College of Liberal Arts and Sciences

Department of Health and Human Physiology

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Project title: Angiotensin II and Chronic Inflammation in Persistent Microvascular Dysfunction Following Preeclampsia

NCT #: 03482440

ang II and inflammation following preeclampsia

PI: Anna Reid-Stanhewicz
IRB ID #: 201909818

Project Details

I. Project Introduction

I.1 ***Project to be reviewed by:***
IRB-01

I.2 ***Project Title:***
Role of angiotensin II and inflammation in persistent vessel dysfunction following preeclampsia

I.3 ***Short Title (optional):***
ang II and inflammation following preeclampsia

I.4 ***Provide a short summary of the purpose and procedures of the study proposed in this IRB application.***

- ***DO NOT include information on studies not proposed in this application.***
- ***Use LAY terminology only. This must be easily understandable by IRB community members and nonscientists.***
- ***DO NOT cut and paste technical abstracts from funding applications that may not be understood by a general audience.***

Women who develop preeclampsia during pregnancy are more likely to develop and die of cardiovascular disease later in life, even if they are otherwise healthy. The reason why this occurs is unclear but may be related to blood vessel damage and increased inflammation that occurs during the preeclamptic pregnancy and persists postpartum. The purpose of this investigation is to determine the mechanisms contributing to this lasting blood vessel damage and chronic inflammation, and to identify factors that may decrease these negative effects in women who have had preeclampsia. Identification of these mechanisms may lead to better clinical management of cardiovascular disease risk in these women.

In this study we use the blood vessels in the skin as a representative vascular bed. Using a minimally invasive technique (intradermal microdialysis for the local delivery of pharmaceutical agents) we examine the blood vessels in a nickle-sized area of the skin and compare the responses of the blood vessels in women who have had preeclampsia to those of our control group, women who have had an uncomplicated pregnancy. We make these measurements after the subjects take a placebo and after they take an anti-inflammatory medication (salsalate) to test the role of inflammation in the differences we see between groups. As a compliment to these measurements, we also draw blood from the subjects and isolate the inflammatory cells to test how sensitive their inflammatory responses are.

I.5 ***Specify your research question(s), study aims or hypotheses (do not indicate "see protocol")***
Specific Aim: To define the mechanistic role of Angiotensin II (Ang II) signaling in microvascular inflammation and associated endothelial dysfunction in women who have had preeclampsia (PreEC) compared to control women who have had a healthy pregnancy (HC).
Using intradermal microdialysis for the in vivo assessment of microvascular signaling mechanisms, we will test the hypotheses that PreEC have (1) increased constrictor sensitivity to Ang II and (2) reduced endothelium-dependent vasodilation, both of which will be normalized post-systemic inhibition of inflammation. We will also utilize peripheral blood mononuclear cells (PBMC) collected pre and post-salsalate treatment to test the hypothesis that (3) immune cells from PreEC have a greater inflammatory response to stimulation with Ang II.

NOTE: This IRB protocol encompasses the projects of aim 1 in the attached NIH K99/R00 award. Aim 2 has been completed and aim 3 will be included in a separate IRB application to come later.

I.6 ***Background and significance and/or Preliminary studies related to this project. (do not indicate "see protocol")***
Angiotensin II Sensitivity Contributes to Endothelial Dysfunction Following Preeclampsia: Women with a history of preeclampsia have an exaggerated presser response to systemic infusion of Ang II. Similarly, compelling in vivo pilot data from our lab demonstrates that this increased sensitivity to Ang II is present in the microcirculation and contributes to endothelial dysfunction postpartum in formerly preeclamptic patients. Ang II mediates several events of the inflammatory process including activation of the vascular endothelium; and AT1R activation plays a large role in the pathophysiology of concurrent inflammatory reactions through this direct action on local vascular cells. Inhibition of Ang II, either through ACE inhibition or AT1-receptor antagonism, reduces tissues inflammation and oxidative injury. Reciprocally, inhibition of inflammatory mediators such as IL-17 producing T cells blunts the pressor response to systemic Ang II infusion in animal models of hypertension. Collectively, increased sensitivity to Ang II coupled with the role of Ang II signaling in chronic inflammation, suggests that these factors are likely reciprocal, and contribute to microvascular dysfunction and elevated CVD risk in women who have had preeclampsia.

Interestingly, women who have had preeclampsia do not have elevated circulating Ang II. However, evidence suggests that activating AT1R autoantibodies (AT1-AAs) develop in women during a preeclamptic pregnancy and remain elevated post-partum. AT1-AAs contribute to the vascular pathology of preeclampsia in animal models, and isolated AT1-AAs induce a dose-dependent vasoconstriction response and the upregulation of pro-inflammatory cytokines. Importantly, these responses are mediated through AT1R binding at the cell membrane, and are inhibited by AT1R blockade. Chronically elevated AT1-AAs post-partum likely contribute to persistent inflammation and endothelial dysfunction in women who have had preeclampsia, independent of endogenous Ang II concentrations. As such, this unique mechanism highlights the relevance of the inflammatory pathway and AT1R sensitivity, as well as the therapeutic potential of physiological (Ang 1-7) and pharmacological (salsalate) inhibitors of AT1R signaling and/or inflammation, in the vascular dysfunction that persists post-partum in women

who have had preeclampsia.

Angiotensin 1-7 is an Endogenous Inhibitor of Ang II: Angiotensin 1-7 (Ang 1-7), a protective component of the renin-angiotensin system, acts on its receptor, mas, to oppose the intracellular actions of Ang II-AT1R signaling. Ang 1-7 – mas receptor binding produces vasodilation, inhibits cell growth, and has anti-inflammatory15, 16 and anti-thrombotic effects. Plasma Ang 1-7 concentrations increase during healthy pregnancy, but are significantly decreased in preeclampsia. There is a negative correlation between plasma Ang 1-7 and both systolic and diastolic blood pressure in preeclampsia19, however few, if any, mechanistic studies examining this relation have been performed in humans. Similarly, to date, there have been no human studies of Ang 1-7 in postpartum women who have had preeclampsia. As an endogenous inhibitor of the intracellular actions of AT1R signaling, Ang 1-7 has emerged as a novel protective pathway in conditions of endothelial dysfunction, including diabetes and essential hypertension. Given the role of increased AT1R signaling – through increased Ang II sensitivity and elevated plasma AT1-AA – in persistent vascular dysfunction post-partum, the actions of Ang 1-7 may be a currently unexplored mechanistic target for the attenuation of inflammation and endothelial dysfunction in women who have had preeclampsia.

The overarching theme of this project – aberrant Ang II signaling and chronic inflammation in the microvasculature of women who have had preeclampsia – is innovative in its delineation of novel mechanisms and interventional targets in an in vivo human microvascular bed. The proposed series of studies utilize state-of-the-art techniques to pharmaco-dissect the deleterious role of Ang II signaling as well as the potentially protective role of Ang 1-7 signaling, in chronic inflammation and endothelial dysfunction in vivo in the human cutaneous microvasculature. Coupled with innovative in vitro measures of immune cell activity and acute systemic inhibition of inflammation, these studies will provide new insight into novel mechanistic targets for intervention.

I.7 ***Literature cited / references (if attaching a grant or protocol enter N/A).***
N/A

II. Research Team

II.1 ***Principal Investigator***

Name	E-mail	College
Anna Reid-Stanhewicz	anna-stanhewicz@uiowa.edu	College Lib Arts and Sciences

II.2 ***Team Members***
UI Team Members

Name	E-mail	College	Contact	Key Prsn	UI COI	VAMC COI	Consent Process Involvement	Deactivated
Anna Reid-Stanhewicz, PHD	anna-stanhewicz@uiowa.edu	College Lib Arts and Sciences	Yes	Yes	No		Yes	No
Kaila Brustkern, BS	kaila-brustkern@uiowa.edu	College Lib Arts and Sciences	No	No	No		Yes	No
Diana Jalal, MD	diana-jalal@uiowa.edu	Carver College of Medicine	No	Yes	No		No	No
Michael Pyevich, AA	michael-pyevich@uiowa.edu	College of Liberal Arts and Sciences	No	No	No		No	No

Non-UI Team Members

Name	Institution	Location	FWA	Role	DHHS Contact	Key Prsn	UI COI	VAMC COI	Consent Process Involvement	Email

Nothing found to display.

II.3 ***The Principal Investigator of this study is:***
Faculty

II.6 ***Identify the key personnel. The system will automatically designate the PI and all faculty members on the project as "key personnel." For information about other team members who should be designated as "key personnel" please click on the help information.***

Name	Is Key Personnel
Anna Reid-Stanhewicz, PHD	Yes
Kaila Brustkern, BS	No
Diana Jalal, MD	Yes
Michael Pyevich, AA	No

II.5 ***Select research team member who is the primary contact for study participants.***
Anna Reid-Stanhewicz

III. Funding/Other Support

III.1 ***Funding Sources***

Type	Source	Grant Title	Name of PI on Grant
Federal Agency	US Department of Health & Human Services, National Institutes of Health	Role of Angiotensin II and Chronic Inflammation in Persistent Microvascular Dysfunction Following Preeclamptic Pregnancy	Anna Stanhewicz

* new source name

III.2 ***What type of funding agreement would be completed?***
Federal/State/Local Agency/Non-Profit Funded/Other

III.3 ***Does any member of the research team have a financial conflict of interest related to this project according to the Conflict of Interest in Research policy? If yes, please indicate which members below.***

Name	Has Conflict of Interest
Anna Reid-Stanhewicz, PHD	No
Kaila Brustkern, BS	No
Diana Jalal, MD	No
Michael Pyevich, AA	No

III.5 ***What is the current status of this funding source?***

Source	Status	Other Status Description
US Department of Health & Human Services, National Institutes of Health	Awarded	

IV. Project Type

IV.1 ***Do you want the IRB to give this project***
Regular (expedited or full board) review

IV.2 ***Enter the date you will be ready to begin screening subjects/collecting data for this project. (If you do not have a specified date, add "upon IRB approval")***
upon IRB approval

IV.3 ***Are you requesting a waiver of informed consent/authorization (subjects will not be given any oral or written information about the study)?***
No

V. Other Committee Review

V.1 ***Does this project involve any substance ingested, injected, or applied to the body?***

- ***Do not answer yes, if the involvement includes a device, wire, or instrument***

Yes

V.1.a ***What is/are the substance(s):***
Note: All of the following research agents used with microdialysis have been approved by the FDA for this study under IND# 124,294.
Acetylcholine, Powder
Angiotensin II, Powder
NG-nitro-L-arginine methyl ester, Powder
Sodium Nitroprusside, Powder
Lactated Ringer's, Liquid

Salsalate, Tablet
Placebo, Tablet

V.1.b ***Are any of these substances defined as a Schedule I - V Controlled Substance?***
No

V.2 ***Are any contrast agents used for any purpose in this study?***
No

V.4 ***Are all drugs or substances in this study being used within the FDA approved population (i.e., children, adults)?***
Yes

V.5 ***Are <u>all drugs or substances</u> in this study being used within the FDA approved indication (i.e., disease, condition)?***
No

V.6 ***Are <u>all drugs or substances</u> in this study being used within the FDA approved dose?***
No

V.7 ***Are <u>all drugs or substances</u> in this study being used within the FDA approved route of administration?***
No

V.8 ***Drugs used in study that are not FDA approved for the population, indication, dose, or route of administration***

Salsalate ()	
Name of Sponsor	Anna Stanhewicz
Investigator's Brochure Version	N/A
Investigator's Brochure Date	N/A
Who is supplying the drug	
Who is dispensing the drug	
IND#	124,294
Dose	3000mg/day for 5 days
Route of administration	oral

acetylcholine ()	
Name of Sponsor	Anna Stanhewicz
Investigator's Brochure Version	N/A
Investigator's Brochure Date	N/A
Who is supplying the drug	
Who is dispensing the drug	
IND#	124,294
Dose	<0.01mg
Route of administration	intradermal microdialysis

NG-nitro-L-arginine methyl ester (L-NAME) ()	
Name of Sponsor	Anna Stanhewicz
Investigator's Brochure Version	N/A
Investigator's Brochure Date	N/A
Who is supplying the drug	
Who is dispensing the drug	
IND#	124,294
Dose	<0.01mg
Route of administration	intradermal microdialysis

angiotensin II ()	
Name of Sponsor	Anna Stanhewicz
Investigator's Brochure Version	N/A
Investigator's Brochure Date	N/A
Who is supplying the drug	
Who is dispensing the drug	
IND#	124,294
Dose	<0.01mg
Route of administration	intradermal microdialysis

sodium nitroprusside (SNP) ()	
Name of Sponsor	Anna Stanhewicz
Investigator's Brochure Version	N/A
Investigator's Brochure Date	N/A
Who is supplying the drug	
Who is dispensing the drug	
IND#	124,294
Dose	<0.01mg
Route of administration	intradermal microdialysis

lactated Ringer's ()	
Name of Sponsor	Anna Stanhewicz
Investigator's Brochure Version	N/A
Investigator's Brochure Date	N/A
Who is supplying the drug	
Who is dispensing the drug	
IND#	124,294
Dose	N/A
Route of administration	intradermal microdialysis

V.9 ***Will any subject be asked to undergo a diagnostic radiation procedure (including radiographic, nuclear medicine, DEXA)?***
No

V.14 ***Will any subject be asked to undergo a radiation therapy procedure (including external beam therapy, brachytherapy, or nuclear medicine therapy)?***
No

V.20 ***Does this project involve the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into one or more human research participant?***
No

V.21 ***Will any portion of this project be conducted in the CRU, or does it use any CRU resources?***
Yes

V.22 ***Will this project use:***

- ***any resource/patients of the Holden Comprehensive Cancer Center***
- ***involve treatment, detection, supportive care, or prevention of cancer***

No

V.25.a ***Will the study involve <u>any</u> of the following activity at UI Health Care, even if subjects or their insurance will not be billed for the item or service, and regardless of the study funding source (including studies with departmental or no funding)?***

- ***Procedures, tests, examinations, hospitalizations, use of Pathology services, use of clinic facilities or clinical equipment, or any patient care services, including services conducted in the Clinical Research Unit; or***
- ***Physician services or services provided by non-physicians who are credentialed to bill (ARNPs, Physician Assistants, etc.)***

Yes

V.25.b ***Will there be any procedures or services that may happen as part of a subject's regular medical care and as part of the study?***
No

V.25.c ***Will any study equipment or devices be supplied by a study sponsor?***
No

V.25.e ***Is there or will there be an internal budget for this study?***
No

V.25.f ***Is there or will there be an external budget for this study?***
Yes

V.26 ***The study involves Department of Nursing Services and Patient Care nursing, nursing resources or evaluates nursing practices at UI Health Care.***
No

VI. Subjects

VI.1 ***How many adult subjects do you expect to consent or enroll for this project?***
40

VI.2 ***What is the age of the youngest adult subject?***
18.0

VI.3 ***What is the age of the oldest adult subject?***
100.0

VI.4 ***What is the percentage of adult male subjects?***
0

VI.5 ***What is the percentage of adult female subjects?***
100

VI.6 ***How many minor subjects do you expect to consent or enroll for this project?***
0

VI.13 ***Describe EACH of your subject populations***

- ***Include description of any control group(s)***
- ***Specify the Inclusion/Exclusion criteria for EACH group***

INCLUSION CRITERIA: Post-partum women who have delivered within two years and who have had a preeclamptic pregnancy diagnosed by their obstetrician and confirmed according to the American College of Obstetricians and Gynecologists criteria for preeclampsia. [This information will be self-reported by the subjects.] Women without a history of preeclampsia matched for age, parity, and time post-partum. 18 years old and older.

EXCLUSION CRITERIA: We exclude participants for skin diseases, current tobacco use, diagnosed or suspected hepatic or metabolic disease, statin or other cholesterol-lowering medication, history of hypertension prior to pregnancy, history of gestational diabetes, current pregnancy, body mass index <18.5 kg/m2, allergy to aspirin or NSAIDs, or known allergy to materials used during the experiment.(e.g. latex). We exclude for renal disease, bleeding disorders and history of gastrointestinal bleeding. Known allergies to study drugs. Taking blood thinners, aspirin or NSAIDS. Women who choose to breastfeed will not participate.

VI.14 ***Provide an estimate of the total number of subjects that would be eligible for inclusion in each of your study populations (include your control population if applicable)***
We will recruit subjects from Johnson and surrounding counties in Iowa. According to the Iowa Department of Public Health, Johnson county has a live birth rate of 11.7/1,000 residents or ~1,750 live births per year. Surrounding counties range from 9.6-12 live births/1,000, adding ~4,000 additional live births annually within the geographical area of reach. Assuming an average rate of 7% for preeclampsia incidence, approximately 400 women would be eligible to participate in the preeclamptic group each year. We will recruit women within 2 years postpartum so we estimate that there are approximately 800 eligible participants in our geographic area at any given time. Approximately 2,900 women will be eligible to participate in the control group.

VI.15 ***Describe how you will have access to each of your study populations in sufficient number to meet your recruitment goals.***
We will advertise for participants by posting fliers in the community and in clinicians offices where permission has been granted(see attached flier). We will also post this flier in digital spaces such as on facebook and twitter. Participants will be recruited from the University of Iowa and Iowa City, Iowa by mass email (see attached mass email text) and the Noon News (see attached Noon news posting) a newsletter available on campus at UIHC. All individuals interested in participating will be directed to contact research staff by phone or email. All individuals interested in the study will be invited to complete the RedCap eligibility survey sent via email. If an individual appears to be eligible for the study based on their RedCap Eligibility survey responses, a member of the research team will contact them via phone or email to tell the individual more about the study, answer any questions, and perform a phone screening to determine eligibility.

VI.16 ***Do you plan to recruit/enroll non-English speaking people?***
No

VI.18 ***Do you propose to enroll any of the following in this study as subjects?***

- ***Employee of the PI or employee of a research team member***
- ***Individual supervised by PI or supervised by member of research team***
- ***Individual subordinate to the PI or subordinate to any member of the research team***
- ***Student or trainee under the direction of the PI or under the direction of a member of the research team***

No

VI.20 ***Will subjects provide any information about their relatives?***
No

VI.23 ***Will anyone (other than the subject) provide you with information about the subject (e.g. proxy interviews)?***
No

VI.26 ***Is this project about pregnant women?***
No

VI.27 ***Will this project involve fetuses?***
No

VI.28 ***Does this project involve adult subjects who may be incompetent or have limited decision-making capacity on initial enrollment into the study?***
No

VI.32 ***Does this project involve subjects whose capacity to consent may change over the course of the study?***
No

VI.37 ***Does this project involve prisoners as subjects?***
No

VII.A. Project Description (A)

VII.A.1 ***Where will project procedures take place (check all that apply)?***

- Other UI campus site - 528 Field House
- CRU

VII.A.2 ***Is this project also being conducted by other researchers at their own sites (e.g. a multi-site collaborative project)?***
No

VII.B. Project Description (B)

VII.B.1 ***Does this project involve any of the following (Check all that apply):***

- ☐ **Registry** – The collection and maintenance of data (not including biologic samples) in which: (1) the individuals in the registry have a common or related condition(s), and/or (2) the individuals in the registry are interested in being contacted for future studies by investigators other than those listed in Section II of this project.(UI Guide)
- ☐ **Repository** – The collection, storage, and distribution of human biologic samples and/or data materials for research purposes. Repository activities involve three components: (i) the collection of data and/or specimens such as blood, tissue, saliva, etc.; (ii) the storage of data or specimens, and data management function; and (iii) the sharing of data/specimens with recipient investigators other than the original investigators. (paraphrased from OHRP)
- ☐ **Expanded Access** – A process regulated by the Food and Drug Administration (FDA) that allows manufacturers to provide investigational new drugs to patients with serious diseases or conditions who cannot participate in a clinical trial. Examples of expanded access include non-protocol access to experimental treatments, including protocol exception, single-patient IND, treatment IND, compassionate use, emergency use, continued access to investigational drug, and parallel track (ClinicalTrials.gov & FDA).
- ☐ **Clinical (or Treatment) trial** – A prospective biomedical or behavioral research study of new treatments, new drug or combinations of drugs, new devices, or new approaches to surgery or radiation therapy. (NIH and ClinicalTrials.gov & FDA)
- ☑ **Physiology intervention/study** – A pharmacologic or measurement study aimed at understanding basic mechanisms of disease and/or of normal human physiology, often without any therapeutic intent (though a clinical trial could include such components, often labeled as "translational" or "basic science" aims.) Measurements in such studies could include, but are not limited to, a blood draw, EKG, EEG, MRI, auditory or sensory testing, checking vital signs, DEXA scans, eye tracking, specimen collection, exercise, fasting, special diets, etc.
- ☐ **Behavioral intervention/study** – May be used to refer to studies of individual or group behavior. This option does not include drugs, biologics, or devices but could include psychotherapy, lifestyle counseling, behavior modification, etc.
- ☐ **Diagnostic trial** – Protocol designed to evaluate one or more interventions aimed at identifying a disease or health condition (ClinicalTrials.gov & FDA)
- ☐ **Non-clinical** – any college/department that would regularly submit to IRB-02
- ☐ **Other**

VII.B.1.b ***Provide the NCT (National ClinicalTrials.gov Identifier) number***
NCT03482440

VII.B.2 ***Does this project involve a drug washout (asking subject to stop taking any drugs s/he is currently taking)?***
No

VII.B.11 ***Is there a separate, written protocol that will be submitted in addition to this IRB New Project form? (Note: a grant application is not considered to be a protocol)***
No

VII.B.18 ***Does this project involve testing the safety and/or efficacy of a medical device?***
No

VII.C. Project Description (C)

VII.C.1 ***Does this project involve any research on genes or genetic testing/research?***
No

VII.D. Project Description (D)

VII.D.1 ***Check all materials/methods that will be used in recruiting subjects (you will need to attach copies of all materials at the end of the application):***

- Website - https://clinicaltrials.gov/ct2/show/NCT03482440?recrs=ab&cond=Preeclampsia&cntry=US&state=US%3AIA&rank=1
- Advertisements -
- Posters -
- E-mail -

VII.D.8 ***Will a member of the research team discuss the study with the subject in person prior to the subject agreeing to participate?***
Yes

VII.D.9 ***Describe the physical location where the consent process will take place:***
The research staff will discuss the study with potential subjects in a conference room or exam room in the ICTS clinical research unit.

VII.D.10 ***Will a member of the research team discuss the study with the subject by phone prior to the subject agreeing to participate?***
Yes

VII.D.11 ***Describe:***

Research staff will discuss the project over the phone with individuals who are interested in participating in the study. (see pre-consent phone script) This will take place in the research office in 528FH. During the call, the research team member will answer any questions and describe the study to the individual as needed. If the subject is still interested in participating they will be given access to the RedCap pre-consent screening survey. If the individual continues to meet eligibility after completing this survey, they will be given information about the study, but will not be consented over the phone. If the subject agrees, they will come to clinical research. and be consented and proceed with Visit 1.

VII.D.12 ***Who will be involved in the consent process (including review of consent document, answering subjects' questions)?***

Name	Consent Process Involvement
Anna Reid-Stanhewicz, PHD	Yes
Kaila Brustkern, BS	Yes
Diana Jalal, MD	No
Michael Pyevich, AA	No

VII.D.15 ***Check all materials that will be used to obtain/document informed consent:***

- Consent Document

VII.D.16 ***Are you requesting a waiver of documentation of consent (either no subject signature or no written document)?***
No

VII.D.19 ***Before the subject gives consent to participate are there any screening questions that you need to directly ask the potential subject to determine eligibility for the study?***
Yes

VII.D.20 ***List any screening questions you will directly ask the potential subject to determine eligibility.***
Eligibility Survey: (see attached pre-consent online survey) This survey will be administered via REDCap link delivered by email. Participants will be invited to complete a survey to determine their eligibility. Age, height, weight and months since they delivered will be collected so that subjects with a history of preeclampsia may be matched with controls.

If subjects' responses to the online screening survey deem them eligible, they will be contacted by a member of the research team via phone to complete an additional screening (see RedCap attachment Additional Info Survey). Some questions are duplicates to the aforementioned online questionnaire and will be asked again to confirm the absence of major exclusion criteria.

VII.D.21 ***Will you keep a screening log or other record that would include information on people who do not enroll in the study?***
Yes

VII.D.22 ***Describe the information being collected and the purpose for keeping this information.***
The following information will be collected in a screening log:
1. Subject's name
2. Age
3. How they heard about study
4. Date that they contacted the study
5. How they contacted us (phone/email)
6. Date of phone screening
7. Phone number
8. Email address
9. Pass online screening; yes or no
10. If did not pass online screening, reason?
11. Pass phone screen? Yes or No
12. If did not pass phone screen, reason?
13. If passed phone screening, date of consent
14. Signed informed consent

Contact information is required to contact the subjects after the phone screening in case the research staff needs to reschedule Visit 1 or for follow up if the subject does not show for the Visit 1. Because this study is recruiting using mass email, subject information including a brief description why they were deemed ineligible (i.e. health history, medication, breastfeeding) will be kept to prevent making contact twice with an individual who is ineligible. Information on why a potential subject is ineligible/not passing the phone screening is to report to NIH and to monitor our recruiting progress. Information on how the subjects heard of the study will help the research team understand the most successful methods for advertising for the study.

VII.D.23 ***Will this information be shared with anyone outside the UI research team members?***
No

VII.D.25 ***After the subject agrees to participate (signs consent), are there any screening procedures, tests, or studies that need to be done to determine if the subject is eligible to continue participating?***
Yes

VII.D.26 ***List and describe screening***
Medical history and physical exam
Resting blood pressure and heart rate

Standard blood chemistries (lipids, complete blood count, basic metabolic panel, liver enzymes)
Urine pregnancy test

VII.D.27 ***Discuss how much time a potential subject will have to agree to consider participation and whether or not they will be able to discuss the study with family/friends before deciding on participation.***
There is no time limit for the subject to agree to consider to be in the study as long as the study is actively recruiting subjects and they are still eligible. Subjects are allowed to discuss the study with family/friends before deciding on participation.

VII.D.28 ***How long after the subject agrees to participate do study procedures begin?***
The procedures in visit 1 (screening visit) can occur on the same day as consent. The experimental visits will begin within 2-3 weeks or less of visit 1.

VII.D.29 ***Provide a description of the enrollment and consent process for adult subjects***

- ***Describe each study population separately including control population***
- ***Include when recruitment and consent materials are used***
- ***Use 3rd person active voice "The Principal Investigator will identify subjects. For example, the principal investigator will identify potential subjects, the study coordinator will discuss the study with subjects over the telephone and schedule the first study visit, etc..."***
- ***Describe the steps that will be taken by the research team to minimize the possibility of coercion or undue influence during the consent process***

The subjects will consist of healthy women (age 18 and older) who have delivered a baby within the past 2 years (24 months). The control group will be women who have had a healthy pregnancy, with no history of cardiovascular or metabolic disease before or during pregnancy. The preeclamptic group will be women who have had a preeclamptic pregnancy, but were free of cardiovascular or metabolic disease prior to pregnancy. These subjects will be enrolled and complete salsalate and placebo treatments in a double-blind randomized crossover design. The PI and research staff will recruit subjects from Johnson county and surrounding areas via flyers, emails, The UI mass-email system, newspaper and newswire ads, and posting on the UIHC website for research volunteers. Subjects will be asked to contact the research staff via phone or email. A study team member will then contact the potential participant by phone to complete the phone screening and schedule visit 1 if they are eligible and wish to participate. Subjects will be sent the consent summary either by e-mail or postal mail (subject preference) before visit 1. Visit 1 will include a detailed description of the study and review of the informed consent, including risks. If subjects are not able to understand the protocol and instructions for any reason, written or verbal, they are not included in the study. Subjects are informed throughout the consenting, screening, and conduction of the study that they may discontinue their participation at any time with no penalty to them. If the subject signs the informed consent, they are given a copy of the signed document to take home with them.

VII.D.37 ***Does the study include any form of deception (e.g., providing participants with false information, misleading information, or withholding information about certain study procedures)?***

Examples:

- ***Procedure includes a cover story that provides a plausible but inaccurate account of the purposes of the research.***
- ***Participants will be provided with false information regarding the particular behaviors of interest in the research.***
- ***Procedures include a confederate pretending to be another participant in the study.***
- ***Participants will be told that the research includes completion of a particular task, when in fact, that task will not be administered.***
- ***Study is designed to introduce a new procedure (or task) that participants are not initially told about.***
- ***If yes, a waiver of informed consent must be requested under question IV.3.***

No

VII.E. Project Description (E)

VII.E.1 ***Will subjects be randomized?***
Yes

VII.E.1.a ***Will any subjects be blinded to which study arm they have been assigned?***
Yes

VII.E.1.b ***Does the protocol permit telling subjects their treatment assignment at the end of the entire study?***
Yes

VII.E.1.c ***Describe the circumstances under which subjects will be told what study arm they have been assigned.***
After the study is completed, subjects can be told which treatment they received at which visit if they wish to know.

VII.E.2 ***Describe randomization scheme/assignment including ratio such as 1:1, 2:1 etc.***
Subjects will be randomized to which treatment they receive first (placebo or salsalate). All subjects will complete both arms of the study. They will be blinded to which treatment they are receiving at the time of the treatments. If they wish to know, subjects can be informed which treatment they received at what time when they complete the study.

VII.E.3 ***Will any questionnaires, surveys, or written assessments be used to obtain data directly from subjects in this study?***
Yes

VII.E.4 ***List all questionnaires, surveys, written assessments and ATTACH each one to the application. (NOTE: You are NOT prohibited from attaching copyrighted materials to this application)***

pre-consent screening survey
additional information survey
health history questionnaire

VII.E.5 ***Does this project involve creating any audiotapes, videotapes, or photographs?***
No

VII.E.6 ***Provide a detailed description in sequential order of the study procedures following the consent process - DO NOT cut and paste from the Consent Document.***

Describe study populations separately if they will be participating in different procedures - include CONTROL population if applicable.

DESCRIBE:

- ***What subjects will be asked to do/what happens in the study (in sequential order)***
- ***The time period over which procedures will occur***
- ***The time commitment for the subject for individual visits/procedures***
- ***Long-term followup and how it occurs***

Visit 1 - Consent and Screening (approximately 1 hour)
The PI or research staff explain the study and go over the informed consent with the participant. After the participant signs the informed consent, the PI or research staff performs the physical screening that includes urine pregnancy test, heart rate (HR), blood pressure (BP), height, waist circumference, and weight measurements. The participant fills out the health history questionnaire. A research nurse performs a standard venipuncture to obtain blood (total = 7.5ml blood) for complete blood count (CBC), chemistry analysis, and lipid profile to be measured at UIHC pathology labs.

Visits 2 and 4- Pick up study medications (approximately 15 minutes)
The PI or research staff give the participant study medication (salsalate or placebo). The PI or research staff goes over the instructions for taking the study medication which includes how many pills to take and at what times of day, potential side effects, and who to call if they have questions or problems while taking the medication.

Visits 3 and 5 - Experimental visits (approximately 4 hours each)
Note: prior to coming to the Field House for the experimental visits, subjects take the oral study medication for 5 days. Subjects will ingest 4 pills in the evening of Day 1, 4 pills in the evening and morning on Days 2,3, and 4, and 4 pills on the morning of Day 5, the same day as the experimental visit (see drug info and schedule handouts). After the first study visit, subjects will undergo a washout period of at least 14 days in which they do not ingest any study medication, before beginning the same 5 day regimen of the other treatment.

Experimental procedures
Subjects will come to the CRU where a research nurse performs a blood draw (14.5 ml, < 1 Tablespoon) for substances of interest (e.g. inflammatory cytokines) and immune cell activity, salsalate level, creatinine, and blood urea nitrogen. Then the the subject will be escorted by a member of the research team to 528 Field House for the intradermal microdialysis experiment.

Intradermal microdialysis experiments will take place in 528 Field House. Please see the attached "intradermal microdialysis" document for general information about intradermal microdialysis, schematic representation of the procedure, and references.

Microdialysis Probe Insertion: The researchers place a tight band around the forearm so they can visualize veins. For each MD site, they make pairs of pen-marks on the arm 2.5 cm (1 inch) apart and away from veins. They remove the tight band. The MD tubing enters and exits the skin at the marks. The researchers clean the arm with povidone iodine and alcohol, and place an ice bag on the site for 5 minutes to numb the skin. Then they insert a thin needle into the skin at each entry mark. The needle's tip travels between the layers of skin for 2.5 cm (1 inch) and exits the skin at the matching exit-mark. They thread the microdialysis tubing through the needle and then withdraw the needle leaving the tubing in the skin. Any hyperemia related to the insertion subsides in about 60 minutes. When the hyperemia induced by inserting the tubing in the skin subsides, the experiment begins.During this time Lactated Ringer's perfuses the tubing. The researchers tape a fiber optic laser Doppler flowmeter probe and its holder over each microdialysis site. The researchers control the temperature of the holders. The holders start at 33°C (91.4°F). During the experiment, the computerized data acquisition system records heart rate, sublingual temperature, skin blood flow, and skin temperatures continuously. The researcher and/or an automated critical care monitor measures blood pressure at 5-7 minute intervals.

Acetylcholine and Angiotensin II Dose Responses: The intradermal microdialysis probes are randomly numbered 1 - 3 and assigned to receive doses of acetylcholine with or without L-NAME (probes 1 and 2) or angiotensin II (probe 3).
Acetylcholine Dose Response: Microdialysis Probe 1. Lactated Ringer's only
Microdialysis Probe 2. Lactated Ringer's + L-NAME
Angiotensin II Dose Response: Microdialysis Probe 3. Lactated Ringer's only
When the skin blood flow is stable, the researchers perform a 10-minute baseline set of measurements. The researchers add identical concentrations of acetylcholine to the perfusate in probes 1 and 2 and Angiotensin II to the perfusate in probe 3. At 5-minute intervals, they increase the concentration of acetylcholine or angiotensin II in the perfusates. The participant receives 10 different concentrations of acetylcholine and 9 different concentrations of angiotensin II. After perfusing the last concentrations, the researchers warm the temperature controllers to 43°C (108°F) and switch perfusates at all sites to lactated Ringer's for about 30 minutes. After 30 minutes they add SNP to the perfusates for approximately 5 minutes. Heating and SNP perfusion causes maximum vasodilation at the microdialysis site. The researchers then de-instrument the subject, remove the microdialysis fibers, and apply sterile dressings. The subject is given verbal and written instructions for how to care for the microdialysis sites.

The researcher measures final vitals before the subject departs. At the end of visit 2, the subject is given instructions for the 14 day washout period. The subject may be given the pills for the second treatment at the end of visit 2(replacing visit 4), along with instructions for when and how to take the pills prior to study visit 5.

VII.E.7 ***Will you attempt to recontact subjects who are lost to follow-up?***

No - followup is not required in this study

VII.E.9 ***Will subjects be provided any compensation for participating in this study?***
Yes

VII.E.10 ***Cash***
No

VII.E.11 ***Gift Card***
No

VII.E.12 ***Check***
Yes

VII.E.13 ***Who will be providing the research compensation check to the subject?***
Accounting Services directly via the e-Voucher system

VII.E.16 ***Other***
Yes

VII.E.17 ***Describe:***
Parking passes will be provided

VII.E.18 ***If you plan to compensate subjects using cash, checks or cash equivalent does your unit have a Cash Handling Procedure in place that has been approved by Accounting Services?***
Yes

VII.E.19 ***Describe the compensation plan including***

- ***Compensation amount and type per visit***
- ***Total compensation***
- ***Pro-rating for early withdrawal from study***

Experimental visits: $75 per visit ($15/microdialysis probe + $7.50/hour for completing the experiment)
Total for completing the study: $150 (two completed study visits)
Parking pass for time involved at study and screening visits.

Pro-rating: Subjects can receive payment for experiments not completed. The researchers pay an amount of money equal to the part completed. For instance, if a subject completes half of Experiment 1, the subject receives $15.00 for each probe inserted plus $7.50 for each hour they completed. The researchers may ask subjects to repeat a trial. If subjects agree to repeat a trial, they receive payment for the repeated trial as stated above.

VIII. Risks

VIII.1 ***What are the risks to subjects including***
- emotional or psychological
- financial
- legal or social
- physical?
Physical Risks:
Microdialysis: Intradermal microdialysis is a specialized research technique. Dr. Stanhewicz is highly skilled in this technique and has been using the procedures in this protocol(including fiber placement, perfusate preparation, subject monitoring, and fiber removal) for over 10 years with no adverse events (see letter of support from Dr. Lacy Alexander)and have been reviewed and approved by Dr. Diana Jalal at the University of Iowa. Similar procedures for the use of intradermal microdialysis are used in other peer institutions around the country (see letter's of support from Dr. Jody Greaney, Dr. Megan Wenner, and Dr. Chris Minson) without regular adverse events.

Cutaneous microdialysis commonly causes some pain and bruising similar to that experienced during venipuncture. There is usually no pain after the probe is in place. The participant may experience mild pain while the researchers remove probe. Minor bleeding may occur. As with any event that breaks the skin, infection is possible. However, no participants in any of the researchers' prior experiments have reported infection. In the unlikely event in which the membrane breaks during removal leaving an isolated piece of membrane under the skin, they treat the piece of membrane in a manner similar to that for a splinter in the skin. In this case, trained personnel make a superficial incision for removal. Such an event has never occurred in any projects overseen by Dr. Stanhewicz.

Microdialysis delivers small amounts of pharmacological substances to a nickel-sized area of the skin. The small quantities used and the extremely localized administration during microdialysis does not produce systemic effects. To the researchers' knowledge, there are no reports of long or short-term side effects of these substances administered through microdialysis. The chance of adverse reactions to these substances is extremely small given the minute amount delivered to the a very small area of skin, the lack of adverse reactions to similar amounts delivered via MD in many other studies, and lack of adverse effects in human cell cultures. There is a slight chance of allergic reaction to these substances that could produce redness, itching, rash, and/or swelling.

Local Heating: The local heating control unit (Moor Instruments) precisely controls and monitors the temperature of the heated probe holders used with the Laser Doppler Flowmeter. The system has programmed maximum temperature limits. To determine the maximal SkBF, the researchers increase the temperature of the heating units slowly (about 0.1°C every 1 second). The skin feels very warm but not painful. Local heating causes temporary redness of the skin that subsides within several hours. This technique is very unlikely to produce long-term ill effects.

Povidone Iodine: Hospitals and researchers use povidone iodine to clean and sterilize the skin. Participants could be allergic to iodine. An allergic reaction could cause redness, itching, rash, and/or swelling. Staff use only alcohol on participants with iodine allergy as identified during screening.

Tape and adhesive disks: Participants could be sensitive to the adhesive of the tape, ECG electrodes, and double-sided adhesive disks used in the study causing redness, rash, tenderness, and/or itching. The researchers remove these items carefully.

Blood draws: Potential risks associated with obtaining blood samples are minimal but include slight bruising, pain, a temporary feeling of faintness, and/or a small risk of infection. All blood draws will be performed by a research nurse or team member trained in drawing blood.

Salsalate: Salsalate is a non-steroidal anti-inflammatory drug (NSAID) in a group of drugs called salicylates. This medicine is used clinically to reduce the pain, swelling, and joint stiffness caused by arthritis.

The most common side effects from the Salsalate medication include (in order of decreasing frequency) tinnitus ('rininging in the ears'), nausea, mild hearing impairment, rash, and vertigo in two studies with combined 280 subjects (percentages not available). Another study of 782 patients with osteoarthritis or rheumatoid arthritis were treated with about 3000 mg/day for 3 weeks, 324 (41%) experience one or more side effects with 234 (30%) requiring discontinuation of Salsalate for GI side effects (13.2%), tinnitus (6.7%), dizziness (1.7%) and 8.5% for other reasons.

Metabolic acidosis: There is a very small risk of metabolic acidosis from salicylate toxicity although no clinical trials above reported any incidence of this. This usually can be treated by stopping salsalate medication and with IV fluid and electrolyte therapy.

Heart attack or stroke: Because Salsalate is an NSAID, people who take NSAIDs (other than aspirin) may have a higher risk of having a heart attack or a stroke than people who do not take these medications. These events may happen without warning and may cause death. This risk may be higher for people who take NSAIDs for a long time and in those who have cardiovascular disease. There is no reported increased risk of heart attack or stroke in individuals on Salsalate so it cannot be determined what the exact risk (if any) is for Salsalate at the dose and duration in this study. Subjects are instructed to get emergency medical help right away if they experience any of the following symptoms during the study: chest pain, shortness of breath, weakness in one part or side of the body, or slurred speech.

Gastrointestinal bleeding: NSAIDs such as salsalate may cause ulcers, bleeding, or holes in the stomach or intestine. These problems may develop at any time during treatment, may happen without warning symptoms, and may cause death. The risk may be higher for people who take NSAIDs for a long time, are older in age, have poor health, smoke, or drink large amounts of alcohol or are on the following medications: anticoagulants ('blood thinners') such as warfarin (Coumadin); aspirin; other NSAIDS such as ibuprofen (Advil, Motrin) and naproxen (Aleve, Naprosyn); or oral steroids such as dexamethasone (Decadron, Dexone), methylprednisolone (Medrol), and prednisone (Deltasone). All of these medication are exclusion criteria in our study. Subjects are instructed to stop taking salsalate and call the study doctor if they experience any of the following during the study: severe stomach pain or heartburn, vomiting a substance that is bloody or looks like coffee grounds, blood in the stool, or black and tarry stools. Because of this risk we exclude for bleeding disorders and history of gastrointestinal bleeding.

Allergic reaction: Subjects could have a mild or severe allergic reaction including hives; difficult breathing; swelling of the face, lips, tongue, or throat. We exclude subjects who have a known allergy to aspirin or NSAID.

Emotional or Psychological risks: There are no foreseeable psychological risks with this study.

Social Risks: There are no foreseeable social risks with this study

Legal Risks: There are no foreseeable legal risks with this study.

Loss of confidentiality is possible.

VIII.2 ***What have you done to minimize the risks?***

- ***If applicable to this study ALSO include:***
  - ***How you (members of your research team at Iowa) will monitor the safety of individual subjects.***
  - ***Include a description of the availability of medical or psychological resources that subjects might require as a consequence of participating in this research and how referral will occur if necessary (e.g. availability of emergency medical care, psychological counseling, etc.)***

Note: All investigational substances used in these protocols are used with general Physician Oversight (see attached Standard Operating Procedures document)

Risks Associated with Salsalate Treatment:
During the study if a subject experiences side effects that are life threatening, the subject will be instructed to call 911. If the subject experiences any mild but tolerable expected side effects such as tinnitus, dizziness, nausea, headache, they will be asked to keep a log of these including the date, the duration, and the severity by rating on a scale of 1 (mild) to 10 (severe/intolerable). If the subject feels the side effects are uncomfortable or intolerable, then they will be instructed to call the Research Nurse or Dr. Stanhewicz. The Research Nurse or Dr. Stanhewicz (although she will be blinded) will instruct the subject to skip the next dose and if the symptoms/side effects have resolved at time of next dose (in 12 hours), they will be instructed to take the next dose. Dr. Stanhewicz will consult with Dr. Jalal if necessary. If symptoms do not resolve or worsen at time of next scheduled dose, the subject will be instructed to not take that dose, and to come to the CRU to have a blood sample to assess serum salicylate concentrations. The subject will be withdrawn from the study procedures but will be followed up as necesary. If serum salicylate concentrations are not in the toxic range (<30 mg/dl), the subject will be re-assessed in 12 hours to confirm that symptoms resolved. If blood plasma salicylate concentrations are in the toxic range (>30 mg/dl), participants will return to the CRU in 12 hours for a follow-up blood draw to confirm that salicylate values are decreasing and to reassess symptoms. Serum electrolytes, urinalysis and respirations may be obtained in the case of suspected metabolic acidosis due to Salsalate toxicity. If metabolic acidosis is suspected, then Dr. Jalal will be notified and determine appropriate medical management in the CRU or referral to the UIHC Emergency Department.

Risks Associated with Intradermal Microdialysis:
Research techniques are only performed by personnel who are trained and approved by Dr. Stanhewicz to complete these procedures. Dr. Stanhewicz has 10 years of experience utilizing intradermal microdialysis without unanticipated adverse events. All laboratory personnel are trained in CPR and basic first aid. In the event of a life threatening emergency, lab personnel call 911. The Field House is equipped with AED and lab personnel are trained in how to use them if necessary.

Prior to placing the microdialysis fibers, the researcher puts on sterile gloves and cleans the skin with iodine and alcohol to reduce the risk of infection. The researchers apply ice to the skin for 5 minutes before placing the microdialysis fibers to reduce any pain associated with placement. Once the fibers are in place, research personnel constantly monitor subjects for adverse reactions (e.g. pain, itching, redness, swelling) to the microdialysis fibers or the perfusates. Participants are never left unsupervised with the fibers in place. An automated monitor measures blood pressure every 5 minutes and constantly records heart rate. The researchers clip the ends of the fibers and clean them with an alcohol swab before removing them from the skin to reduce the risk of infection. They stop any bleeding by applying mild pressure to the sites with a sterile gauze pad. The researchers apply a clean, sterile bandage to the area before the participant leaves. Participants are given verbal and written instructions on how to care for the sites and to call Dr. Stanhewicz or the research nurse if they have any questions or concerns about the sites after they have left the study visit.

Risk of loss of confidentiality:
The investigators collect the minimum amount of confidential data in order to complete the aims. All subjects are assigned a code and data and specimens are collected using the code only. The only time subject names and codes appear together is on their file which is kept in a locked cabinet in the PI's office. Only IRB approved personnel have access to these files. All data files are stored on password protected computers and servers and only IRB approved personnel have access. Extensive details on how the risk of loss of confidentiality is minimized are provided in section X. Privacy and Confidentiality.

VIII.3 ***Does this study have a plan to have an individual or committee review combined data from all subjects on a periodic basis (such as summary or aggregate safety and/or efficacy data)?***
Yes

VIII.4 ***Describe the plan to review combined data from all subjects, such as summary or aggregate safety and/or efficacy data. Include the following:***

- ***Describe what data will be summarized and reviewed***
- ***Describe how frequently data will be reviewed.***

The local Data Safety Monitoring Board performs bi-annual reviews of the study protocol, subject enrollment information, and aggregate data collected to that point. The DSMB does not have access to individual identification of subjects. The DSMB then makes recommendations regarding the progress of the study.

VIII.5 ***Will overall safety monitoring be performed by individual(s)/committee at The University of Iowa. (NOTE: If this study involves more than minimal risk, in most cases these should be individuals who are not members of the study research team.)?***
Yes

VIII.6 ***List names:***
Mark Santillan, MD; Melissa Swee, MD; Bridget Zimmerman, PhD

VIII.7 ***Will overall safety monitoring be performed by individuals or committee not associated with The University of Iowa (such as a study Data Safety Monitoring Board)?***
No

IX. Benefits

IX.1 ***What are the direct benefits to the subject (do not include compensation or hypothesized results)?***
The study procedures and/or findings do not provide direct benefits to the subjects participating in the study. Subjects receive a medical screening that could inform them about their health. They learn their blood pressure and blood cholesterol levels. This is important knowledge. High blood pressure and blood cholesterol contribute to many serious health problems. We advise those with high blood pressure or blood cholesterol to follow-up with a health care provider. They also learn of the connection between preeclampsia and cardiovascular disease.

IX.2 ***What are the potential benefits to society in terms of knowledge to be gained as a result of this project?***
Preeclampsia, a disorder of pregnancy effecting ~5-8% of pregnancies in the United States, and ~8 million pregnancies worldwide. Otherwise healthy women who develop preeclampsia during pregnancy have 2-4 times greater risk for developing cardiovascular disease later in life. Cardiovascular disease is the leading cause of death among women worldwide, a fact lending even more gravity to the need for elucidating the mechanisms mediating the vessel dysfunction associated with preeclampsia and the increased risk for cardiovascular disease. The information to be gained is essential to the understanding of the related cardiovascular pathology. This knowledge could suggest and aid the development of novel therapeutic strategies for the management of risk in this population.

X. Privacy & Confidentiality

X.1 ***What are you doing to protect the privacy interests of the subjects?***
The minimum amount of data necessary to complete the aims will be collected during the study. The informed consent process will be conducted in a private exam room in the CRU with the door closed. All screening and experimental procedures will be conducted in the laboratory in 528 FH with the door closed and locked. Only personnel directly involved in the study will be allowed in the rooms.

X.2 ***Are you collecting the Social Security Number of any subjects for any purpose?***

No

X.4 ***How will information/data be collected and stored for this study (check all that apply):***

- Electronic records (computer files, electronic databases, etc.) - Data will be entered using subject ID code into the ICTS REDCap web-based database application that is password protected. No personal identifiable data will be entered. Only research staff on the IRB approved study will be allowed access this database. The ICTS REDCap staff are responsible for maintaining security of the data. Some data using subject ID code will also be entered into a Microsoft Excel and SPSS datasheets that will be kept in a shared server for CLAS that is password protected. Only research staff on the IRB approved study will have access to the folder the study on the server.
  - Name - Bryan Ringen
  - Title - IT Support Consultant
  - University Job Classification - Faculty/Staff
- Paper/hard copy records (hard copy surveys, questionnaires, case report forms, pictures, etc.) - Most of the data are coded and do not contain personal identifying information. Some data will temporarily contain the subject's name, address, and/or telephone number (Phone Interview Form). Documents allowing identification of participants do not leave our labs and are only available to authorized persons. Only authorized personnel may access the lab computer. Data forms containing identifiable information are shredded when no longer needed (within 5 years after publication of results). We keep data in the laboratory in locked cabinets, the password-protected folder on the secured University of Iowa RDSS server, and on password-protected computers maintained in locked laboratory rooms. Only authorized personnel have access. Coded data shared with unauthorized persons cannot be traced to individuals. The list linking code numbers to participants is not shared with unauthorized persons and destroyed when the project is completed and within 5 years of publication of the data. Screening data from subjects who are not accepted into the study are shredded when the project ends. Subjects may give permission to have their contact information retained in the investigator's secured files if they wish to be considered for participation in future studies. After we complete the study, we remove all identifiers from the data and store the data indefinitely. Individual data may be used without identifying the subject to illustrate representative responses. Any hard paper copy of subject lists or data will be kept in a locked cabinet in the locked office of the PI. Subjects will be assigned a code for the study. Files will be labeled with this code for confidentiality. Study data including laser-Doppler flowmetry data will be stored on password protected computer hard-drives and password protected server folders which are only accessible to research team members via password. Subject confidentiality will be maintained in all presentations and publications and information/records pertaining to subject participation in the research project will not be released without prior authorization by the subjects.
- Biologic samples (blood draws, check swabs, saliva samples, tissue samples, etc.) - Basic blood chemistries will be sent to the UIHC pathology lab for analyses. Remaining biological specimens, such as blood, will be labeled with subject code, date collected and IRB protocol number and transported to the PIs laboratory (528 Field House) in a secure unbreakable biohazard container. Samples will be stored in the PIs laboratory in a -80C freezer in S503 FH. All samples will be labeled with date collected and subject ID code only. No personal identifiable information will be labeled on the sample. Only the PI and her research staff will have access to the samples.
  - Name - Anna Stanhewicz
  - Title - Assistant Professor
  - University Job Classification - Faculty/Staff

X.5 ***Do the confidentiality protections indicated above allow only members of the research team to access the data/specimens?***
Yes

X.7 ***Does your study meet the NIH criteria for a Certificate of Confidentiality or will you be applying for Certificate of Confidentiality?***
Yes

X.8 ***If yes, provide rationale:***
Per Section 2012 of the 21st Century Cures Act as implemented in the 2017 NIH Certificates of Confidentiality Policy, all ongoing or new research funded by NIH as of December 13, 2016 that is collecting or using identifiable, sensitive information is automatically issued a CoC.

XI. Data Analysis

XI.1 ***Describe the analysis methods you will use, including, if applicable, the variables you will analyze***
The primary outcome variable will be cutaneous vascualr conductance, which is calculated from laser-Doppler flux/mean arterial pressure. Three-way repeated measures analysis of variance will be performed to examine group differences (PrEC vs. control) or systemic treatment differences (salsalate vs. placebo), and local microdialysis treatment differences across the doses of pharmacological stimuli. Appropriate post-hoc analyses with corrections for multiple comparisons will be performed when main effects are identified, including potential confounding variables as covariates.

XI.2 ***Provide the rationale or power analysis to support the number of subjects proposed to complete this study.***
Using previously published data with similar primary outcomes we determined by power analysis (2 group ANOVA, power=0.80, α=0.05) that 12 subjects per group will be sufficient to measure a meaningful physiological difference of at least 15% between microdialysis sites (within-subject comparison) and groups (between subject comparisons). Conservatively, we recommend increasing to 16 subjects per group to account for potential dropout during the salsalate trials.

XII. Future Research

XII.1 ***Do you wish to keep any information about subjects involved with this research project so that members of the current research team may contact them in the future for your own research projects?***
Yes

XII.2 ***Do you wish to keep any information about subjects involved with this research project so that other researchers may***

contact them for future research?
No

XII.3 ***List the data or information you will keep:***
Name
Race/ethnicity
Age
Pregnancy history
Contact information (mailing address, e-mail address, phone number)

XII.4 ***Does this project involve storing any data, tissues or specimens for future research?***
Yes – contribution for future use is optional

XII.5 ***Describe how you will keep track of those who consent to future use and those who do not and how you will prevent future use for those who do not consent.***
We will keep track of who consents to future use on our enrollment log.