU Health Sciences IRB, or hospital accrediting agencies, in the course of carrying out their duties. If your record is inspected or copied by the study sponsor (and/or its agents), or by any of these agencies, the University of Missouri will use reasonable efforts to protect your privacy and the confidentiality of your medical information. The results of this study may be published in a medical journal or used for teaching purposes. However, your name or other identifying information will not be used in any publication or teaching materials without your specific permission.

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WHAT IF I AM INJURED?

It is not the policy of the University of Missouri to compensate human subjects in the event the research results in injury. The University of Missouri, in fulfilling its public responsibility, has provided medical, professional and general liability insurance coverage for any injury in the event such injury is caused by the negligence of the University of Missouri, its faculty and staff.
The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to subjects who suffer injuries while participating in the research projects of the University of Missouri. In the event you have suffered injury as the result of participation in this research program, you are to contact the Risk Management Officer, telephone number (573) 882-1181, at the Health Sciences Center, who can review the matter and provide further information. This statement is not to be construed as an admission of liability.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Participation in this study is voluntary. You do not have to participate in this study. Your present or future care will not be affected should you choose not to participate. If you decide to participate, you can change your mind and drop out of the study at any time without affecting your present or future care at the University of Missouri. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. In addition, the investigator of this study may decide to end your participation in this study at any time after she has explained the reasons for doing so and has helped arrange for your continued care by your own doctor, if needed. You will be informed of any significant new findings discovered during the course of this study that might influence your health, welfare, or willingness to continue participation in this study.

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

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions regarding your rights as a participant in this research and/or concerns about the study, or if you feel under any pressure to enroll or to continue to participate in this study, you may contact the University of Missouri Institutional Review Board (which is a group of people who review the research studies to protect participants' rights) at (573) 882-3181 or email muresearchirb@missouri.edu.

If you want to talk privately about your rights or any issues related to your participation in this study, you can contact University of Missouri Research Participant Advocacy by calling 888-280-5002 (a free call), or emailing MUResearchRPA@missouri.edu. You may ask more questions about the study at any time.

For questions about the study or a research-related injury, contact Dr. Camila Manrique at (573) 529-1141. A copy of this consent form will be given to you to keep.

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SIGNATURE

I confirm that the purpose of the research, the study procedures, the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Alternatives to my participation in the study also have been discussed. I have read this consent form and my questions have been answered. My signature below indicates my willingness to participate in this study.

_____ _____

Subject Date

Signature of Study Representative

I have explained the purpose of the research, the study procedures, identifying those that are investigational, the possible risks and discomforts as well as potential benefits and have answered questions regarding the study to the best of my ability.

_____ _____

Study Representative Date

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