BIOMEDICAL RESEARCH PROTOCOL
UNIVERSITY OF MISSOURI

Project Title: Estrogen receptor alpha signaling in endothelial cells exacerbates arterial stiffening via upregulation of ENaC in insulin resistant females
IRB Number: 2012990
Version Number: 24
Version Date: October 30, 2023
Principal Investigator: Camila Manrique
Funding Source: ***NHLBI***

Clinical Trial Phase: *Phase II*
Clinicaltrials.gov Number: NCT03837626
Study Drug/Study Device: *Amiloride*

I. Research Objectives/Background

Hypothesis: Estrogen action, through the endothelial cell (EC) estrogen receptor alpha (ERα) upregulates EC ENaC expression and activity exacerbating endothelial and arterial stiffening in overweight and obese, insulin resistant (IR) premenopausal females. The corollary to this hypothesis is that ENaC inhibition will have a greater impact on arterial stiffness in overweight and obese, premenopausal insulin resistant women than in overweight and obese IR postmenopausal women or age matched obese IR men.

To test our hypothesis, we will use in vivo measures of endothelial function and arterial stiffness in a cohort of overweight and obese IR subjects treated with amiloride, an ENaC inhibitor, to accomplish the follow aim:

To determine whether treatment with the ENaC inhibitor, amiloride, improves endothelial function and arterial stiffness in overweight and obese IR subjects in a randomized placebo-controlled trial examining pre and postmenopausal women and age-matched men.

II. Drugs/Biologics/Devices

Following screening, male and female subjects will be randomized to a doubled blinded placebo control trial. Subjects will start on either a 24-week treatment with oral medication, either amiloride, a max dose of 5 mg daily, or placebo intervention.
Amiloride therapy: In previous clinical trials with higher doses (10 mg) amiloride was well tolerated.[1] Nevertheless, our team of investigators is well aware of the potential side effects (1-10%): dizziness, fatigue headache, hyperkalemia. Subjects will be closely monitored during the drug/placebo treatment intervention. After each safety visit, the PI will review blood pressure and heart rate readings, as well as results of renal function and potassium levels. The treatment will be held in cases of hyperkalemia > 5.5 mEq/L, creatinine increases more than 30% above baseline, hypotension or severe dizziness. If the subject exhibits hyperkalemia or if creatinine increases more than 30% above baseline, the study team will repeat safety labs until they return to baseline. If a subject exhibits potassium > 5.5 mEq/L, creatinine increases more than 30% above baseline, hypotension or severe dizziness on the 5 mg dose, medication will be held for 1 week and a lower dose of amiloride will be started (half of a 5mg tablet). Once the dose of a half of a 5mg tablet has been started, a safety visit will be completed 1 week later. If no clinically significant abnormalities are documented, safety visits will proceed as scheduled for the 5 mg dose. In the event of a severe adverse event the subject will be unblinded to facilitate medical care.

Placebo therapy: The use of placebo and blinding of study team prevents bias of the investigator and study team as well as providing a control for the study. There are two components to the placebo, one being the cellulose found inside the capsule and the other being the gelatin pill capsule used for all study pills being dispensed.

An Investigational New Drug (IND) is not included as part of this protocol because the study medication, amiloride is being used as an FDA approved dose in a population that is not anticipated to have increased risk or decreased acceptability of risk. Lastly, no information will be submitted to the FDA to support a labeling indication, a labeling change, or a change in advertising, as this is not the intent of the current study.

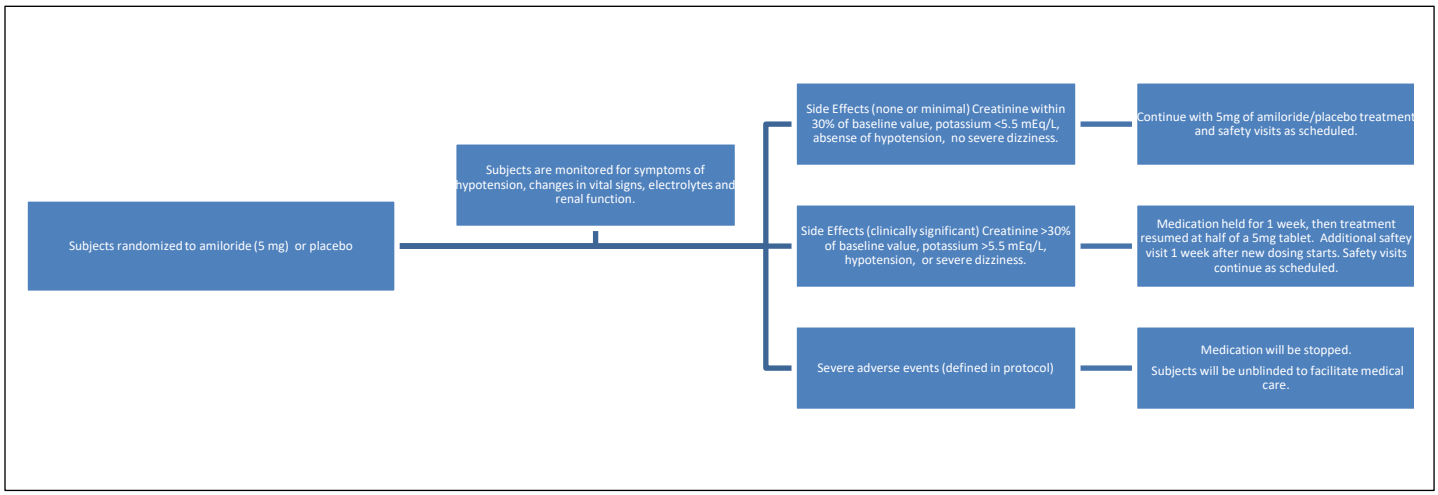


Figure 1. Treatment Flow Chart based on management of side effects and adverse events.

III. Recruitment Process

Subjects will be recruited by a number of means.

1. A separate informatic screen will be completed to identify clinic patients from the Division of Endocrinology, the Department of Medicine and the Department of Family Medicine who are between the ages of 30 and 70, overweight or obese and prediabetic. A letter will be sent notifying them of their eligibility to participate in a research study designed to lower future risk of heart disease risk.
2. Subjects may also respond to the recruitment flyer or advertisement. For all recruitment, subjects will be given an email address and/or a phone number to contact. Subjects may also be given a link to complete an informational screening via Qualtrics with their waiver of documentation of consent.
3. The recruitment material will be posted around University of Missouri campus, as well as other public places such as swimming pools, churches, and grocery stores. We will advertise through MU Info, TV slides throughout the University of Missouri, other MU provided resources, local newspapers, local TV stations and via multiple social media sources. Additional avenues of advertisement might become available throughout the study, and we will utilize only the approved recruitment material information. We also plan to use the service called Studykik (https://studykik.com/), which is a clinical trial recruitment site. No data are collected using this site - it is only used to provide potential subjects with information about the study and provide staff contact information. We will use Washington University School of Medicine StudySearch Website (an online listing of research studies). In addition, we will use Washington University Research Participant Registry (RPR) -an IRB approved database of participants who have consented to be contacted for research studies and social media I.E. Facebook for assistance with recruitment. We will used Research, Innovative and Impact website for additional recruitment

(https://research.missouri.edu/). We will use Research Match. We will used BuildClinical to manage digital marketing recruitment for this study. BuildClinical is a data-driven digital marketing company that helps academic researchers recruit participants for research studies more efficiently using social media, software, and machine learning. They will ensure that all IRB-approved guidelines and procedures are followed during recruitment. They use study-specific advertisements to engage participants on digital platforms such as Facebook, Google, WebMD, etc. Participants who click on the advertisements will be redirected to a study-specific landing page. On the landing page, the person can complete an online pre-screen questionnaire. Pre-screening data is then routed to BuildClinical's Secure Socket Layer (SSL) software, which encrypts all information to keep it private and HIPAA compliant. Their backend servers are stored in secure data centers in the USA. All currently approved advertisement could potentially be used with any of the above listed means of recruitment.

IV. Consent Process

One consent form will be used that describes in detail the amiloride/placebo study.

Telephone/Qualtrics screening
Subjects who contact us via phone or email to inquire about participation will answer screening questions via phone or Qualtrics questionnaire, with their waiver of documentation of consent. These questions are for preliminary screening only and are not used as study data since the data represent self-report. The questions include queries about health history/habits, age, sex, height, weight, tobacco use, medications/ supplements, over the counter medications use, illness, chronic conditions, and family history of diabetes or heart disease. The study design is described in general terms to subjects, with mention of factors most likely to impact subject interest in participating. If the subject is interested in being screened for participation, we then make an appointment for a screening visit.
Consenting/screening visit
The first visit to the Clinical Translational Science Unit (CTSU) will take up to an1 hour and include a detailed written consent for the study. Review of the consent form will occur with study personnel in a quiet, unhurried setting. Comprehension will be assessed by asking the subjects to explain the study in their own words. Participants will have adequate opportunity to review the informed consent and to ask any questions they may have about the research protocol, compensation, risks, and benefits of taking part in the study.
After signing the consent form, medical information is obtained by the nurse or physician including DOB, gender, ethnic/racial category, height, body weight (history of body weight gain or loss), vital signs, waist circumference, and medical history is obtained using a questionnaire. A fasting blood draw is taken to test for biochemistries (volume no more than 25 mL). Eligibility will be communicated with subjects following the screening visit and baseline appointments will be scheduled accordingly. Upon request subjects will be provided with a copy of the results.

V. Inclusion/Exclusion Criteria

Inclusion criteria:

- 30 to 70 years of age at randomization
- Body mass index (BMI) 25.1-50 kg/m2 or waist circumference > 88 cm (> 35 in) in women and >102 cm (>40 in) in men. [3, 4]
- One other characteristic of metabolic syndrome (elevated triglycerides ≥150 mg/dl; HDL cholesterol <40 mg/dl in men and <50 mg/dl in women; blood pressure ≥130/85 mm Hg or treatment for hypertension; impaired fasting glucose (≥100 mg/dl)) or fasting insulin level >10 mU/L (correlates with insulin resistance).

Subjects will be excluded if there is evidence of:

1. History of type 1 or type 2 diabetes
2. Known cardiovascular events within the last 12 months (stroke, acute coronary event, revascularization, heart failure hospitalization).
3. History of uncontrolled thyroid disease, chronic liver disease (cirrhosis) or GFR <50 ml/min.
4. Use of potassium sparing medications (angiotensin II receptor blockers, angiotensin converting enzymes inhibitors or mineralocorticoid receptor blockers) or use of potassium supplements.
5. Active cancer (This criterion does not apply to those subjects with basal cell carcinoma or stage 1 squamous cell carcinoma of the skin)
6. Excessive alcohol consumption (>14 drinks/week for men, >7 drinks/week for women)
7. Current tobacco use
8. Non controlled hypertension
9. Participation in regular exercise > 3 days/wk per week at a moderate or vigorous intensity
10. Pregnancy or lactation in women (or women not using contraceptives)
11. Women who plan to become pregnant during the duration of the trial
12. Chronic use of NSAIDs
13. Potassium level > 5.0 mqE/L at time of screening
14. Blood pressure at screening <110/70

VI. Number of Subjects

Men (n=72) and women (n=87) (total=159 overweight/obese subjects) with IR will be recruited for this study. Postmenopausal women are those who have not menstruated for at least **6 months.**[5, 6]

Table 1 presents the estimated sample sizes necessary to test the response to amiloride and compare the

Table 1. Repeated-measures design for amiloride treatment	**Baseline and treatment cfPWV (m/s)**	**SD of the Δ**	**Power**	**Alph**	**Calculated sampled size**	**Final recruitment target 2:1 Amiloride**	**Placebo**
1. Premenopausal women	7.2 --> 6.2	1.2	90%	0.05	30	34	17
2. Young men (30-50 y)	7.1 --> 6.6	0.5	90%	0.05	21	24	12
3. Old men (51-70 y)	8.0 --> 7.5	0.5	90%	0.05	21	24	12
4. Postmenopausal women	8.0 --> 7.5	0.5	90%	0.05	21	24	12

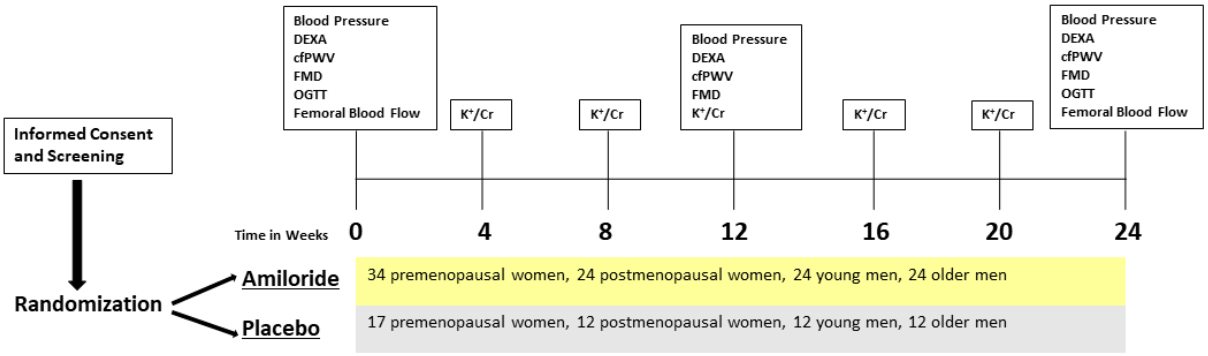
responses of men and women. We hypothesize that the improvement in carotid femoral pulse wave velocity (cfPWV) in men will be intermediate between the two female groups. Because of the negative effect of estrogen-stimulated ENaC activation, we believe that amiloride will benefit premenopausal women more (a Δ, reduction of 1.0 m/s) than the other groups. Our sample size also allows for a greater variation in the response of premenopausal women due to the effects of reproductive hormones in this group[7] (SD of the change of 1.2 m/s, **Table 1**). By contrast, we anticipate that the response of post-menopausal women (**Table 1**) will be equal to that of the older men or could even be the lowest of all the groups, given a lesser contribution of estrogen signaling in the activation of ENaC in this population. In summary, the final sample sizes listed below will give 90% power to detect each group's changes in cfPWV.

VII. Study Procedures/Design/Treatment Plan

Planned visits:

Screening visit: Written consent, DOB, gender, ethnic/racial category, height, waist circumference, body weight (history of body weight gain or loss, vital signs, medical history and screening laboratories. Entire visit

Figure 2



is expected to take up to an 1 hour.

Visit #1: Vital signs (blood pressure and heart rate), weight, DEXA, assessment of brachial and popliteal artery flow-mediated dilation (FMD), cfPWV, femoral artery blood flow, and oral glucose tolerance test (OGTT) (amount of blood drawn not exceeding 55 mL). OGTT will be done when glucose beverage is available. Entire visit is expected to take up to 6 hours. Women of childbearing age will undergo a pregnancy test prior to the DEXA scan and excluded if pregnancy is confirmed. Subject is dispensed the bottle of study drug/placebo and instructed how to take them.

Visit #2 (1 week after starting the medication +6 days/-2 days): Vital signs, safety questionnaire for side effects, and blood draw for safety laboratories (basic metabolic panel). Entire visit is expected to take up to 30 minutes.

Visit #3 (4 weeks after starting the medication +/- 6 days): Vital signs, safety questionnaire for side effects, and blood draw for safety laboratories (basic metabolic panel). Entire visit is expected to take up to 30 minutes.

Visit #4 (8 weeks after starting the medication +/- 6 days): Vital signs, safety questionnaire for side effects, and blood draw for safety laboratories (basic metabolic panel). Entire visit is expected to take up to 30 minutes. If the prior safety visit labs have been within the protocol parameters this visit may be completed as a phone call visit to collect safety questionnaire for side effects and any changes in the subject's health.

Visit #5 (12 weeks after starting the medication +/- 6 days): Vital signs, weight, DEXA, safety questionnaire for side effects, and blood draw for safety laboratories (basic metabolic panel) and general biochemistries. Subject also will have assessment of brachial and popliteal artery FMD and cfPWV. Women of childbearing age will undergo a pregnancy test prior to the DEXA scan and withdrawn from study if pregnancy is confirmed. Subject will be dispensed a new bottle of study drug/placebo. Entire visit is expected to take up to 3 hours.

Visit #6 (16 weeks after starting the medication +/- 6 days): Vital signs, safety questionnaire for side effects, and blood draw for safety laboratories (basic metabolic panel). Entire visit is expected to take up to 30 minutes. If the prior safety visit labs have been within the protocol parameters this visit may be completed as a phone call visit to collect safety questionnaire for side effects and any changes in the subject's health.

Visit #7 (20 weeks after starting the medication +/- 6 days): Vital signs, safety questionnaire for side effects, and blood draw for safety laboratories (basic metabolic panel). Entire visit is expected to take up to 30 minutes. If the prior safety visit labs have been within the protocol parameters this visit may be completed as a phone call visit to collect safety questionnaire for side effects and any changes in the subject's health.

Visit #8 (24 weeks after starting the medication +/- 6 days): Vital signs (blood pressure and heart rate), weight, DEXA, waist circumference, safety questionnaire for side effects, and blood draw for safety laboratories (basic metabolic panel, lipid panel, and hemoglobin and hematocrit). Subject will also have assessment of brachial and popliteal artery FMD, cfPWV, femoral artery blood flow and oral glucose tolerance test (OGTT) (amount of blood drawn for safety labs, other biochemistries and OGTT not to exceed 70 mL). OGTT will be done when glucose beverage (75g) is available in a volume within 50ml of what was used in the baseline visit. These will minimize variability of the measurements related to changes in the volume of liquid ingested. Women of childbearing age will undergo a pregnancy test prior to the DEXA scan and withdrawn from study if pregnancy is confirmed. Entire visit is expected to take up to 6 hours.

Additional Safety Visits/unscheduled visits might be scheduled in cases of down titration of amiloride dose. Vital signs, safety questionnaire for side effects, and blood draw for safety labs (amount not to exceed 15mL). Unscheduled visits may also occur if safety lab specimens are compromised for any reason or questionable accuracy of the result. Safety labs will then be repeated (amount of blood drawn not to exceed 15 mL).

Medication changes and adverse events collected through out subjects' participation.

Special considerations in case of unforeseeable hardships such as public health emergencies or weather-related events.

In the event of subjects not being able to complete study visits for the reasons described above the following remedial steps will be taken:

- Visits #3,4, 6 and 7 can be completed via a phone interview during which the subject will answer the safety questionnaire (no changes in compensation).
- In case that subject cannot complete safety visit #2, every effort will be made to complete this visit within 7 days of the original scheduled date. In case that the visit cannot be completed, the subject will be instructed to discontinue study medication and his/her participation in the study will be completed. Safety officer will be informed.
- In case that study visit #5 cannot be completed and providing patient has completed at least 2 in-person visits since the medication was started, study medication will be mailed by investigational pharmacy for subject to continue treatment. Safety officer will be informed. Compensation will be unchanged.

In the event of the Clinical Translational Science Unit (CTSU) not being available or if it is considered by the study physician that performing studies there can increase risk of exposure to infectious agents or related hazards, the following alternative sites will be made available for screening, safety and study visits.

- MU Physical Activity and Wellness (PAW)
- University hospital clinic rooms

These locations are not carpeted, and we do not anticipate an increased risk for the subjects given that medical supervision and nursing staff assistance will be unchanged.

Detail on procedures:

DEXA scan: to assess body composition with dual-energy X-ray absorptiometry.

Oral glucose tolerance test: After an overnight fast, a catheter will be inserted into an antecubital vein for sampling of venous blood. Additional blood will be collected every 15-30 mins over the next 120 mins after consuming the glucose beverage (75 grams of dextrose).

Arterial Stiffness and Blood Pressure: The SphygmoCor XCEL device will be used to assess blood pressure and aortic pulse wave velocity, a marker of arterial stiffness. A blood pressure cuff will be wrapped around the upper arm and upper leg. They will periodically inflate to ~200 mmHg for less than 60 seconds. A pressure sensor (tonometer) will be placed over the skin of the neck region to obtain the pressure wave form in the carotid artery. This measure will be performed in duplicate.

Brachial and Popliteal artery FMD: Arterial measurements will be performed by imaging the brachial and popliteal artery longitudinally using high-resolution duplex ultrasonography. Brachial and popliteal artery vasodilatory responses to hyperemia (i.e. flow mediated dilation) will be examined by inflating a cuff, distal to the antecubital fossa up to 250 mmHg for 5 minutes. Before, during and after rapid release of the cuff, brachial artery blood flow velocity and diameter will be continuously measured. Before, during and after rapid release of the cuff popliteal, artery blood flow velocity and diameter will be continuously measured. This measure will be performed in duplicate.

OGTT with leg blood flow: After an overnight fast, a catheter will be inserted into an antecubital vein for sampling of venous blood. Blood will be collected every 15-30 mins over the next 120 mins after consuming the glucose beverage (75 grams of dextrose). Moreover, before and during the OGTT, we will be using an ultrasound to measure femoral artery blood flow.

VIII. Potential Risks/Adverse Events

Phlebotomy: The total blood withdrawn will not exceed 550 mL in any 24-week period. Hematocrit is measured during screening and before the interim and final visits to check that it is within safe limits. All subjects will be advised to refrain from donating blood in public blood drives, during their participation in this project.
These activities are associated with a small risk of phlebitis, bruising and minor pain. Antiseptic technique will be used by nurses who are experts in phlebotomy and catheter placement. If phlebitis occurs, it will be treated conservatively with heat and when appropriate, with antibiotics.
Carotid-femoral PWV and femoral artery blood flow: These procedures pose no risks.
Brachial and Popliteal artery FMD: FMD is a measurement of conduit artery endothelial function. There is risk of minor discomfort in the arm during the five minutes of cuff inflation.
Oral Glucose Tolerance Test (OGTT): This procedure could cause possible nausea from glucose beverage.
DEXA and anthropometrics: Radiation exposure during DEXA is equivalent to about two percent of the average radiation dose from all sources (natural background radiation, consumer appliances, radon gas, medical tests, etc.) that a person in the United States receives each year. Subjects who have participated in any other research study or medical procedure involving significant ionizing radiation exposure (e.g., multiple chest x-rays) in the past 12 months will be excluded. For female subjects, a urine pregnancy test is administered before the DEXA. Body weight is measured to the nearest 0.1 kg and height to the nearest 0.1 cm.
Amiloride therapy: In previous clinical trials with higher doses (10 mg) amiloride was well tolerated.[1] Nevertheless, our team of investigators is well aware of the potential side effects (1-10%): dizziness, fatigue headache, hyperkalemia. Subjects will be closely monitored during the drug/placebo treatment intervention. After each safety visit, Dr. Manrique and Dr. Bostick will review blood pressure and heart rate readings, as well as results of renal function and electrolyte levels. The treatment will be discontinued in case of hyperkalemia > 5.5 mEq/L, creatinine increases more than 30% above baseline, hypotension or severe dizziness. If the subject exhibits hyperkalemia or if creatinine increases more than 30% above baseline, the study team will repeat safety labs until they return to baseline. If a subject exhibits potassium > 5.5 mEq/L, creatinine increases more than 30% above baseline, hypotension or severe dizziness on the 5 mg dose, medication will be held for 1 week and a lower dose of amiloride will be started (half of a 5mg tablet). Once the dose of half of a 5mg tablet has been started, a safety visit will be completed 1 week later. If no clinically significant abnormalities are documented, safety visits will proceed as scheduled for the 5 mg dose. If there are clinically significant abnormalities

documented after down titration of amiloride, subjects will discontinue medication and participation in the clinical trial is terminated. In the event of a severe unexpected adverse event the subject will be unblinded to facilitate medical care.
Placebo: Pill Capsule: There is a potential risk for an allergic reaction to the gelatin capsule.
Placebo: Cellulose: These are no known risks.

Protection Against Risks
Risks to loss of confidentiality are reduced by assigning all subjects a data identifier code. Hard copies of data are stored in locked file cabinets, and only the PI and study coordinator have access to the locked files. Individual names or initials are not used in any discussions or publications of the data. We have assembled a research team which includes scientists, physicians, and clinician-scientists with significant experience in human research and metabolic diseases to help anticipate and reduce the risks to subjects. The specific protocol to minimize risk associated with each procedure is described below.

Psychological stress: The psychological stress from participation in this study is minimal. However, some of the questions about food intake and physical activity may make the subjects feel uncomfortable. Subjects will be told they may skip any portion of a questionnaire if they feel uncomfortable about answering the questions.
Blood collection and IV lines: Aseptic technique is used to collect blood using butterfly needles and, if necessary, through an IV. Only the amount of blood necessary for analyses is withdrawn and the staff members performing venipuncture are nurses and trained phlebotomists. Risk of bleeding is reduced by applying pressure at the site of puncture. Bruising is treated with ice. Fainting is prevented by drawing blood in the semi-recumbent position.
Arterial stiffness, flow mediated dilation, and blood pressure: When assessing carotid-femoral PWV, the blood pressure cuff will squeeze the arm and leg tightly; however, any discomfort will be alleviated as soon as the pressure in the cuff is released.
DEXA: Risks include a small radiation for the DEXA, equivalent to about 2% of the average radiation dose from all sources that a person in the U.S. receives each year. Subjects who have participated in other research studies or medical procedures involving significant ionizing radiation exposure in the past 2 months will be excluded. A urine pregnancy test will be performed before the DEXA.
There are no alternative methods that will allow us to test our proposed hypotheses.

Plan for Reporting Study Deviations
We will report all noncompliance per the IRB's noncompliance policy.

Stopping Rules
We will stop an individual study in the event of a serious adverse event. If 4 or more subjects experience serious adverse events (as described below), the study will be stopped and the events will be discussed with the IRB to determine whether it is appropriate to continue and/or determine appropriate modifications to the protocol to avoid further adverse events. Adverse events will be considered unanticipated problem if it is unexpected, serious, and would have implications for the conduct of the study.
All adverse events will be submitted for review according to current protocols.

Definition of serious adverse event (SAE)
An SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria (a – f):

a. Results in death
b. Is life-threatening
The term 'life-threatening' in the definition refers to an event in which the subject was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization
A hospitalization or prolongation of hospitalization will not be regarded as a serious adverse event if at least one of the following exceptions is met:

- The admission results in a hospital stay of less than 12 hours
- The admission is pre-planned
- The admission is not associated with an adverse event related to the study medication or study procedures as determined by the study safety officer and data safety monitoring board.

d. Results in persistent or significant disability / incapacity
Disability means a substantial disruption of a person's ability to conduct normal life's functions.
e. Is a congenital anomaly / birth defect
f. Is another serious or important medical event as judged by the investigator.

Breach of Confidentiality

Subject confidentiality will be rigorously maintained. The data collected as part of this study will be for research only. It will be de-identified after collection. Confidentiality of data will be assured by coding of unique subject identities and that coding will be known only to the research team, including the use of secure files, locked filing cabinets, and a unique subject coding system. The original study data will be kept in locked cabinets (hard copy) or entered into a secure computer database password protected under a secure server space allocated for use by only the study team (electronic). Furthermore, data analysis will be appropriately blinded, and any individual data presented in manuscripts will also be presented in an anonymous nature. No identifying information will be disclosed. *Confirming with University of Missouri policy, all research records will be retained for a period of 7-years following completion of the study.*
All protocols and techniques to be used will be approved by the Institutional Review Board (IRB) prior to initiation of any studies. Each subject will give written informed consent after all questions have been answered by a study team member. The consent form will also include a statement guaranteeing confidentiality. Adverse event reports and annual summaries will not include subject-identifiable material. No information will be given to anyone without permission from the subject. Electronic communication with study team members will involve only coded, unidentifiable information. *Any unanticipated breach of confidentiality will be summarized and reported to the IRB within 5 working days of awareness*

IX. Anticipated Benefits

There may be no benefit to the subjects in this study. These data will aid in the understanding of how epithelial cell sodium channel (ENaC) contributes to the genesis of arterial stiffness in obese and insulin resistant subjects, preferentially in women. From the screening and baseline tests, all subjects will gain health information about themselves. At the end of the study, results collected will be shared with the subject in face-to-face meetings. In summary, the risks of the treatments are minimal, as witnessed by millions of Americans who are treated with similar drugs each year. The health testing offers some risk, which includes the blood draws. It is possible however, that the benefits to the subjects may be substantial, and the new information on characteristics that increase cardiovascular risk in insulin resistance and obese women will benefit society.

X. Compensation

We have completed multiple clinical studies involving human subjects with adherence rates of greater than 95% and subject retention rates of greater than 85%.[8-12] We utilize a validated retention strategy published by Jeffrey et al.[13] that has been successful. With respect to subject compensation, as shown in **Table 2**, subjects will be paid $30 for screening visit, $100 for baseline visit, $50 for week 1, $100 for week 4, $75 for weeks 8, 16 and 20, $150 for week 12 (interim), and $350 for week 24 (final visit). Any additional unscheduled safety visits will be compensated $50. Thus, a total compensation of $1005 will be provided as study events are completed not including any additional visits.

Table 2. Subject compensation

Visit	Compensation
Screening	30
Baseline test	100
Week 1	50
Week 4	100
Week 8, 16 & 20	75
Week 12 Interim visit	150
Week 24 Final test	350
Unscheduled visit	50

XI. Costs

Subjects will not be charged for any procedures that are part of this research study. The costs of the study will be covered by a NHLBI R01 grant.

XII. Data Safety Monitoring Board

Overall framework for safety monitoring and what information will be monitored:

The drug treatment could pose risk to participants. The data safety monitoring plan (DSMP) for this trial focuses on close monitoring by the **PI safety officer**, **local IRB** and the **Data Safety Monitoring Committee (DSMC)**. Monitoring will include review of the protocol and statistical analysis plan, enrollment, attrition, efficacy end-points, and adverse events. PI will submit an annual progress report to the NHLBI that will include: confirmation of adherence to data safety and monitoring plan, summary of any data or safety monitoring issues, description of any changes to the research protocol or the DSMC if applicable and new/continuing IRB approvals.

Plan for data management:

A password-protected database will be used to manage all study data. To ensure confidentiality only subject ID numbers will be entered into the database. Signed informed consent forms are kept in a locked filing cabinet. Participants will not be individually identified in any publication. Participants' right to privacy will be protected.

Frequency of monitoring, including any plans for interim analysis and stopping rules:

In addition to monitoring by the PI, study coordinator, and safety officer monitoring, the MU IRB monitors all aspects of the project on an annual basis. The DSMC will conduct scheduled reviews on a semiannual basis. Two members external to the PI institution are included in the DSMC. The frequency of the structured data review for this study can be summarized in the following table:

Data type	Frequency of review
Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion)	At the end of each recruitment

Adverse event rates (injuries)	semi-annually
Compliance to treatment	semi-annually
Out of range laboratory data	semi-annually
Stopping rules report regarding statistical power implications of dropouts and missing data	semi-annually
IRB review	annually
DSMC review	semi-annually

Process for Managing and Reporting Adverse Events, Serious Adverse Events and Unanticipated Problems:

Unanticipated events will be reported to MU IRB, and both serious and non-serious adverse events will be reported to the MU School of Medicine Data and Safety Monitoring Committee. For reporting to the DSMC, adverse events will be categorized and classified according to Common Terminology Criteria for Adverse Events Scale (CTCAE v3.0). Safety reports will be sent to the safety officer. The PI will be responsible for assembling the data and producing these reports, as well as assuring that all parties obtain copies of these reports.

Qualifications and responsibilities of the Safety Officer:

The safety officer for this trial will be Brian Bostick, MD-PhD. **Dr. Bostick is an Assistant Professor in the Division of Cardiology**. In addition to practicing medicine and cardiology, he is a clinician scientist. As Safety Officer, Dr. Bostick will review eligibility criteria with Dr. Manrique, and be unblinded as to treatment assignment. He will review all reports sent by the study coordinator to determine whether there is any corrective action, trigger of an ad hoc review, or stopping rule violation that should be communicated to the University of Missouri IRB and the Data and Safety Monitoring Committee. In addition, the safety officer may comment on whether the study investigator needs to report any specific out-of-range laboratory data.

MU Data and Safety Monitoring Committee (DSMC):

The DSMC provides education for investigators, research teams, and faculty regarding data and safety monitoring; reviews proposed Data & Safety Monitoring Plans; establishes Data & Safety Monitoring Boards; conducts independent, interim reviews of study safety and progress; and makes recommendations concerning the continuation of studies, including recommendations regarding the modification, suspension, or termination of a study. The data safety monitoring board minutes will summarize the topics discussed and list the recommendations. Minutes will be signed by the board chair. For the current study the DSMC has 3 voting members representing: Endocrinology, Diabetes and Metabolism, Cardiovascular Diseases and Echocardiology, and Biostatistics. Additional members may be consulted ad hoc as needed and would be reflected in the minutes. In addition, among these members and **for the purpose of this clinical trial, 2 voting external members have been included: Dr. George Bakris (University of Chicago) and Dr. Linda Peterson MD (Washington University)**.

XIII. Multiple Sites

N/A

XIV. References/Appendices

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