MicroRNA Activation of LOX-1 Mechanisms in Endometriosis

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Informed Consent

CONSENT FOR RESEARCH
The Pennsylvania State University

Title of Project: Role of microRNA activation of lectin-like oxidized LDL receptor (LOX-1) mechanisms in microvascular dysfunction in women with endometriosis (IRB# 9584)

Principal Investigator: Lacy M. Alexander, Ph.D.
Address: 113 Noll Laboratory
Telephone Number: 814-867-1781

Subject's Printed Name: _____

We are asking you to be in a research study. This form gives you information about the research.

Whether or not you take part is up to you. You can choose not to take part. You can agree to take part and later change your mind. Your decision will not be held against you.

Please ask questions about anything that is unclear to you and take your time to make your choice.

1. Why is this research study being done?
Endometriosis is a disorder that occurs in women. With endometriosis, tissue that should be found in the womb is found in sites outside of the womb. This disorder impairs the function of the cells that line the body's blood vessels (endothelium). The endothelium helps to control blood flow in healthy vessels. Women with this disorder have an increased risk for high blood pressure and high cholesterol. They have a higher risk for cardiovascular disease, too.

With this study, we will learn how endometriosis impairs the lining of blood vessels and increases the risk for disease.

We are asking you to be in this research because you fit our criteria for being a subject.

2. What will happen in this research study?

You participate on the circled days or procedures. Please read the descriptions of the circled items. Then write your initials by the circled days or procedures.

We may ask you to repeat a trial, procedure, or test. This could happen for many reasons such as equipment failure, power outage, inconclusive test results, etc. You do not have to repeat a trial, procedure, and/or test if you do not wish to do so.

Note: This study involves the use of drugs that are not approved by the FDA to treat disease. All of the drugs have been used in humans by us or others. The FDA approved the use of the drugs for this study. We dilute the drugs in Lactated Ringer's, a type of saline fluid like that found throughout your body. The drugs are:
Acetylcholine (ACh) – like a substance made by your body; causes blood vessels to dilate
Atorvastatin – blocks a substance made by your body that impairs blood vessel function.

L-NAME – blocks the body from making nitric oxide; causes blood vessels to get smaller.
Poly I:C - blocks a substance made by your body that impairs blood vessel function.
Sodium nitroprusside (SNP) – supplies nitric oxide; causes blood vessels to dilate

_____ initial A. Screening Visit

1. You drink only water, and do not eat for 12 hours before the screening.
2. The research nurse and/or Clinical Research Center (CRC) staff perform the screening. The staff measures your height and weight, blood pressure (BP), and heart rate (HR). They measure waist circumference. The staff place sticky tabs on your chest. Wires connect the tabs to an ECG machine. The ECG measures your heart's function. The staff reviews your medical history. Women of childbearing age have a urine pregnancy test.
3. The staff draws 30 ml (2 Tbsp) of blood from a vein in your arm. We send some of the blood to a lab to see if the proteins, blood cells, electrolytes, etc. are within normal levels. We may test the blood for other substances of interest.
4. If you take a thyroid drug, please tell the nurse your thyroid stimulating hormone (TSH) level. If you do not know your TSH level and/or you have not had it measured within 6 months, we will test for TSH in the blood sample.
5. The researchers do not perform genetic tests on the blood nor look for disease (e.g. HIV).
6. This study includes taking Lipitor. If you participate, you need to tell your doctor. The researchers give you a note that tells your doctor that you take Lipitor as part of this study.

_____ initial B. Preparation for all experiments

1. We give you printed and verbal instructions listing what to do before you arrive at the lab. Please follow the instructions with care. If you have questions, please contact us right away.
2. Do not drink alcohol 12 hours before the experiment.
3. Do not drink caffeine (ex. coffee, tea, Coca Cola, chocolate) for 12 hours before the experiment.
4. On the day of the experiment
    a. Refrain from hard exercise, physical labor, and other tasks in which you to exert yourself more than you would on an easy walk.
    b. We measure your blood pressure, heart rate, and oral temperature.
    c. Women of childbearing age have a urine pregnancy test if they have not had a test within 2 weeks of the experiment.
    d. The nurse draws 10 ml blood to analyze for relevant substances of interest.
    e. We insert the microdialysis (MD) probes:
        i. You wash the skin on your forearm. We place a tight band around the forearm so we can see the veins.
        ii. For each MD site, we make pairs of pen-marks on the arm 2.5 cm (1 inch) apart and away from veins. The marks serve as entry and exit points for the MD tubing. We remove the tight band.
        iii. We clean the arm with an orange fluid called "povidone iodine" and alcohol. We place an ice bag on the site for 5 minutes to numb the skin.
        iv. Then we insert a thin needle into the skin near each entry mark. The needle's tip travels between the layers of skin for 2.5 cm (1 inch). The needle exits the skin near the matching exit-mark.
        v. We thread the MD tubing through the needle and then withdraw the needle leaving the tubing in the skin.
        vi. We prepare 5 MD sites.

vii. Any skin redness caused by the insertion fades in about 60 minutes. During this time Lactated Ringer's flows through the MD-tubing. Lactated Ringer's is saline-fluid like that found throughout your body.

f. Skin Blood Flow (SkBF):
   i. We tape a thin fiber optic laser Doppler flowmeter probe and its holder over each MD site.
   ii. The thin probe measures skin blood flow with a weak laser light. We measure skin blood flow throughout the experiment.
   iii. We control the temperature of the holders. The holders start at 34°C (93°F).

g. Heart Rate: We place 3 ECG tabs on your chest and connect them to an ECG machine.

h. Blood pressure: We may use two methods to measure blood pressure. Both methods use a cuff that inflates on your upper arm. In one, we listen with a stethoscope at the inside of your elbow. Another method, a critical care machine, makes the measure. The critical care machine also measures heart rate. During the experiment, we record blood pressure and heart rate every 5 to 7 minutes.

_____ initial B. MD Experiment 1 – Pre-Statin Therapy

Acetylcholine (ACh) Dose Response
Probe 1. Lactated Ringer's only (control)
Probe 2. Lactated Ringer's + LNAME
Probe 3. Lactated Ringer's + Poly I:C
Probe 4. Lactated Ringer's + atorvastatin
Probe 5. Lactated Ringer's + (LNAME + Poly I:C) or (LNAME + atorvastatin)*
*****Note: You will have one of the two treatments listed at MD Probe 5.

1. We add the PolyE:C or atorvastatin to Probes 3, 4, and 5 as shown above.
2. After 30 minutes, we add LNAME to Probes 2 and 5.
3. After 30 minutes we add ACh to all sites. All sites receive the same amount of ACh.
4. We increase the amount of ACh every 5 minutes. Each site receives 6 different amounts of ACh.
5. After we complete the last amount of ACh, we stop all test substances.
6. Then only Lactated Ringer's flows through the tubing at all sites. At the same time, we warm the probe holders at all sites to 43°C (108°F).
7. After 30 minutes, we add SNP to all sites while heating stays at 43°C (108°F). Heating and SNP causes the blood vessels at those sites to dilate as much as they can.
8. After 10 minutes, we remove the MD tubing from your skin. We place sterile bandages over the sites. If you wish, we place a bag of ice on the sites for 10 minutes to reduce the chance of bruising.
9. We measure blood pressure and heart rate before you depart.

_____ initial C. Statin (Lipitor) Therapy – You have a pregnancy test before starting Lipitor.

1. You take one 10 mg tablet of atorvastatin (Lipitor) each morning for 6-9 days.
2. We give you the bottle of tablets to take home with you.
3. We give you verbal instructions about the therapy. We give you written instructions and information sheets to take home with you.
4. If you have side effects from the Lipitor such as dark urine or muscle soreness, etc.
   a. Call:
      i. Research Nurse, Susan Slimak RN (W: 814-863-8556, M: 814-880-4396)
      ii. Study head, Lacy M. Alexander, Ph.D. (W: 814-867-1781)

b. You return to the lab for the nurse to draw a 10 ml (<1 Tbsp) blood sample to check wellness and liver function markers.
   i. If the tests show that your liver function markers are too high, you stop the Lipitor and withdraw from the study.
   ii. We check your liver function markers at intervals until they return to normal. This requires extra 10-ml (<1 Tbsp) blood draws.
c. See Section 3 below and separate Lipitor handout for more information.

_____ initial C. MD Experiment 2 – Post-Statin Therapy

1. Return to the lab to repeat the MD experiment between 6-9 days of statin therapy.
2. Take your final dose of Lipitor on the morning of the experiment before coming to the lab.
3. Bring any remaining Lipitor tablets with you.
4. All other preparations are the same as that for MD Experiment 1 (see item B).
5. MD Experiment 2 is the same as MD Experiment 1 (see item C).

3. What are the risks and possible discomforts from being in this research study?

There is a risk of loss of confidentiality if your information or your identity is obtained by someone other than the investigators, but precautions will be taken to prevent this from happening. The confidentiality of your electronic data created by you or by the researchers will be maintained to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed.

Microdialysis: The risks are less than that for a blood draw because microdialysis uses only a small, local area of skin. In contrast, a blood draw involves not only skin, but also large blood vessels and blood. You are likely to have some pain and bruising like that from a blood draw. However, we use ice to numb your arm when we insert the tubing. Also, the small needle reduces pain when we insert the tubing. You are not likely to have pain after the tubing is in place. You may feel a little pain when we remove the tubing from your skin. Needles make some people feel lightheaded or cause them to faint. Although rare, the tubing could break as we remove it from the skin. Then we remove the tubing still in your skin by pulling on the other end of it. This presents no added risk for you. Even more rare, the tubing could break so that a piece of the tubing is left under your skin. In this case, we treat any tubing still in your skin like a splinter. We stop any mild bleeding with mild pressure and sterile gauze. Infection is possible. We keep the risk of infection very small by using sterile techniques and supplies like those used with blood draws. We apply a sterile bandage to the site after the experiment. We tell you how to take care of the site.

Fluid flowing through the tubing: The substances flowing through the tubing only go to a 2.5 cm^2 (0.4 $inch^2$) area of skin at each tubing site. The amount that enters the skin is very small. However, there is a chance of having a bad reaction to the substances. This reaction could produce redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse, convulsions, and/or fainting. Although rare, you could feel lightheaded. You could feel sick to your stomach or vomit. You could become flushed or feel like your heart is pounding. We end the MD procedure if you have any of these signs and symptoms. We and other researchers have used these substances with microdialysis in skin. There have been no reports that these substances caused lasting bad reactions. If a bad reaction should occur, we summon medical help.

Lactated Ringer's Solution: This fluid is similar to the natural fluids in your skin. This fluid contains salt, potassium, lactate, and chloride. The acid content is like that of your body's natural fluids. A bad reaction to this fluid is highly unlikely.

ACh, Atovastatin, Poly I:C, LNAME, SNP: These substances stop or mimic the action of your body's natural chemicals upon the blood vessels in the skin. A small amount of these substances enter the skin around the tubing. This only affects the blood flow in the vessels in a nickel-sized area of skin. The effect of these substances is gone within an hour after the experiment.

Laser Doppler Flowmetry: Weak lasers can hurt your eye if you stare into the light for a long time. We do not turn on the laser until the probes are taped to a surface. The tape may irritate your skin.

Blood Pressure (manual, critical care monitor): The researchers measure blood pressure with the method used in a doctor's office and/or they can use a machine. A cuff inflates on the upper arm. As the cuff slowly deflates, the researchers listen with a stethoscope at the bend in the elbow. In like manner, the critical care monitor takes a reading. During the short time the researchers inflate the cuff, your arm may feel numb or tingly. The cuff could cause mild bruising.

Povidone Iodine: Researchers and hospitals use this orange-colored fluid to clean the skin. You could have a bad reaction to this fluid if you are allergic to iodine. You inform us if you have this allergy. In this case, we use only alcohol instead. A bad reaction could cause redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse, convulsions, and/or fainting.

Blood Draw: Blood draws often cause mild pain, bruising, swelling, or bleeding. There is also a slight chance of infection or a small clot. If you are nervous about needles, blood pressure and heart rate may increase for a little while. You may also feel lightheaded, sick to your stomach, or may faint. Using the same techniques used in hospitals keeps the chance of infection minimal. Do not exercise hard for 24 hours before a blood draw.

Tape and sticky disks: The tape or sticky disks could cause a rash. During screening, you tell us if you are sensitive to tape. If a disk sticks very strongly, removing the disk could cause an abrasion like a rug-burn on your skin. An abrasion can feel tender or slightly painful, and can increase risk of infection. If you are sensitive to tape, you may have an increased chance for abrasion. An abrasion has occurred only twice during the years that the disks have been used in similar studies in our lab. We may use an adhesive remover like that used in a doctor's office to remove the disks. If you get an abrasion a nurse checks the site. Antibiotic ointment and a sterile bandage are applied. We tell you how to take care of the site. You could have an allergic reaction to the adhesive remover. The reaction could include rash, itching, fever, or breathing problems. Also, it could include changes in pulse, and/or blood pressure, convulsions, shock, and/or fainting. If a bad reaction should occur, we summon medical help right away.

Medical Screening: You may feel shy about giving health information. The staff collects the information in a private and professional manner. You may feel shy about being measured. You may request someone of the same sex to conduct the screening.

Initial screening form: Only members of our lab group use this form. We use the form to help decide whether you are a good candidate for the study. You may feel shy about answering questions. You may request someone of the same sex to ask you the questions. We collect the information in a private and professional manner. We keep the completed confidential and secure.

Local heating: We measure the temperature of your skin under the holders. During heating, the skin feels very warm but will not hurt. The heating makes the skin under the holder red like when you take a hot bath. The redness goes away within several hours. Some people may be more sensitive to heating. If your arm feels too hot, tell us, and we reduce or stop the heating.

ECG: This machine measures the electrical activity of your heart. You have 3 – 10 wires from the machine taped to spots on your chest. There have been no adverse effects. The tape may irritate.

Lipitor Therapy: We will give you verbal and written information about Lipitor. See the handout for more details about Lipitor, its use, and side effects. Lipitor is a drug often prescribed by doctors for people who have high blood cholesterol. Lipitor lowers blood cholesterol. In this study, the approved clinician directs the Lipitor therapy. Lipitor could cause you to have a headache. You will stop taking Lipitor if you get blurred vision or decreased or rust-colored urine. You will stop taking the drug if you have an allergic reaction. This could include problems with breathing, closing of your throat or a rash. Also an allergic reaction could include swelling of the lips, tongue, or face. There have been rare cases of muscle or liver problems with Lipitor use. We will check your liver function with a blood draw before you start Lipitor. We also check your liver function if you have symptoms of liver problems while taking the Lipitor therapy. If the blood test shows a rise in certain liver markers, we will tell you to stop taking Lipitor. Then we would take more blood to test for the markers' to return to normal. Early symptoms of muscle or liver problems include muscle pain, soreness, or weakness. You could also have fever or flu-like symptoms. You may have yellowing of your skin or eyes, stomach pain, or feel tired. You may have dark colored urine or pale colored stools. If you have any of these symptoms, stop taking Lipitor. Then contact the approved clinician right away. Lipitor could harm a developing fetus. If you are a woman who becomes pregnant while taking Lipitor, you must stop taking Lipitor right away. Then tell the researcher and your health care provider right away that you became pregnant while on Lipitor. While taking Lipitor, you should not drink more than two servings of alcoholic beverages (i.e. beer, wine) per day. Grapefruit and grapefruit juice may interact with this drug. Do not eat grapefruit nor drink grapefruit juice while taking Lipitor. You should see a health care provider or Centre Volunteers in Medicine for follow-up after stopping Lipitor.

Latex: Some gloves and medical materials are made of latex rubber. You will inform us if you are allergic to latex and decline to participate in the study.

4. What are the possible benefits from being in this research study?

4a. What are the possible benefits to you?

You will receive a medical screening that could inform you about your health. You will learn the cholesterol level in your blood. Also, you learn your blood pressure. This is important knowledge. High blood pressure and blood cholesterol can lead to many serious health problems. If you have high blood pressure or blood cholesterol, we suggest that you to follow-up with a health care provider. You can also feel good about helping to identify the reasons for the increased risk for CVD in women with endometriosis.

4b. What are the possible benefits to others?

Endometriosis causes chronic pelvic pain and pain during intercourse. It also can reduce or end a woman's ability to bear children. This disorder affects 6% - 10% of women of childbearing age. It can occur as often as 35-50% in women who have pain or cannot bear children. However, little research have been devoted to this disorder. This research can help to draw attention to the importance of this malady to women's health.

In addition, endometriosis impairs the function of the "endothelium" or the lining of blood vessels. Impaired endothelium increases the risk for getting high blood pressure and high blood cholesterol. It is also a hallmark of cardiovascular disease (CVD). CVD is the leading cause of death in women. Women with endometriosis have an increased risk for these diseases. This study will expand the knowledge base regarding the mechanisms by which endometriosis increases risk for these diseases in women. The data from this study serves as a foundation for further investigation.

Also, this study provides experience, education and degree-work for students of The Pennsylvania State University.

5. What other options are available instead of being in this research study?

You may decide not to participate in this research.

6. How long will you take part in this research study?

You visit the lab 3 times over about two weeks to complete this study.

Screening (1 Visit)	less than 1.5 hour
MD Experiments (2 Visits)	5 hours
Total:	~11.5 Hours

7. How will your privacy and confidentiality be protected if you decide to take part in this research study?

We make efforts to limit the use and sharing of your personal research information to people who have a need to review this information.

- We keep the list that matches your name with your code number in a locked file or password protected file on a computer in a room that is locked when unoccupied. Only authorized members of the lab have access to the list.
- We label your research records with your code number and keep them in a locked file or password protected computer in a room that is locked when unoccupied.
- We label your research samples with your code number. We keep some samples in a dedicated ultralow freezer in Noll Lab until analysis. We send some samples to Quest Labs for analysis.

In the event of any publication or presentation resulting from the research, we do not share your personally identifiable information.

We do our best to keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may find out about your participation in this research study. For example, the following people/groups may check and copy records about this research.

- The Office for Human Research Protections in the U. S. Department of Health and Human Services
- The Food and Drug Administration
- The Institutional Review Board (a committee that reviews and approves research studies) and
- The Office for Research Protections.

Some of these records could contain information that personally identifies you. We make reasonable efforts to keep the personal information in your research record private. However, we cannot guarantee absolute confidentiality.

8. What are the costs of taking part in this research study?

8a. What will you have to pay for if you take part in this research study?

None

8b. What happens if you are injured as a result of taking part in this research study?

In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

9. Will you be paid or receive credit to take part in this research study?

MD Experiments: ($ 15.00 / MD probe inserted + $20.00 completing MD experiment.)

Experiment	$ 95.00 each (5 MD probes; 2 experiments: 1 pre-treatment, 1 post-treatment)
Total	$ 190.00

For each experiment, we pay you the amount of money equal to the part of the trial that you complete. For instance, if you complete half of a MD experiment, we pay you for each probe that we insert plus $10.00 for that trial. This is because $10.00 is one-half of $20.00. We may ask you to repeat a trial. If you agree to repeat a trial, we pay you for the repeated trial as stated above. We reimburse you for gasoline if you live more than 20 miles from Noll Lab.

10. What are your rights if you take part in this research study?

Taking part in this research study is voluntary.

- You do not have to be in this research.
- If you choose to be in this research, you have the right to stop at any time.
- If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.
- If you choose to withdraw from the study, all data collected up to the point of withdrawal will remain part of the study and may not be removed.

The person in charge of the research study can remove you from the research study without your approval. Possible reasons for removal include adverse reaction to the Lipitor. Other possible reasons for removal from the study include if the researcher deems that your health or behavior adversely affects the study or increases risks to you beyond those approved by the Institutional Review Board and agreed upon by you in this document. You may decline to answer certain questions. You may decide not to comply with certain procedures. However, your being in the study may be contingent upon answering these questions or complying with the procedures.

During the course of the research you will be provided with any new information that may affect your health, welfare or your decision to continue participating in this research.

12. If you have questions or concerns about this research study, whom should you call?

Please call:

- Study head, Lacy M. Alexander, Ph.D. (W: 814-867-1781)
- The research nurse, Susan Slimak RN (W: 814-863-8556, H: 814-880-4396)
- Dr. Alexander's assistant, Jane Pierzga (W: 814-865-1236, H: 814-692-4720)

if you:

- Have questions, complaints or concerns about the research.
- Believe you may have been harmed by being in the research study.

You may also contact the Office for Research Protections at (814) 865-1775, ORProtections@psu.edu if you:

- Have questions regarding your rights as a person in a research study.
- Have concerns or general questions about the research.
- You may also call this number if you cannot reach the research team or wish to offer input or to talk to someone else about any concerns related to the research.

INFORMED CONSENT TO TAKE PART IN RESEARCH

Signature of Person Obtaining Informed Consent

Your signature below means that you have explained the research to the subject or subject representative and have answered any questions he/she has about the research.

_____ _____ _____

Signature of person who explained this research Date Printed Name

(Only approved investigators for this research may explain the research and obtain informed consent.)

Signature of Person Giving Informed Consent

Before making the decision about being in this research you should have:

- Discussed this research study with an investigator,
- Read the information in this form, and
- Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

Signature of Subject

By signing this consent form, you indicate that you voluntarily choose to be in this research and agree to allow your information to be used and shared as described above.

_____ _____ _____

Signature of Subject Date Printed Name