Study Title: **Estrogen mediated vascular endothelial dysfunction in Diabetes**

Brief Title: **Estrogen and Diabetes**

Clinical Trials: **NCT03436992**

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Principal Investigator: **Ryan Harris, PhD**

1. Objectives

Describe the purpose, specific aims, and hypothesis:
The purpose of this proposal is to investigate whether or not estrogen contributes to vascular dysfunction in premenopausal women with diabetes. Specific Aim 1. To test the hypothesis that estrogen contributes to vascular endothelial dysfunction in premenopausal women with diabetes.

2. Background

Describe the background and rationale for the study:
There is an extensive body of knowledge that indicates premenopausal women have an innate protection from cardiovascular disease and estrogen is what contributes to this protection. In contrast to these protective findings in humans, using an animal model, estrogen in diabetes has been indicated to contribute to vasoconstriction.

3. Inclusion and Exclusion Criteria

List the inclusion/exclusion criteria:
Inclusion criteria:

- *Both boys/men (with T1DM) and girls/premenopausal women (with or without T1DM)*
- ≥ 7 years of age
- *Normal menstrual cycle interval of 25-35 days for at least 3 previous cycles*
- *All races*
- *Clinical diagnosis of insulin-dependent type 1 diabetes (patients only)*

Exclusion criteria:

- *Clinical diagnosis of hepatic, cardiovascular, or renal disease*
- *Uncontrolled Diabetes (HbA1c > 12 %)*
- *Diabetic complications (i.e. macrovascular, microvascular, or autonomic)*
- *Proteinuria*
- *Uncontrolled Hypertension (>140/90 mm Hg on therapy)*
- *Pregnancy*
- *Oligomenorrhea*
- *Direct vasoactive medications (i.e. nitrates)*
- *Anti-estrogens (i.e. SERMs)*
- *Polycystic ovarian syndrome (defined by NIH guidelines – hyperandrogenic anovulation)*
- *Undetectable Anti-Mullerian Hormone (AMH) following screening*

4. Number of Subjects/Records/Samples Collected

Indicate the total number of subjects to be accrued/records reviewed/samples collected across all sites:
198 total subjects to be accrued

5. Recruitment Methods

Describe when, where, and how potential subjects will be recruited:

Subjects will be recruited through word of mouth and flyer placement. Word of mouth recruitment will take place through ordinary interactions by the principal and sub-investigators with individuals in the community and in clinic. Once informed of the study, individuals will be asked to call Dr. Harris or Ms. Lange at the GPI if interested in participating. The telephone screening form will be used for when subjects contact the GPI to verify eligibility prior to the consent visit. If the subject chooses not to enroll in the study during that time, the telephone screening form (with any PHI) will be shredded.

The current study will NOT recruit vulnerable participants whom include: fetuses, pregnant women, human in vitro fertilization, prisoners, or other institutionalized individuals. MCG employees who wish to participate may only do so if they are not on the protocol or working for any investigators listed on the protocol.

6. Procedures Involved

a. *Describe the procedures involved to include those procedures that are standard evaluation and/or care and those that are solely for research purposes:*

- *Blood Pressure – Standard non-invasive blood pressure techniques will be used to measure subject's blood pressure. Participants will be asked to sign a consent form to obtain blood pressure measurements prior to reviewing the study consent document.*

- *Questionnaires- Participants will be asked to fill out a questionnaire to gather contact information, personal information, and family and health history. The health history questionnaire will ask questions about family history of certain medical conditions, current medications, heart disease, and the type(s) of exercise performed.*

- *Body Composition – Body fat will be measured at the beginning of the study with a low level x-ray machine (DXA for short). The subject will be asked to remove all items that may contain metal including: jewelry, belts, shoes, ear and body piercings, etc. Gowns will be available if he/she needs to remove clothing. For the scan, the subject will be asked to lie still on a table with your eyes closed while the table moves, and a mechanical arm moves above their body measuring the fat in your body. The DXA gives the body about 1 extra days' worth of the type of background radiation (about 0.4 mRem) that everyone receives from the environment. Body Mass Index (BMI) will also be calculated using measurements from height and weight. Waist to Hip Ratio (WHR) will be measured using a vinyl tape measuring around the waist and hips.*

- *Muscle Function Test - For this test, you will lie on your back. We will place a cuff around your upper thigh and place an oxygen measuring device and 2-4 electrodes on the*

middle of your thigh muscle and/or calf. These electrodes will be used to stimulate your muscle using a commercial electrical stimulation unit. The stimulation will be set so that your muscles contract strongly, but we will adjust the stimulation to a level that you can tolerate. We will perform at least two measurement trials (but no more than 4 trials), each consisting of a short electrical stimulation period (ranging from 15 seconds to 5 minutes) followed by a series of 15 short cuff inflations to block the blood flow to the leg. There will be a 2-5 minute rest period between trials.

- *Urine Sample - A urine sample will be collected to rule out any unknown condition that may exclude the individual from participating in the study.*

- *Blood Sample - Blood samples will be taken from a vein by the P.I., phlebotomist, or research assistant that is certified in drawing blood. At the preliminary visit, up to 18 ml of blood (approximately 1-2 tablespoons) will be taken to test for blood counts, the amount of sugar in the blood and certain hormones. For **ADULTS**: Up to 75 ml of blood (approximately 4 - 5 tablespoons) will be taken during each experimental day (approximately 44 ml pre- treatment and 31 ml post-treatment). This amount is less than 1/5 of the amount that is withdrawn during a normal blood donation (500 ml or 15 fluid ounces). For **CHILDREN**: Up to 50 ml of blood (approximately 3 – 4 tablespoons) will be taken during each experimental day (approximately 30 ml pre- treatment and 20 ml post-treatment). This amount is about 1/10 of the amount that is withdrawn during a normal blood donation (500 ml or 15 fluid ounces). To prevent the number of needle sticks for the blood draws, a sterile plastic catheter tube will be placed for the duration of experimental day 2. We will use this blood to test for blood fat (cholesterol), blood sugar, and the concentration of certain hormones. **NOTE**: Healthy controls will not receive treatment, and therefore the blood draw amount will be the "pre-treatment" volume listed above.*

- *Arterial Stiffness Evaluation (PWV/PWA) – A device will be used to measure how fast blood travels. This test is called pulse wave velocity or PWV. How fast blood travels is also a measure of how stiff the arteries are. Stiff blood vessels/arteries are linked to high blood pressure and heart disease. To start the test, a blood pressure cuff is placed on the subject's arm and leg. A pen-like device is gently applied on the carotid artery (neck) and the radial artery (wrist) to record how fast the blood flows between each of the points.*

- *Arterial Function (FMD) - Measurements of arterial health will be completed using ultrasound technology to detect how well the artery expands with changes in blood flow. This technique is used to characterize the health of arteries. The subject will lie down for 20 minutes to become fully rested. After the 20 minute resting phase, a water based gel will be put on the ultrasound transducer and then put on the subject's skin. The gel helps achieve better pictures of the artery. We will then test the artery function. After baseline measurements, a blood pressure cuff placed around the forearm will be inflated to 250 mm Hg to stop blood flow for 5 minutes. Data will be recorded for 2 minutes following the*

release of the cuff. The health of the artery will be expressed as a percentage of the artery expansion with the increased blood flow, compared to the resting diameter.

- *Nitroglycerin spray (EID) – Nitroglycerin is a fast acting drug that causes blood vessels to dilate (or get bigger). After we perform the post FMD test and while the subject is still laying down, Dr. Harris or his research associate will spray one dose (0.4 mg/ spray) of nitroglycerin under the tongue. We will continue to monitor heart rate, blood pressure, and the blood vessel through ultrasound for up to 15 minutes. This test measures arterial health and the maximal capability of the artery to expand.*

- *Microvascular Function (MVF) - Using a low frequency laser imaging camera and/or a small skin probe, skin blood flow will be measured non-invasively in the arm. We will place ring-shaped probes that are about 1.5 inches wide on the forearm with sticky tape. One ring will be filled with ~2 ml (about 1/2 teaspoon) of water for local heat and the others will be used for iontophoresis. For local heat, we will heat the water in the ring to between 42°C (107°F) and 44°C (111°F) and wait ~30 minutes for the increase in skin blood flow to become stable. Iontophoresis is a technique that delivers a vasoactive drug about 1 mm deep into the skin using a low level electrical current. The advantage of this technique is that it is non-invasive and the drug does not go into the body, it just remains at the surface of the skin. We will use a 1-2% solution of either acetylcholine, sodium nitropruside, or L-NAME (vasoactive substances) to increase or decrease skin blood flow. Multiple short currents (10-180 seconds) will be performed approximately 1 minute apart. We will monitor skin blood flow through the ring during the artery function test with the cuff (described above) as well as during local heat and iontophoresis.*

- *Ovulation Predictor Kit – Female subjects will be given an ovulation predictor kit to take home at the end of experimental day 1 in order to determine peak estrogen level. This test requires a urine sample and the subject will use it in a similar way that she would use a pregnancy test. The kit comes with several disposable tests that she will use daily. We want to schedule the subject's final study visit roughly 3 days after the digital screen shows a flashing "smiley face." The purpose of this study involves testing women (on experimental visit 2) when their estrogen levels are highest. Because the menstrual cycle can vary between individuals, using this predictor kit allows us to estimate which day their estrogen levels will peak.*

- *Physical Activity Monitoring/Accelerometry – You will be asked to wear an accelerometer for the periods of time between experimental visits. Accelerometers will be used to collect information about daily physical activity and sleep quality, even during evenings and weekends. The monitoring device will attach to your wrist.*

- *Continuous Glucose Monitoring (CGM) -- For patients that use a Dexcom for clinical evaluation of continuous glucose monitoring, we will ask him/her for permission to share their data using the Clarity software provided by Dexcom. To do this, we will send them a link that integrates*

their individual continuous glucose data with the Clarity software to give us real time access of the data throughout the duration of the study. The Clarity software is HIPAA compliant, and patients using this software have already agreed to share their health information with Dexcom. Our study team will discontinue our access to the patient's data through the Dexcom Clarity app within one week of the participant's final visit. This will remove all future access to the participant's data. Access can only be granted if the participant re-shares their data with our research team.

- *Randomized Treatments – After enrollment, the subject will be randomized to receive either an antioxidant cocktail, resveratrol, or a placebo. The antioxidant cocktail is comprised of over the counter vitamins* ***(vitamin C 1,000 mg, vitamin E 600 IU, and alpha lipoic acid 600 mg)*** *that will be given in two doses, 30 minutes apart. Resveratrol (1500 mg of trans-resveratrol) is a natural polyphenol that is found in multiple food sources. Nicotinamide riboside (1,000 mg) is an over-the-counter supplement. The placebo will consist of lactose or sucrose (sugar pills).*

b. *Describe and explain the study design:*

   Brachial artery flow-mediated dilation and a small blood sample (10 ml) will be performed in the girls/women during the menses (days 1-5 of the menstrual cycle) and late follicular (day 12-14) phases, whereas the men will be studied twice at the same frequency as the girls/women. Participant subject medical information will be assessed during the first visit.

c. *Describe the procedures performed to lessen the probability or magnitude of risks:*

   All procedures in this study have been routinely performed in research studies in the past and are of low risk to participants. Dr. Harris is a trained clinical exercise physiologist with experience performing high-risk exercise in patient populations. In an effort to maintain subject safety but also investigate endothelial dysfunction from smooth muscle dysfunction, we will administer nitroglycerin only in adults, and only one time at baseline.

d. *Describe the duration of an individual subject's participation in the study and the time involved:*

   Participation in the study will involve 3 testing visits to the GPI (one preliminary testing visit at the beginning of the study and 2 experimental that for females are based on their menstrual cycle. Prelim visit will take approximately 2 hours, experimental visits 1 and 2 will last approximately 5 hours. Those subjects serving as controls will not receive randomized treatments. Participants may or may not be asked to conduct all visits or even take treatments and/or perform all procedures during each experimental testing day depending on time and availability. If a subject recently participated in a study with us that involved these same tests, we may use data from that study to avoid redundant testing measures.

7. Data and Specimen Management

a. *Describe the data analysis plan, including any statistical procedures:*
This is simply a pilot experimental study. Therefore, descriptive statistics will be generated and paired t-tests and linear regression analyses will be performed among the dependent variables; FMD, hormones, and lipids.

☐ N/A

b. *When applicable, provide a power analysis:*

There is no formal power calculation because this proposal is designed just to obtain preliminary data for a grant submission.

☐ N/A

c. *Describe how data and specimens will be handled:*

☐ N/A

i. *What information will be included in that data or associated with the specimens?*

All data sheets and stored specimens will be de-identified.

ii. *Where and how data and/or specimens will be stored?*

Data are stored in subject folders, specimens are stored de-identified in -80 freezers

iii. *How long will the data and/or specimens be stored?*

Click here to enter text.

iv. *Who will have access to the data or specimens?*

Only investigators that will be involved with the data will have access to the data or specimens

v. *Who is responsible for receipt or transmission of the data and/or specimens?*

Dr. Harris is responsible

vi. *How will data and/or specimens be transported?*

Specimens will be transported according to the biosafety procedures and regulations

8. Provisions to Monitor the Data to Ensure the Safety of Subjects

This study involves no more than minimal risk study and this section is not required. ☐ **N/A**

The plan might include establishing a data monitoring committee and a plan for reporting data monitoring committee findings to the IRB and the sponsor.

a. *Describe the plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe.*

   Dr. Harris will monitor all human studies on a daily basis and evaluate collected data to ensure subject safety. In the event that Dr. Harris is unavailable, Dr. Layman (study physician), will serve in his place. In order to determine if there are changes needed to the human protocol, Dr. Harris will notify the IRB as soon as possible per their policies and procedures. We have assembled a data safety monitoring plan which outlines ongoing quality control in which protocol compliance checks will be performed by Dr. Harris.

b. *Describe what data are reviewed, including safety data, untoward events, and efficacy data.*

   Ongoing Quality Control will include regular data verification and protocol compliance checks to be performed by Dr. Harris. Required administrative reports that describe study progress to include accrual, demographics, and participants' status will be prepared and reviewed with assistance from the study coordinator. These reports will also identify adherence to inclusion/exclusion criteria, study protocol, and any adverse events. Summary reports that are reviewed will always be void of personal identifiers to protect confidentiality.

c. *Describe how the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with participants).*

   Vital signs will be collected continuously throughout the experiments. If we notice any significant changes, we will stop the protocol immediately. Data and specimen collection will be conducted by trained personnel (e.g. only those trained in phlebotomy will conduct blood draws).

d. *Describe the frequency of data collection, including when safety data collection starts.*

   Data collection, including that which pertains to safety, will be collected throughout each visit in accordance to the protocol.

e. *Describe who will review the data.*

   Drs. Harris and Layman will be responsible for review of the data.

f. *Describe the frequency or periodicity of review of cumulative data.*

   Data will be monitored and reviewed during each visit.

g. *Describe any conditions that trigger an immediate suspension of the research.*

If the PI has reason to believe the study is causing harm to a participant, testing will be stopped and the participant will be withdrawn from the study. This may include significant changes to vital signs during implementation of the protocol.

9. Withdrawal of Subjects ☐ N/A

a. *If applicable, describe anticipated circumstances under which subjects will be withdrawn from the research without their consent.* *In the event that participating in the study might be harmful to the subject's health and well-being, we will end their study participation. In addition, if we feel that the participants are not adhering to the treatment we have the right to end their study participation. At any time, the subjects also have the right to stop participating in the study.*	☐ N/A
b. *If applicable, describe any procedures for orderly termination.* Click here to enter text.	☒ N/A
c. *If applicable, describe procedures that will be followed when subjects withdraw from the research, including partial withdrawal from procedures with continued data collection.* *Whether or not the withdrawal from the research is voluntary or done by the investigative team, participants will be compensated for their time per the subject compensation guidelines set forth in the protocol.*	☐ N/A

10. Risks to Subjects

a. *List the reasonably foreseeable risks.*
Venous Blood Draw. Potential risks related with taking a blood sample are few, but include slight bruising, pain, a temporary feeling of faintness, and phlebitis. Rarely, there may be a small blood clot or infection at the site of the needle puncture. All blood draws will be performed by a research team member or a nurse who is certified in drawing blood; in either case sterile techniques will be used.

Arterial Function. The risk associated with the flow-mediated dilation (FMD) test is minimal, if not none. Potential risks associated with the FMD technique may include redness of the skin, bruising, numbness, pain, tingling of the fingers and discomfort while the cuff is inflated. The risks associated with the lubricant gel are skin irritation and possible break out of rash.

Body Composition. A DXA scanning machine gives a small amount of radiation (about 0.4 mRem per scan), about the same amount of radiation exposure you get from less than one day of natural background radiation, the exposures that we receive from natural radiation found all around us. This radiation exposure is not necessary for your medical care and is for research purposes only. This use involves minimal risk and is necessary to obtain the research information desired. Although high doses of radiation are linked to an increased risk of cancer, the effects of the low doses of radiation from diagnostic imaging are not well known. No one is certain if any real risks are involved. Even though we believe there are no known risks associated with this amount of radiation, the possibility that the small amount of radiation might have an effect on a developing fetus makes it necessary to rule out the possibility of pregnancy. Therefore, females in this study may be asked to provide a urine sample for a pregnancy test. This discussion of radiation risk does not address radiation that you may have received from other medical tests outside of this study. You may wish to discuss prior radiation exposures with the study doctor or with your physician, as the adverse effects of radiation exposure are cumulative over time.
Pregnancy. Radiation at large doses is known to have an effect on a developing fetus. Although radiation doses from these studies are extremely small, a urine pregnancy test will be conducted on all female participants to rule out pregnancy.

Muscle Function Test: Electrical stimulation of the muscles may be uncomfortable and could be painful. If the muscle stimulation is too painful, we will adjust the stimulation to a level that you can tolerate. The study does not require the stimulation to be painful for the study to be successful. Because the stimulator "exercises" the muscle, the muscle may be sore for a few days following the protocol.

Nitroglycerin spray (EID): The most common side effects associated with nitroglycerin spray include headache (~50%) and hypotension (less common). We will minimize the risks of NTG by only using 1 spray (0.4 mg) and keeping the subject in the supine position with knees bent. The half-life of the drug is 1-3 minutes which means it is out of the system in that time. Additional risks that have been reported include passing out, dermatitis, burning sensation, flushing, chest pain, nausea, vomiting, weakness, restlessness, and rash. In the event the subject passes out, we will give him/her oxygen and continue to monitor your blood pressure.

Microvascular Function (MVF): The laser imaging camera is a special camera that shines a low energy laser light on the surface of the skin to measure blood flow. The FLPI makes graphs, photos, and movies of skin blood flow. The subject may be able to see a harmless red light on the

skin. There are no known risks associated with the laser imaging camera. The small skin probes can hurt the eye if stared into for a long time. We do not turn on the laser until the probes are taped to a surface.

Iontophoresis: Acetylcholine, sodium nitropruside and L-NAME are vasoactive substances that will be used to increase and decrease skin blood flow. The amount of drug delivered is based on the amount of current being delivered. Using this technique with such a low level current, there should be no drug that enters the body. Although the current being used is very small (less than 200mA), the subject may feel mild skin irritation on the hand or the forearm where the chambers are placed.

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

Accelerometry: There are no known risks associated with physical activity monitoring.

Treatments: There are occasional side effects associated with ingestion of the antioxidant cocktail and Resveratrol that include nausea, upset stomach and diarrhea. These side effects are more common with higher doses. Risks for the antioxidants used in this study will be minimized by not exceeding the recommended dosing. Possible side effects of nicotinamide riboside are typically mild and may include skin flushing, hypotension, nausea, and bloating.

Reproductive Risks: Being a part of this study while pregnant may expose the unborn child to potential risks, some of which may be currently unforeseeable. Therefore, pregnant women will be excluded from the study. If the participant is a girl/woman of childbearing potential, a pregnancy test will be done and it must be negative before she can continue in this study.

If sexually active, the participant must agree to use appropriate contraceptive measures for the duration of the study. Medically acceptable contraceptives include:

1. *surgical sterilization (such as a tubal ligation) [ADULTS ONLY]*
2. *approved hormonal contraceptives (such as birth control pills or patches),*
3. *barrier methods (such as a condom or diaphragm) used with a spermicide, or*
4. *a copper intrauterine device (IUD).*

If the participant does become pregnant during this study, she should inform the study staff immediately.

b. *If applicable, describe any costs that subjects may be responsible for because of participation in the research.* *No cost other than transportation to the GPI.*	☒ N/A
c. *If applicable, describe risks to others who are not subjects.* Click here to enter text.	x N/A

11. Potential Benefits to Subjects

Describe the potential benefits that individual subjects may experience from taking part in the research.

Although there is no direct benefit to the participant, access to the results and information on blood lab values, blood pressure, and the functional and structural measurements of the health of the participants' arteries will be available. They may also choose whether they receive the results of these assessments; however, if the participant choose to receive the results, Dr. Harris or another qualified research member will explain the results to the participant in detail.

12. Confidentiality

Describe the procedures for maintenance of confidentiality.

To minimize the risk of the subject confidentiality, no testing material will be linked to the name, SSN, medical record number, or any other identifiable markers. Instead, each participant will be assigned a random identification number (ID). Those unique ID numbers will be used in the data file. A list of participants' names and their ID numbers is kept in a separate file and only the Principal Investigator and collaborating investigators named on the informed consent document will have access to this file. E-mail addresses and phone numbers are not shared with outside parties.

13. Consent Process

If you are obtaining consent of subjects describe the consenting process.

Informed consent will be obtained. Only sub investigators or the principal investigator named on the informed consent documents for this study will seek consent. The potential participant will have the study explained to them in a language that they can understand, be asked to read each page of the documents and initial at the bottom of each page after reading it. The burden of participation, potential risks, and any costs that may be incurred as a result of their participation will be discussed with the prospective participant. After reading the detailed consent documents and explaining all aspects of the study they will be given an opportunity to ask questions and have them answered. The participant will acknowledge that participation in the study is voluntary and that he/she may withdraw at any time and for any reason.

After having all their questions answered, and if the potential participant chooses to participate, then they will sign and date the consent form. The consent document will then be signed by a witness and signed by the investigator seeking consent. After signing consent forms, testing will commence. The plan for the testing procedures will be explained to the participant (continued consent) and he/she will have an opportunity to ask questions about the procedure and decline participation.

If the environment is perceived by the participant to not provide adequate privacy, accommodations will be made to ensure participant feels and knows sensitive materials are safeguarded.

14. Compensation for Research-Related Injury

This section is not required when research involves no more than Minimal Risk to subjects. ☒ **N/A**

a. *Describe the available compensation in the event of research related injury.*

Click here to enter text.

15. Resources Available ☐ N/A

a. *Describe the availability of medical or psychological resources that subjects might need as a result of an anticipated consequences of the human research.*
N/A

b. *Describe your process to ensure that all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions.*

All research staff are required to sign by their duties associated with each study that they understand and know how to carry out their duties in any event. These documents can be found in the Study binders in the office of the PI.