Inflammation Inhibition in Prediabetic Humans (INCITE)

NCT01977417

2/15/23

Inflammation Inhibition in Prediabetic Humans

PI: Gary Pierce
IRB ID #: 201209707

Project Details

I. Project Introduction

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

I.2 ***Project Title:***
Inflammation Inhibition for Microvascular and Autonomic Dysfunction in Obese Prediabetic Humans (INCITE)

I.3 ***Short Title (optional):***
Inflammation Inhibition in Prediabetic Humans

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

Prediabetes, characterized by elevated fasting blood sugar or exaggerated blood sugar response to sugar ingestion, affects over 79 million adult Americans and is a precursor to the development of Type 2 diabetes. Importantly, approximately 42% of Iowans (950,000) have diabetes and 32% (670,000) have prediabetes with the majority of those with prediabetes going undiagnosed. Adults with prediabetes demonstrate early signs of cardiovascular and nervous system abnormalities and are at high risk for developing overt diabetes unless aggressive lifestyle (weight loss, exercise) or pharmacological interventions are employed. Interestingly, data in recent years has linked obesity and diabetes to chronic inflammation of the blood vessels and brain areas that regulate blood pressure. Therefore, the current study will test whether a commonly used aspirin-like anti-inflammatory drug called salsalate, will improve blood vessel health and nervous system dysfunction in adults with prediabetes. Eligible subjects will have measurements of blood pressure, blood vessel function in the arms and eyes, assessments of nerve activity, and blood samples taken before and after 4 weeks of ingesting an FDA approved aspirin-like drug called salsalate. The study is important because it will identify a potentially new pharmacological strategy to treat vascular and nervous system abnormalities in overweight and obese adults with early stage type 2 diabetes using an inexpensive, generically available drug with an excellent safety record that has been used for decades to treat chronic inflammatory conditions such as rheumatoid arthritis. If proven effective, this will provide preliminary support for the concept of targeting inflammation as a new clinical approach to treating early diabetes related complications. Furthermore, the current pilot study will provide support for developing a larger clinical trial using salsalate that could potentially then be extended to patients with type 2 diabetes and cardiovascular disease, as well as lead to the development of new anti-inflammatory agents with greater specificity for selective inflammatory pathways.

I.5 ***Specify your research question(s), study aims or hypotheses (do not indicate "see protocol")***

Aim 1: To measure microvascular function and aortic wall stiffness in obese prediabetic adults before and after 1 month of salsalate or placebo.

Hypothesis 1: *Chronic inflammation inhibition will improve microvascular and large elastic artery function in obese adults with prediabetes.*

Aim 2: To measure muscle sympathetic nervous system activity (MSNA) and baroreflex sensitivity in obese prediabetic adults before and after 1 month of salsalate or placebo.
Hypothesis 2: *Chronic inflammation inhibition will decrease MSNA and improve baroreflex sensitivity in obese adults with prediabetes.*

I.6 ***Background and significance and/or Preliminary studies related to this project.***
(do not indicate "see protocol")

Prediabetes is characterized by insulin resistance and glucose intolerance and is estimated to affect ~79 million Americans (data from 2011 National Diabetes Fact Sheet uses both fasting glucose and A1C levels to derive estimates for undiagnosed diabetes and prediabetes) including ~35% of adults 20- 50 years of age.1 Prediabetes is a precursor to the development of overt type 2 diabetes and is typically associated with obesity. Obesity-related prediabetes is a major risk factor for the cardiovascular disease (CVD) and adults with prediabetes are at high risk of progressing to Type 2 diabetes unless aggressive lifestyle or pharmacological intervention is implemented. 2 Even before overt Type 2 diabetes is present, adults with prediabetes demonstrate signs of microvascular endothelial dysfunction and increased large elastic artery stiffness and remodeling. 3 In addition, obese prediabetic adults develop tonically elevated sympathetic nervous activation 4, likely as a compensatory mechanism to promote increased thermogenesis. However, this has long-term clinical cardiovascular consequences including the development of hypertension and reduced baroreflex sensitivity. Prevention of the progression to type 2 diabetes and associated vascular and autonomic dysfunction by implementing lifestyle changes (e.g., exercise, weight loss) remains the first line therapeutic strategy for adults with prediabetes. Unfortunately, many adults cannot sustain these lifestyle changes in the long-term, therefore identifying novel therapeutic agents aimed at treating vascular and sympathetic nervous system dysfunction in obese prediabetic humans is a high biomedical priority.

Although the mechanisms for vascular and autonomic dysfunction with prediabetes are not completely understood, strong experimental evidence implicates inflammation as a common molecular link between them. Specifically, chronic activation of the nuclear factor kappa B (NFkappaB), a key pro-inflammatory transcription factor that regulates hundreds of inflammatory genes and is regulated by its upstream activator IkappaB kinase beta (IKKB), is hypothesized to be a central feature of vascular inflammation that promotes atherosclerosis in obesity and diabetes. 5, 6 Moreover, recent evidence strongly links IKKB/NFkappB axis activation in the mediobasal hypothalamus to obesity-mediated hypertension in rodents primarily from enhanced sympathetic nervous system activation. 7 Taken together, these data suggest that the IKKB/NFkappaB axis may be a common molecular target to treat vascular and autonomic dysfunction in obese prediabetic humans.

Salsalate, a non-acetylated salicylate prodrug that has been used for decades to treat chronic inflammatory diseases such as rheumatoid arthritis, inhibits the IKKB/NFkappB pro-inflammatory pathway. 8 Accordingly, we recently demonstrated that acute high-dose salsalate improved vascular endothelial function in older obese adults 5 via reduced vascular NFkappaB. We also found reduced blood pressure, fasting glucose and HOMA score, suggesting favorable effects on autonomic function and insulin sensitivity. Indeed, a recent clinical trial demonstrated improved dysglycemia and HbA1c after 3 months of salsalate therapy in adults with diabetes9, however, there have been no studies

investigating the effects of chronic salsalate administration on cardiovascular and autonomic function in these groups of adults. Therefore, the current pilot proposal will test the central hypothesis that short-term salsalate administration will improve vascular and autonomic dysfunction in obese adults with prediabetes. We chose to study adults with prediabetes rather than overt Type 2 diabetes in order to isolate the efficacy of salsalate on vascular and autonomic dysfunction in the early stages of diabetes without the confounding effects of anti-diabetic medications and advanced diabetic co-morbidities.

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

II. Research Team

II.1 ***Principal Investigator***

Name	E-mail	College
Gary Pierce	gary-pierce@uiowa.edu	College of Liberal Arts and Sciences

II. *Team Members*
2

UI Team Members

Name	E-mail	College	Contact	Key Prsn	UI COI	VAMC COI	Consent Process Involvement	Activity Location	Subjects consented	Deactivated
Gary Pierce, MS, PHD	gary-pierce@uiowa.edu	College of Liberal Arts and Sciences	Yes	Yes	No		Yes			No
Michael Abramoff, PHD, MD	michael-abramoff@uiowa.edu	Roy J. & Lucille A. Carver College of Medicine	No	Yes	No		No			No
Marcelo Correia, MD	marcelo-correia@uiowa.edu	Admin - University Hospitals	No	No	No		No			No
Kaitlyn Dubishar, BA	kaitlyn-dubishar@uiowa.edu	Graduate College	No	No	No		Yes			No
Jess Fiedorowicz, MD, PHD	jess-fiedorowicz@uiowa.edu	Roy J. & Lucille A. Carver College of Medicine	No	Yes	No		No			No
Victoria Guzman, ARNP	victoria-guzman@uiowa.edu		No	No	No		No			No
William Haynes, MD	william-g-haynes@uiowa.edu	Roy J. & Lucille A. Carver College of Medicine	No	Yes	No		No			No
Veronica Howsare, AA	veronica-howsare@uiowa.edu		Yes	No	No		Yes			No

Kyle Siefers, High School	kyle-siefers@uiowa.edu	Graduate College	No	No	No	Yes		No
Christine Ann Sinkey, BSN	christine-sinkey@uiowa.edu		No	No	No	No		No
Harald Stauss, MD, PHD	harald-stauss@uiowa.edu	College of Liberal Arts and Sciences	No	Yes	No	No		No
Ericka Tank, High School	ericka-tank@uiowa.edu	College of Liberal Arts and Sciences	No	No	No	No		No
Lauren Wegman, PHD	lauren-wegman@uiowa.edu	College of Liberal Arts and Sciences	No	No	No	No		No
Harold Winnike, AE-C, BS, RRT	harold-winnike@uiowa.edu		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	Activity Location	Subjects consented

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
Gary Pierce, MS, PHD	Yes
Michael Abramoff, PHD, MD	Yes
Marcelo Correia, MD	No
Kaitlyn Dubishar, BA	No
Jess Fiedorowicz, MD, PHD	Yes
Victoria Guzman, ARNP	No
William Haynes, MD	Yes
Veronica Howsare, AA	No
Kyle Siefers, High School	No
Christine Ann Sinkey, BSN	No
Harald Stauss, MD, PHD	Yes
Ericka Tank, High School	No
Lauren Wegman, PHD	No
Harold Winnike, AE-C, BS, RRT	No

III. Funding/Other Support

III.1 ***Funding Sources***

Type	Source	Grant Title	Name of PI on Grant	Status	Status Description
Federal Agency	US Department of Health & Human Services, National	University of Iowa Clinical and Translational Science Program	Gary Rosenthal	Awarded	

	Institutes of Health				
Private Foundation/Association	Fraternal Order of Eagles	Inflammation Inhibition for Microvascular and Autonomic Dysfunction in Obese Prediabetic Humans	Gary L. Pierce	Awarded	

* new source name

III.2 ***Which office will process the agreement for this project***
Sponsored Programs - Federal/State/Local Agency Funded

III.3

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	
Fraternal Order of Eagles	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.***
9/22/12

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):***
salsalate, sublingual nitroglycerin, acetylcholine, sodium nitroprusside, ascorbic acid, inhalation of FiCO2=2%CO2,4%CO2, 6%CO2 for 3 min each (all balanced by 21% O2 and N).

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 all drugs or substances in this study being used within the FDA approved indication (i.e., disease, condition)?***
No

V.6 ***Are all drugs or substances in this study being used within the FDA approved dose?***
Yes

V.7 ***Are <u>all drugs or substances</u> in this study being used within the FDA approved route of administration?***

Yes

V.8 ***Drugs Used In Study:***

Nitroglycerin (Nitrostat)	
Name of Sponsor	
Investigator's Brochure Version	
Investigator's Brochure Date	
Planned Use in this Study	
Condition/Disease Indication(s)	Vascular dysfunction
Subject Population	Obese adults age 18-49 with prediabetes
Dose(s)	0.3 mg
Administration	Other: sublingual
Dosing Regimen	0.3 mg once
FDA Approved Use	
Approved Condition/Disease Indication(s)	Refief of angina pectoris pain
Approved Patient Population	Angina pectoris
Approved Dose(s)	0.3mg
Approved Administration	Other: sublingual
Approved Dosing Regimen	0.3 mg
Is this study intended to be reported to the FDA as a well-controlled study in support of a new indication or a significant change in the labeling for this product?	No
Is this study intended to support a significant change in the advertising for this product?	No
Does this planned use of the product in this study, taking into consideration the route of administration, the dosage level, and the subject population, significantly increase the risk (or decrease the acceptability of the risk) associated with the use of this product?	No
Rationale:	This is used to test the responsiveness of vascular smooth muscle to an exogenous nitric oxide vasodilator. Our subjects are healthy you and older adults. The dose is small and has a fast onset of action (1-3 mins) duration of action of 30 min. reference: Hardman JG, limbrid LE, eds. Gilmans The Pharmacolgical Basis of Therapeutics. 9th ed. New yourk: McGraw Hill, 1996.

Sodium nitroprusside (Nitropress AE)	
Name of Sponsor	
Investigator's Brochure Version	
Investigator's Brochure Date	
Planned Use in this Study	
Condition/Disease Indication(s)	Forearm vascular endothelial dysfunction
Subject Population	Obese adults age 18-49 years with prediabetes
Dose(s)	1 and 10 µg/mL/min intra-arterially
Administration	Other: intra-arterial via brachial artery
Dosing Regimen	1 µg/mL/min intra-arterially for 6 min; 10 µg/mL/min intra-arterially for 6 min
FDA Approved Use	

Approved Condition/Disease Indication(s)	Hypertension
Approved Patient Population	Adults with hypertension
Approved Dose(s)	1 µg/mL/min intra-arterially for 6 min
Approved Administration	Intravenous, Other: intra-arterial via brachial artery
Approved Dosing Regimen	0.3 µg/kg/min up to 10 µg/kg/min IV
Is this study intended to be reported to the FDA as a well-controlled study in support of a new indication or a significant change in the labeling for this product?	No
Is this study intended to support a significant change in the advertising for this product?	No
Does this planned use of the product in this study, taking into consideration the route of administration, the dosage level, and the subject population, significantly increase the risk (or decrease the acceptability of the risk) associated with the use of this product?	No
Rationale:	Intra-arterial infusion at the proposed dose results in no change in systemic hemodynamics (e.g., heart rate and blood pressure). Raghuveer G, Sinkey CA, Chenard C, Stumbo P, Haynes WG: Effects of vitamin E on resistance vessel endothelial dysfunction induced by methionine. Am J Cardiol 88(3): 285-290; 2001.; 3) Gudmundson S, Sinkey CA, Chenard C, Stumbo P, Haynes WG: Resistance vessel endothelial function in healthy humans during transient postprandial hypertriglyceridemia. Am J Cardiol 85:381-385, 2000. Pierce et al. Hypertension 2008;52:72-79.

Acetylcholine (Michol-E-System pack)	
Name of Sponsor	
Investigator's Brochure Version	
Investigator's Brochure Date	
Planned Use in this Study	
Condition/Disease Indication(s)	Forearm vascular endothelial dysfunction
Subject Population	Obese adults age 18-49 with prediabetes
Dose(s)	3-30 mcg/ml/min intra-arterially via brachial artery
Administration	Other: intra-arterial via brachial artery
Dosing Regimen	3 mcg/ml/min for 6 min x 2; 30 mcg/ml/min for 6 min x 2
FDA Approved Use	
Approved Condition/Disease Indication(s)	vasodilation; gastorintestinal tract stimulant
Approved Patient Population	Adults
Approved Dose(s)	30 mcg/ml/min
Approved Administration	Intravenous, Other: intra-arterial via brachial artery
Approved Dosing Regimen	3 mcg/ml/min for 6 min x 2; 30 mcg/ml/min for 6 min x 2
Is this study intended to be reported to the FDA as a well-controlled study in support of a new indication or a significant change in the labeling for this product?	No
Is this study intended to support a significant change in the advertising for this product?	No

Does this planned use of the product in this study, taking into consideration the route of administration, the dosage level, and the subject population, significantly increase the risk (or decrease the acceptability of the risk) associated with the use of this product?	No
Rationale:	The dose used in this study is intended to cause local vasodilation of resistance arteries in forarm vasculature and does not cause any systemic cardiovascular hemodynamics (blood pressure and heart rate). Gundmundsson et al. Am J Cardiol 2000;85:381-385. Pierce GL et al. Hypertension 2008;52:72-79.

salsalate (Disalcid, Amigesic, Salsitab, Argesic-SA, Marthritic, Salflex)	
Name of Sponsor	Gary L. Pierce
Investigator's Brochure Version	673
Investigator's Brochure Date	7/8/09
IND#	118676
Dose	3000 mg day (taken 1500 mg in the a.m. and 1500 mg in the p.m.)
Route of administration	Oral

Carbon dioxide gas ()	
Name of Sponsor	Praxair
Investigator's Brochure Version	Material Data Safety Sheet
Investigator's Brochure Date	July 2007
Planned Use in this Study	
Condition/Disease Indication(s)	Reduced middle cerebral artery reactivity
Subject Population	obese prediabetic adults
Dose(s)	FiCO2:2%, 4%, 6%, balanced by 21%O2 and nitrogen
Administration	Other: inhalation
Dosing Regimen	2%, 4%, 6% FiCO2 for 3 min each
FDA Approved Use	
No approved use	
Is this study intended to be reported to the FDA as a well-controlled study in support of a new indication or a significant change in the labeling for this product?	No
Is this study intended to support a significant change in the advertising for this product?	No
Does this planned use of the product in this study, taking into consideration the route of administration, the dosage level, and the subject population, significantly increase the risk (or decrease the acceptability of the risk) associated with the use of this product?	No
Rationale:	The potential side effects of CO2 breating of 2, 4 and 6% FiCO2 are minor (see risks) and reversible upon breathing room air. Yang Y, et al.(1997). The effect of moderately increased CO2 concentration on perception of coherent motion. Aviat Space Environ Med 68:187-191. Sun M, et al (1996). Effect of low-

	concentration CO2 on stereoacuity and energy expenditure. Aviat Space Environ Med 67:34-39. Wagner JA, et al. (1983). Effects of carbon dioxide inhalation on physiological responses to cold. Aviat Space Environ Med 54:1074-1079.

Ascorbic acid injection (vitamin c) (Ascorbic acid)	
Name of Sponsor	
Investigator's Brochure Version	
Investigator's Brochure Date	
Planned Use in this Study	
Condition/Disease Indication(s)	Vascular dysfunction
Subject Population	Obese adults 18-49 with prediabetes
Dose(s)	25 mg/min intra-arterial infusion
Administration	Other: Intra-arterial via brachial artery
Dosing Regimen	Intra-brachial infusion at 25 mg/min at rate of 1 ml/min for a 10 minute. Then, co-infused with acetylcholine for 6 min at 3 mcg/min , 6 min at 30 mcg/min. After a 20 min washout, intra-brachial artery infusion at 25 mg/min at a rate of 1 ml/min for 10 minutes and co-infused with sodium nitroprusside at rate 1 ml/min for 6 min at 1 mcg/min and then 6 min at 10 mcg/min.
FDA Approved Use	
Approved Condition/Disease Indication(s)	Scurvy
Approved Patient Population	Patients with scurvy
Approved Dose(s)	300-1000 mg/day
Approved Administration	Oral, Intravenous, Other: Intra-arterial via brachial artery
Approved Dosing Regimen	300-1000 mg/day
Is this study intended to be reported to the FDA as a well-controlled study in support of a new indication or a significant change in the labeling for this product?	No
Is this study intended to support a significant change in the advertising for this product?	No
Does this planned use of the product in this study, taking into consideration the route of administration, the dosage level, and the subject population, significantly increase the risk (or decrease the acceptability of the risk) associated with the use of this product?	No
Rationale:	Intra-arterial vitamin C via the brachial artery has been administered for over a decade safely into humans by many laboratories without any adverse events (Taddei et al. Circulation. 2000 Jun 27;101(25):2896-901; Kirby BS et al. J Physiol. 2009 May 1;587(Pt 9):1989-2003; Crecelius AR et al. Am J Physiol Heart Circ Physiol. 2010 Nov;299(5):H1633-41).

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 HCCC?***
No

V.23 ***Will any part of this project be conducted on VAMC premises?***
No

V.24 ***Does this project involve VAMC patients or records?***
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)?***
(for studies conducted entirely at the VAMC, the answer to this question is "no")

- ***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?***
Yes

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

V.26 ***The study involves nursing, nursing resources or evaluates nursing practices.***
No

VI. Subjects

VI.1 ***How many adult subjects do you expect to consent or enroll for this project?***
60

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

VI.3 ***What is the age of the oldest adult subject?***
49.0

VI.4 ***What is the percentage of adult male subjects?***
50

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

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***

A total of 30 healthy men and women ages 18-49 years who are obese defined as body mass index (BMI) of 30 kg/m2 or greater and with prediabetes (defined as fasting blood glucose between 100-126 mg/dl, a fasting blood glucose of 140-199 mg/dl at 120 min during an oral glucose tolerance test; or HbA1C of 6-6.5%) will be enrolled and randomized to 4-5 weeks of salsalate or placebo in the study. CONTROL GROUP: Another n=10 healthy obese men and women age 18-49 WITHOUT prediabetes will be enrolled for baseline testing but will not undergo randomization to the salsalate/placebo intervention. Therefore, total enrollment will be n=40.

All subjects will have no history of cardiovascular, metabolic or pulmonary disease as determined from medical history and physical exam, and a resting 12-lead ECG. Subjects may be on anti-hypertensive medications but will be asked to hold the medication on the morning of testing. Subjects on medications for Type I or II diabetes mellitus or for hyperlipidemia will be excluded (see complete list below). Subjects will be non-smokers or quit smoking at least one year ago. Women will have regular menses and will be tested during the early follicular phase of their menstrual cycle (within 8 days of onset of menses) to control for differences in circulating estradiol concentrations.

Inclusion criteria:
• Willing and able to provide written, signed informed consent after the nature of the study has been explained, and prior to any research-related procedures.
• Age is > or = 18 and < or = 49 years (older)
• Obese defined as body mass index > or = 30 kg/m2
• Prediabetic defined as fasting blood glucose 100-126 mg/dl, blood glucose between 140-199 mg/dl at 120 min of oral glucose tolerance test
•healthy, as determined by health history questionnaire, medical history and physical examination by physician or nurse practitioner, blood chemistries, resting blood pressure and exercise 12-lead ECG
• blood chemistries indicative of normal renal function (creatinine <2.2 mg/dl), liver (<3 times upper limit for ALT, AST), and thyroid function (TSH between 0.4 - 5.0 mU/L)
• If currently receiving treatment with or taking any of the following supplements, be willing and able to discontinue taking them for 2 weeks prior and throughout the treatment period: Vitamin C, E or other multivitamins containing vitamin C or E; nutraceuticals containing vitamin C or E
• No history of cardiovascular disease (e.g., heart attack, stroke, heart failure, valvular heart disease, cardiomyopathy), Type 1 or 2 diabetes mellitus, or peripheral arterial disease
• Sedentary or recreationally active defined as performs regular aerobic exercise (30 min or more of vigorous walking, jogging, swimming, cycling, etc) less than 2 days/week or less than 12 days/month over the last year
• Non-smokers, defined as no history of smoking, no smoking for at least the past 1 year
• Normal resting 12-lead ECG.

Exclusion Criteria:
• History of cardiovascular disease such as myocardial infarction, stroke, heart failure with or without LV ejection fraction <40%, cardiomyopathy, valvular heart disease,

cardiomyopathy, heart transplantation, Type 2 diabetes and Type 1 diabetes
• Smoking or history of smoking within past one year
• History of gastric ulcers, bleeding disorders, dyspepsia, severe gastroesophageal reflux disease (GERD), or metabolic acidosis
• History of asthma or lung disease (chronic obstructive pulomonary disease, COPD)
• Abnormal resting 12-lead ECG (e.g., evidence of myocardial infarction, left ventricular hypertrophy, left-bundle branch block, 2nd or 3rd degree AV block, atrial fibrillation/flutter)
• Serious neurologic disorders including seizures
• History of renal failure, dialysis or kidney transplant
• Serum creatinine > 2.2 mg/dL, or hepatic enzyme concentrations > 3 times the upper limit of normal
• History of HIV infection, hepatic cirrhosis, other preexisting liver disease, or positive HIV, Hepatitis B or C test at screening.
• Use of any investigational product or investigational medical device within 30 days prior to screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
• History of recent chicken pox, shingles or influenza (ie., risk of Reye's syndrome) Recent flu-like symptoms within the past 2 weeks
• Pregnant or breastfeeding at screening, or planning to become pregnant (self or partner) at any time during the study. A urinary pregnancy test will be done on all females. If test is positive, the subject will be excluded.
• Women with history of hormone replacement therapy within the past 6 months
• History of rheumatoid arthritis, Grave's disease, systemic lupus erythamatosis, and Wegener's granulomatosis;
• Taking medications for diabetes mellitus, kidney disease, liver disease, asthma, sepsis or seizure disorders;
• Taking lipid lowering (e.g., statins, niacin), glycemic control (e.g. metformin, insulin), anticoagulation, anti-seizure, anti-depression or antipsychotic agents
• History of co-morbid condition that would limit life expectancy to < 6 months.
• It is unknown if Salsalate is transferred in seminal fluid of men. However, it is recommended that proper protection such as a condom be used during intercourse during the study.
• Concomitant treatment with: aspirin, baby aspirin, indomethacin, naproxen (Aleve), acetaminophen (Tylenol), ibuprofen (Advil, Motrin), any other non-steroidal anti-inflammatory drugs; cox-2 inhibitors (Celebrex, Vioxx, etc); allopurinol (Zyloprim, Lopurin, Alopurin; coumadin (Wafarin), enoxaparin (Lovenox); clopidogrel (Plavix); dypyridamole (Persantine); heparin; diabetic medications (Metformin, glyburide, insulin, etc), TZDs (Avandia, Rezulin, Actos); corticosteroids (prednisone); methotrexate, infliximib (Remicade), etaneracept (Enbrel); levothyroxine (Levoxyl, Synthroid, Levoxyl, Unithroid); Levodopa; Phosphodiesterase (PDE) 5 inhibitors (e.g., Viagra®, Cialis®, Levitra®, or Revatio®); PDE 3 inhibitors (e.g., cilostazol, milrinone, or vesnarinone); lithium
• May participate if use of the following medications are discontinued 2 weeks prior to participation: salicylate medications, aspirin, antioxidants, herbal supplements, vitamins, omega-3 fatty acids; cox-2 inhibitors (Celebrex, Vioxx, etc)
• May participate if no use of the following medications in the 48 hours prior to experimental visits: naproxen (Aleve), acetaminophen (Tylenol), ibuprofen (Advil, Motrin), other any non-steroidal anti-inflammatory drugs
• Vulnerable populations (prisoners, etc.) are not included in this study because we are studying healthy middle-aged/older adults.
• Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
• Hemoglobin <12 mg/dl for men; < 10 mg/dl for women
• History of alcohol abuse or >10 alcoholic units per week (1 unit= 1 beer, 1 glass of wine, 1 mixed cocktail containing 1 oz alcohol)
• Low platelets (<100,000 cu mm)
• On weight loss drugs (e.g., Xenical (orilistat), Meridia (sibutramine), Acutrim

(phenylpropanol-amine), or similar over-the-counter medications) within 3 months of screening
• Any surgery within 30 days of screening

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)***

The 2010 U.S. census indicates that there are 139,165 adults between the ages of 18-39 in Johnson county. Of these based on rates of obesity (BMI >/= 30 kg/m2)in the State of Iowa, we expect that approximately 1/3 or 45,924 adults are obese in Johnson County. Given that approximately 42% of Iowans (950,000) have diabetes and 32% (670,000) have prediabetes, we estimate that ~44,532 adults between age 18-39 have prediabetes in Johnson County.

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 via mass email to University of Iowa community, post flyers on buildings on University of Iowa and UIHC campus, advertise in the Daily Iowan newspaper, the 'volunteer research' clinical trials website on UIHC website (http://www.uihealthcare.org/ClinicalTrials.aspx/), post flyers in the community, and in the 'Noon news' in UIHC (see attachments).

Training controls for microneurography will be recruited from the community via flyers posted on campus and emails to UI community. Subjects will be asked to contact the research staff via phone or email. No undergraduate students associated with the PI will be recruited for this training.

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)?***

- CRU
- Other UI campus site - 522 Field House (PIs lab)
- UIHC - Ophthamology Clinic, Pomerantz Pavillion

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:***

- ***clinical intervention***
- ***pharmacologic intervention***
- ***therapeutic intervention***
- ***physiology studies (e.g. studying the functions of organs, tissues, or cells)***

Yes

VII.B.2 ***Does this project involve a drug washout (asking subject to stop taking any drugs s/he is currently taking)?***

Yes

VII.B.3 ***Describe the management plan, including when you would stop the subject's participation in the event the subject worsens during the washout period.***

If subjects are on salicylate medications, aspirin, antioxidants, herbal supplements, vitamins, omega-3-fatty acids, they may be in the study but will be asked during the consenting process to go off of these medications for 2 weeks before participating. If subjects are unwilling or medically unable to go off these drugs for the 2 weeks and during the course of the study, they will be ineligible to participate in the study and not consented. If they are willing to go through the 2 week washout, they can sign the consent during visit 1 but will have the Visit 1 procedures rescheduled to be completed in 2 weeks. If the subject is unclear if they can medically go off of the medications they will be required to contact their personal physician to confirm they can go off any of the prescription medications listed above for the study duration.

VII.B.4 ***Describe the method (phone/in person) and frequency of contact with the subject during the washout period.***

After the subject's sign the informed consent document and agree to go off of the aforementioned drugs for 2 weeks, they will be called by the study coordinator on the phone after 2 weeks of the washout and invited back to the CRU for Visit #2.

VII.B.5 ***Who (list names) will be available on a 24/7 basis for questions or emergencies during the washout period?***

Gary Pierce, PhD; William Haynes, MD, PhD

VII.B.6 ***Will any subjects receive a placebo in this study when, if they were not participating, they could be receiving an FDA-approved treatment for their condition?***

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?***
Yes

VII.C.2 ***What information will be obtained from the DNA samples?***
DNA will be isolated from whole blood monocytes and used to determine nuclear factor kappa B DNA binding activity before and after Salsalate treatment.

VII.C.3 ***What data will be stored with the DNA samples? (e.g., identifiers, code numbers linked to identifiers, diagnoses, other clinical information, etc.)***
The only data to be stored with DNA samples are subject ID codes, the date collected and protocol number. No personal health information will be stored with the samples.

VII.C.4 ***Will subjects be able to request at a later time that samples be destroyed?***
Yes

VII.C.5 ***Where will the DNA and any associated information be stored?***
DNA will be stored in a -80C freezer in the PIs laboratory (N400 Field House). Stored DNA samples will be labeled only with subject ID code, date sample was collected and the IRB protocol number. A key (spreadsheet) with subject's ID code and their name will be stored in a folder on the CLAS server that is password protected. Only the PI and his research staff that are on the IRB approved protocol will have password access to this spreadsheet.

VII.C.6 ***Describe the mechanisms for maintaining confidentiality at the storage location.***
The tubes of DNA samples that only contain subject ID, date and protocol number written on them. No personal identifiers will be on the tubes. The freezer will be kept in a locked laboratory in N400 FH.

VII.C.7 ***Could the DNA and/or associated information be shared in the future with other researchers?***
No

VII.C.9 ***Will the subjects have the option of receiving any DNA testing results?***
No

VII.C.11 ***Is the laboratory that will be performing the DNA testing CLIA certified?***
No

VII.C.12 ***Will the DNA samples be destroyed at the conclusion of the study?***
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):***

- Advertisements -
- Posters -
- E-mail -
- Letter -
- News releases -
- Use of any information available to the researchers or their colleagues because this person is a patient OR use of any information considered to be Protected Health Information (PHI) OR review of patient/clinic records - Reviewing the medical record for study eligiblity.
- Website - http://www.uihealthcare.org/ClinicalTrials.aspx/

- Other - The initial approach will be by a member of the research team by email, phone, or in person.
- Research Match.org -

VII.D.2 ***List the individual data elements you will need to access/use from the patient or clinic records to identify potential subjects for recruitment***

The research team will review the following for eligibility:
Age 18-49
BMI > 30
Fasting glucose >100 mg/dl < 126
No Type 2 or Type I diabetes
No history of cardiovascular disease (e.g., heart attack, etc) They can be on some medications for high blood pressure, but not statins for cholesterol or diabetes meds (insulin, metformin). No SSIDs pysch meds.
No other disease e.g., lung, kidney, cancer,

VII.D.3 ***Describe why you could not practicably recruit subjects without access to and use of the information described above***

The use of electronic medical records would make preliminary screening as efficient as possible and would avoid errors in the screening process. We cannot practically recruit subjects without use of PHI because we want to ensure we approach subjects who meet the inclusion and exclusion criteria for study.

VII.D.4 ***Describe why you could not practicably obtain authorization from potential subjects to review their patient or clinic records for recruitment purposes.***

It is not practical to have each potential subject sign a release to review their electronic medical record for study participation. It is more efficient to screen for patient in EPIC and then ask for their consent if they want to participate in the study. We only want to approach subjects who meet the inclusion and exclusion criteria for study

VII.D.5 ***Describe plans to protect the identifiers from improper use or disclosure***

Identifiers used from the PHI will be name and address to mail the potienal subject a recruitment letter.

All patient identifiers will be kept on a password protected computer that only the research team will have access to. All paper documents with patient identifiers will be kept in a locked filing cabinet in a locked office and only the research team will access.

VII.D.6 ***Describe plans to destroy identifiers at the earliest opportunity consistent with conduct of the research***

After study closure all study documents will be stored in a locked storage facility at the ICTS and will be destroyed 3 years after study closure. The UIHC has outsources a document destruction company that will shred and recycle all documentation. All electronic data will be placed on a Zip drive and stored with the paper documents and destroyed.

VII.D.7 ***Does the research team agree that the requested information will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the study, or for other research for which the use or disclosure of the requested information would be permitted by the HIPAA Privacy Rule***

Yes

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:***

Study staff will discuss the study with potential subject and answer questions in a conference 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:***

The subjects will call, email or in person the research staff in response to seeing one of our research advertisements or by the colleague referral. The potential subject will leave a contact telephone number for the coordinator or staff and the best day and time to contact them. The staff will give study information over the phone to the potential subject or in person and then ask the subject the questions on the phone screening form (see attachment) to determine eligibility. If it appears that the subject is eligible, the subject will be invited to the CRU for the informed consent. Informed consent will not be done over the phone.

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

Name	Consent Process Involvement
Gary Pierce, MS, PHD	Yes
Michael Abramoff, PHD, MD	No
Marcelo Correia, MD	No
Kaitlyn Dubishar, BA	Yes
Jess Fiedorowicz, MD, PHD	No
Victoria Guzman, ARNP	No
William Haynes, MD	No
Veronica Howsare, AA	Yes
Kyle Siefers, High School	Yes
Christine Ann Sinkey, BSN	No
Harald Stauss, MD, PHD	No
Ericka Tank, High School	No
Lauren Wegman, PHD	No
Harold Winnike, AE-C, BS, RRT	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.***

1. What is your name?
2. What is your gender?
3. What is your age?
4. What is your address?
5. What is your phone number and email address?
6. Do you take any prescription medications? If so, which ones, how frequent, and the dose?
7. Do you take any over-the-counter medications, supplements, vitamins, minerals? If so, which ones?
8. Do you have any of the following conditions or diseases(answer yes or no):
-Cancer?

-Kidney disease or failure?
-Thyroid disease/disorder?
-Diabetes type I?
-Diabetes type II?
-Severe GI/gastric reflux/GERD?
-Quit smoking in the last 12 months?
-Liver disease?
-HIV/AIDS?
-Graves disease/Granulomatosis?

8. Do you have any of the following conditions or diseases(answer yes or no):
-Brain tumor?
-Seizures?
-Tinnitus or 'ringing in the ears'?
-Brain injury?

10. Do you have any of the following conditions or diseases(answer yes or no):
-Heart attack?
-Angina (i.e., chest discomfort/pain/pressure upon exertion)
-Congestive heart failure?
-Heart angioplasty/stent or bypass surgery?
-Heart valve surgery/replacement or valve disease?
-Pacemaker/defibrillator?
-Peripheral artery or vascular diease in legs?
-Atrial fibrillation/flutter?

11. Do you have any of the following conditions or diseases(answer yes or no):
-Fibromyalgia/lupus?
-Organ transplant?
-Lung disease-emphasema or chronic bronchitis?
-Rheumatoid arthritis?
-Vasculitis?
-Currently using investigational medical device or drug?
-Chicken pox, shingles or flu in last 2 weeks?

12. Questions for women only: Do you have any of the following conditions or diseases(answer yes or no):
-Pregnant?
-Trying to get pregnant?
-Postmenopausal?
-If postmenopausal, on hormone replacement therapy in the last 6 months?

13. Salsalate-specific questions.
Do you have any of the following conditions or dieases? Answer Yes or No.
a. Allergy to aspirin?
b. Have now or ever had stomach, esophageal, or GI bleeding or ulcer?
c. Do you have any bleeding disorders?
d. Do you have any GI disorders (e.g., colitis, Chrohn's, etc.)?
e. Taking now blood thinning medication (e.g., warfarin, coumadin)?
f. Drink more than 10 alcoholic drinks per week?

14. Subject tolerance questions.
Are you willing to do the following? Answer Yes or No
a. Fast overnight for 8 hours?
b. Take study medication?
c. Willing to have your blood drawn?
d. Record your food intake for 3 days?

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 the screening log:
1. subject's name
2. age
3. gender
4. address
5. date that they contacted the study
6. how they contacted us (phone/email)
7. how they heard of the study
8. date of phone screening
9. Phone number
10. email address
11. pass phone screening; yes or no
12. if did not pass phone screening, reason?
13. If passed phone screening, date of consent
14. Signed informed consent

The purpose for collecting and keeping this information is to keep track of number and demographics of subjects phone screened in order to determine the level of success of recruiting strategies. 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.

Information on how the subjects heard of the study will help the research team understand the most successful methods for advertising for the study. Keeping information on reasons for not passing the phone screening is to report to the NIH (if/when the study recieves extramural funding) and to monitor our recruiting progress

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

VII.D.25 ***<u>After</u> 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
Demographics and Contact information questionnaire
Health history questionnaire
Resting heart rate, blood pressure
Resting 12-lead ECG
Standard blood chemistries (lipids, complete blood count, basic metabolic panel, HbA1C, thyroid stim hormone (TSH), liver enzymes (ALT/AST)
Oral glucose tolerance 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. Subjects are allowed to discusss the study with family/friends before deciding on participation.

VII.D.28 ***How long after the subject agrees to participate do study procedures begin?***
Screening tests can begin on the same day as consent (Visit 1). The subject will return for an additional screening visit (Visit 2) within 1 week or less. The

experimental procedures will be completed within 1-2 weeks of Visit 2.

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 obese adults with prediabetes(age 18-49 years) who will undergo randomization to salsalate or placebo and baseline and post-intervention testing. Healthy control obese adults (age 18-49 years) without prediabetes will undergo only baseline testing and not the intervention. The PI and his research staff will recruit subjects from the community and UIHC through reviewing medical records for eligiblity criteria, via flyers posted on campus, emails to UI community, flyers to eligible partcipants on ResearchMatch.org,the UIHC "Noon news" Subjects will be asked to contact the research staff via phone or email. A study team member will then contact subjects by phone and perform a phone screening (see attached phone screening form) to determine their eligibility. If they are determined to be eligible and they are interested in the study, the research staff will schedule them for Visit 1 which will include a detailed explanation of the study and review of the informed consent with the potential subject.

we will also contact patients by a recruitment letter (attached) that will contain a check box for the potential subject to check whether they would like to be contacted about the study or if they would prefer not to participate. We will provide a self-addressed stamped envelope for the subject to return the letter to us by mail. If they indicate (in the check box) in the return letter that they are not interested we will not contact them again. If we do not hear from the potential subject in 2-3 weeks, we will contact them by phone to determine whether or not they are interested. We will call them until we can personally talk to them. Otherwise, they can contact us directly at 319-467-5677 and express their decision about participating in this study. They can leave a message on the voice mail if no investigator is available to answer the call and we will call them back.

The study coordinator or research staff will answer all questions asked by the potential subject and subject will be informed of all potential risks before the he/she signs the consent.

Subjects will in no way be coerced to sign the consent form and will be informed that it is their choice whether to volunteer for this study. Even after the subjects sign the consent they are free to withdraw from the study at any time for any reason.

Microneurography training controls: The subjects will consist of healthy adults age 18-49 years who will undergo microneurography of the leg peroneal nerve. The PI and his research staff will recruit subjects from the community via flyers posted on campus and emails to UI community. Subjects will be asked to contact the research staff via phone or email.

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.***
At the end of the study, or after an adverse event that the subject is withdrawn from the study.

VII.E.2 ***Describe randomization scheme/assignment including ratio such as 1:1, 2:1 etc.***
Subjects will be randomized 2:1 to salsalate or placebo, respectively so their is 2/3 chance they will be randomized to salsalate and 1/3 to placebo. This scheme was chosen to ensure proper effect sizes can be determined in the salsalate group in this pilot study. The obese non-prediabetc group will not be randomized and only undergo baseline testing.

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)***
Phone screening questionnaire
Demographics and Contact Info questionnaire
Health History questionnaire
Modifiable Activity Questionnaire (MAQ)
3 Day dietary record (CRU bionutrition)
Salsalate screening log

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***

Experimental design. A total of 30 obese (defined as body mass index >30 kg/m2) adults age 18-49 years with prediabetes will be enrolled and randomized to 4-5 weeks of Salsalate or placebo in a randomized (2:1), double-blind, placebo controlled, parallel study design. Subjects will orally ingest 3000 mg/day (1500 mg in a.m. and 1500mg in the p.m.) of Salsalate (n=20) or placebo (n=10) for 28-35 days. A total of n=10 obese NON-prediabetic CONTROL subjects will be enrolled to establish prediabetes-related differences in obese adults primary outcomes at baseline. Therefore, a total of 40 subjects will be enrolled. The non-prediabetic control subjects will undergo screening (Visits 1 and 2) and baseline experimental testing (Visits 3, 4) but will NOT undergo the Salsalate/placebo intervention or visits 5, 6 or 7. Prediabetes will be defined as fasting (at leaset 8 hour fast) blood glucose between 100-126 mg/dl, a fasting blood glucose of 140-199 mg/dl at 120 min during an oral glucose tolerance test, or HbA1C between 6.0 – 6.5%.

Order of visits. Subjects will undergo experimental testing at baseline and again after the 4-5 weeks of Salsalate or placebo. Subjects will be studied between 7:00 and 10:00 am in the Clinical Research Unit (CRU) in the Institute for Clinical and Translational Science (ICTS) at the University of Iowa following an 8 hour overnight fast. The order of visits are as follows and will occur over a period of 7-8 weeks (see TABLE 1 attachment for summary of visits):

Visit 1 (3.5 hours): Consent and Screening
a. Explanation of the study; reading and signing of written informed consent
b. If subject consents but requires a 2-week washout for aspirin, salicylate, NSAID, vitamin, supplements, they will be scheduled to complete the Visit 1 in 2 weeks.
If subject consents and no washout is needed, screening tests will be performed same day to determine eligibility by the following:

i. Research staff will obtain resting vitals (blood pressure, heart rate)
ii. Research staff will obtain anthropometrics including weight, height, waist and hip circumference with tape measure.
iii. Research staff will obtain resting 12-lead ECG
iv. Subject will fill out the Demographics and Health History survey, and the Modified Activity Questionnaire (MAQ)
v. Research nurse or trained staff will obtain venous blood draw using butterfly needle for UIHC pathology labs:
• Creatinine, Lipid panel, TSH(4.5 ml blood- 1 light green PST tube)
• Complete blood count(4.5 ml blood- 1 lavender tube)
• Subject will undergo oral glucose tolerance test (OGTT): Subject will consume a beverage containing 75 grams of glucose. Blood samples will be collected at timed intervals of baseline, 1 hour and 2 hours after consuming beverage for glucose and insulin. Total blood collected for insulin and glucose for OGTT: 13.5 ml blood (4.5ml x 3 PST tubes).

• TOTAL blood collected for Visit 1: 22.5 ml (1.5 Tablespoons)

Visit 2 (2 hours): Dietary food record, cerebral blood flow velocity
a. Meet with CRU bionutritionist for instruction on 3-day dietary record to return on visit 3
b. Middle cerebral artery blood flow velocity using transcranial dopper during the following protocol to measure cerebral vasoreactivity to hypercapnia:
-After 3 min of normal breathing, subjects will perform 2, 30 second breathholds sparated by 3 min.
-Next, After 3 min of room air breathing, 3 min of breathin 2%CO2, 3 min of breathing 4%CO2 (balanced with 21%O2 and N) will be performed followed by 3 min of room air
breathing.
c. Subject will be instrumented with 24 hour blood pressure monitor to wear home for 24 hours. Subject will return it to the investigators when come in for Visit 3. There is no long term follow up.

Visit 3 (3 hours): 1st Baseline experimental visit (Blood, Endothelial cells, FMD, PWV and Retina scan)
a. Subject arrives at the CRU between 7-10 am after overnight 8 hour fast
b. Subjects on vasoactive medications will be asked to hold medication on the morning of experimental visits but to bring the medications with them to be taken after the visit.
c. Return 3-day dietary recall
d. IV catheter, endothelial cell collection and blood sample: Subject will lie supine and research nurse will insert venous 18 G catheter into antecubital vein.
e. Research staff or physician will perform J wire endothelial cell collection (3 wires) via 18 G catheter.
f. After 20 min, nurse practitioner will then obtain blood samples thru catheter:
• Lipid panel, insulin, hs-CRP, salicylate, creatinine, AST/ALT (4.5 ml blood- light green PST tube)
• Catecholamines (8 ml blood- 2 x 4 ml Na+ Hep green top tubes)
• HbA1c (4.5 ml blood- 1 x 4.5 ml lavender top tubes)
• Extra blood collected for specialized labs performed in PIs lab:
Serum: 17 ml blood- 2 x 8.5 red/gray top SST tube) for interleukin-6, tumor necrosis factor-alpha.
Whole blood (17 ml blood- 3 x 8.5 ml Citrate PPT tube) for mononuclear cell DNA isolation.

f. After 15 min supine rest the following non-invasive vascular measurments are obtained by the PI and his research staff:
1) Radial, brachial, carotid and femoral waveforms non-invasively for PWV assessment
2) Carotid artery compliance using ultrasonography
4) Brachial artery flow-mediated dilation (FMD) with ultrasound
5) Brachial artery dilation after 0.3 mg sublingual nitroglycerin with ultrasound

g. Subject escorted to the CRU Human Cardiovascular Research Lab and a physician or research staff trained in microneurography will insert needle into peroneal nerve for measurement of muscle sympathetic nerve activity
h. Subject will meet with bionutritionist and review 3-day food record with subject
i. Subject receives meal/snack from CRU bionutrition and then can leave

**TOTAL blood collected for Visit 3= 68 ml (~4.5 tablespoons)

Visit 4 (4.5 hours): 2nd Baseline experimental visit (Forearm Blood Flow and Microneurography)
a. Subject comes to the CRU Human Cardiovascular Research Lab in the CRU between 7-10am after 8 hour overnight fast
b. Subject will be instrumented with upper arm venous occlusion cuffs and wrist cuffs

for measurements of forearm blood flow using venous occlusion plethysmography
c. A physician trained in brachial artery catheterization in the Human Cardiovascular Research Lab will then perform brachial artery catheterization with 27 G needle and undergo incremental infusions of saline, acetylcholine, acetylcholine + vitamin C (ascorbic acid), and sodium nitroprusside + vitamin C (see Expermental Procedures for details)
d. Subject will be escorted to Dept of Ophthalmology in Pomerantz Bldg and have picture of retinal performed (scan takes ~10 min)
e. Subject will be given snack or lunch in CRU.
f. Subjects will be randomized in a 2:1 scheme to Salsalate or placebo
g. Nurse will dispense 35 day supply (210 tablets) of Salsalate (dose 3000 mg/day= 500 mg tablets x 6/day x 35 days= 210 tablets) or placebo
h. Subject will take first dose of Salsalate (500 mg x 3 pills) or placebo at CRU and 2nd dose (1500 mg) at home in evening- this is Day 0

Visit 5 (45 min) ~Day 14: Subject will return to CRU for venous blood sample to measure serum salicylate (4.5 ml x 2 PST tubes: total 9 ml or ~0.5 tablespoons), Blood pressure, HR and survey of any side effects. If any side-effects/symptoms present, nurse coordinator will follow instructions as outlined in section VIII.2 "Plan for Managing Risks". Subject will receive 3-day food record booklet from CRU bionutritionist

Days 25: Coordinator or research staff will call subject in the evening to remind subject to start 3-day dietary record on days 26, 27, 28.

Visit 6 (5 hours) Betwee Day 26-34: Post-experimental visit #1 (FMD, PWV and Retina)
a. Subject will take morning dose of Salsalate/placebo and return to CRU between Day 26 -35 for venous blood sample to measure serum salicylate, blood pressure, HR; and survey of any side effects
b. All vascular measurements of Visit 3 will be repeated
c. Middle cerebral artery blood flow velocity using transcranial dopper during the following protocol to measure cerebral vasoreactivity to hypercapnia:
-After 3 min of normal breathing, subjects will perform 2, 30 second breathholds sparated by 3 min.
-Next, After 3 min of room air breathing, 3 min of breathin 2%CO_2, 3 min of breathing 4%CO_2 (balanced with 21%O_2 and N) will be performed followed by 3 min of room air
breathing.
d. Subject escorted to the CRU Human Cardiovascular Research Lab and a physician or research staff trained in microneurography will insert needle into peroneal nerve for measurement of muscle sympathetic nerve activity
e. Subject will be instrumented with 24 hour blood pressure monitor to wear home for 24 hours. Subject will return it to the investigators when come in for Visit 7. There is no long term follow up.

Total blood collected for visit 6: 68 (~4.5 Tablespoons)

Visit 7 (3 hours) Between Day 27-35: Post-experimental visit #2
a. Subject will take morning dose of Salsalate/placebo return to CRU on Day 27 or 28 for venous blood sample to measure serum salicylate, AST/ALT and creatinine (0.5 ml blood)
b. All measurements from Visit 4 will be repeated
c. Blood draw for salicylate, insulin, lipid panel, creatinine, ALT/AST (1 x 4.5 ml lt green PST tube)
d. Total blood collected for visit 7: 4.5 ml (~1/3 tablespoon)

GRAND TOTAL Blood drawn over course of study: 172 ml (~11.5 tablespoons)

Screening Methods.

a) Blood pressure, ECG. Subject will undergo resting BP in a private CRU exam room after 15 minutes of rest. BP will be measured in supine position in triplicate separated by 2 min with an automated oscillometric BP machine under quiet, comfortable laboratory conditions. All BPs throughout study will be performed in this identical manner. A resting supine 12-lead ECG will be recorded by placing 10 electrodes on chest and recording the ECG.

b) History and Physical exam. Prior to the experimental visit (Visit 3), subjects will undergo a complete medical history and physical examination by Dr. Haynes or a CRU nurse practitioner.

c) Blood chemistries: After passing the physical exam standard blood chemistry analysis will be obtained under fasting conditions including a basic metabolic panel, lipid panel, HbA1C, complete blood count, TSH and FSH (for women only).

d) 3-day dietary records. Because any differences between subjects in diet could influence between-subject comparisons, dietary composition (macro- and micro-nutrients) and caloric intake will be determined from 3-day food intake records recorded for the 3 days leading up to baseline Visit 3 and Visit 6 experimental measurements. The subject will meet with a CRU bionutritionist to instruct the subject how to record food intake for 3 days. The subject will be instructed to eat as he/she normally does and not to make any dietary changes. Nutrient composition of food records will be analyzed using the most current version of Nutrition Data System for Research (NDSR) (Nutrition Coordinating Center, University of Minnesota, Suite 300, 1300 South Second Street, Minneapolis, MN 55454-1087- http://www.ncc.umn.edu/).

e) Physical activity surveys. To document the habitual physical activity status of our subjects over the past 12 months, daily energy expenditure will be estimated from the Modifiable Activity Questionnaire (MAQ).

Experimental Methods for Visit 3.

a) Vascular endothelial cell protein expression and J wire collection procedure. Endothelial cells will be collected from an antecubital vein, washed, isolated fixed to slides, and stained with primary and secondary (immunofluorescence) antibodies for quantification of NFkappaB, IkappaBalpha nitrotyrosine, AMPkinase expression. Briefly, under sterile conditions a CRU nurse will insert an 18G catheter into an antecubital vein followed by a 0.018- or 0.021-inch mesh St. Jude 3 mm flexible guide wire (Daig Corp., Minnetonka, MN) with a J-shaped tip that is advanced 3-4 cm into and retracted 2-3 times through the catheter. The CRU nurse practitioner will be trained by the PI by watching a detailed video of the procedure and instructed by the PI who has observed >100 of these procedures at the University of Colorado-Boulder Clinical Translational Research Center between 2005-2009. The distal portion of the wire is then transferred to a 50-ml conical tube containing a buffer solution. Cells will be taken to the PIs lab (522 Field House) and cells are recovered by centrifugation and fixed to poly-lysine slides with formaldehyde and frozen at -80C until analysis. After blocking non-specific binding sites with 5% donkey serum (Jackson Immunoresearch), cells will be incubated with monoclonal antibodies for proteins of interest and a specific AlexaFlour488-conjugated secondary antibody (Research Diagnostics). Slides are then cover slipped with a VECTASHIELD DAPI (4',6'-diamidino-2-phenylindole hydrochloride) fluorescent mounting medium (Vector Labs) and stored at 4 degrees C overnight. Slides are viewed using a fluorescence microscope (Eclipse 600, Nikon) and 30 individual endothelial cell images are digitally captured by a Photometrics CoolSNAPfx digital camera (Roper Scientific). These

endothelial cells are documented by cell staining of vWF and nuclear integrity is confirmed using DAPI staining. Once endothelial cells with intact nuclei are identified, they were analyzed using Metamorph Software (Universal Imaging; Downingtown, PA) to quantify the intensity of primary antibody-dependent AlexaFlour488 staining (i.e., average pixel intensity). The number of cells typically recovered from each guide wire results in approximately 50-100 cells per slide. Eight slides and two control cultured human umbilical vein endothelial cell (HUVEC: passage 6-9 processed identically to the sample cells) slides are selected for each staining batch. Values are reported as a ratio of sample endothelial cells to HUVEC average pixel fluorescence intensity to reduce variability between staining batches. A singe technician will be blinded to the identity of the subject during the staining and analysis procedures.

b) Pulse wave velocity. Carotid-femoral, carotid-brachial, and carotid-radial PWV will be measured non-invasively by recording carotid, femoral, brachial and radial artery pressure waveforms sequentially with an applanation tonometer (Non-invasive Hemodynamics Workstation, Cardiovascular Engineering, Inc.). Pressure waveforms are gated to the ECG R wave in order to calculate the transit time (t) between the foot of the carotid and the respective peripheral (femoral, brachial, radial) waveforms. The carotid-femoral transit distance (CFTD) is estimated between the 2 anatomical sites as the difference between the suprasternal notch (SSN) to carotid (SSN-C) and femoral (SSN-F) sites. Thus, the CFTD is calculated as CFTD= (SSN-F)-(SSN-C) and PWV calculated as CFTD/t. This approach accounts for parallel transmission of the pulse wave up the brachiocephalic and carotid arteries, and simultaneously along the aortic arch using the SSN as a fiducial point where parallel transmission begins (e.g., bifurcation site of aortic arch and brachiocephalic artery). The intra-subject reproducibility of carotid-femoral PWV is excellent with a coefficient of variation of 2.1% for triplicate measurements on non-consecutive days in 7 young adults.

c) Middle cerebral artery velocity reactivity to hypercapnia: Using non-invasive transcranial doppler ultrasound (TOC Neurovision, Multigon Industries, Inc.), blood flow velocity of the middle cerebral artery (MCA) velocity will be obtained using a 2 MHz doppler probe attached to custom headgear to maintain stable doppler signal. The basal portion of the MCA will be insonated by placing the probe over the temporal bone just above the zygomatic arch between the frontal process and front of the ear. Basal resting velocity will be obtained for 3 minuted followed by 2, 30-second breathholds separated by 1 min. Next, subjects will breathe room air through a Hans-Rudolph mouthpiece with one-way valve (to prevent rebreathing), followed by 3 stepwise increases in end-tidal CO2 (ETCO2) by adding 2%CO2, 4%CO2 and 6%CO2 fractional concentration of CO2 (FiCO2) for 3 minutes each while oxygen content is maintained at 21% and balanced by nitrogen(N). Immediately after the 9 min stepwise increase in FiCO2 trial, the subjects will breathe room air for 3 min to measure MCA velocity recovery. Cerebrovascular reactivity is calculated from the slope of the relation between increases in MCA velocity and ETCO2 in response to stepped increases in FiCO2. ETCO2 will be monitored with a Novametix NICO CO2 monitor attached to the Hans Rudolph mouthpiece, and 3 lead ECG recorded throughout testing.

c) Brachial artery FMD (endothelium-dependent dilation, EDD) and endothelium-independent dilation. Brachial artery FMD and endothelium-independent dilation will be determined non-invasively using high-resolution ultrasonography (LOGIQ E9, GE Healthcare) as described originally by Celermajer et al. (1992) and more recently by the PI (19, 50, 60, 61). While supine, the subject' s arm will be abducted and positioned comfortably on a side table and a pediatric cuff will be secured on the upper forearm (i.e., below the antecubital fold). After selecting a segment of the brachial artery ~3-6 cm above the antecubital fold with clear anterior and posterior intimal-luminal interfaces, the ultrasound probe will be clamped in place to avoid any

involuntary movement. Baseline ECG-gatedto R wave (i.e., end-diastolic) ultrasound images and Doppler flow velocity of the artery will be acquired in duplex mode (B Mode/Pulsed Doppler) simultaneously for 30 seconds. For FMD, brachial artery reactive hyperemia will be produced by inflating the pediatric forearm blood pressure cuff to 250 mmHg for 5 minutes followed by rapid deflation. ECG-gated end-diastolic ultrasound images and pulsed doppler of the brachial artery will be acquired during the last 30 seconds of the cuff occlusion and for two minutes after the release of the cuff. Ten minutes after FMD, endothelium-independent dilation will be determined by measuring brachial artery dilation in response to sublingual nitroglycerin tablet (0.3 mg) and images will be acquired for 10 minutes as described previously (19, 60). Subject will have blood pressure monitored every at baseline, and at 3, 5, 7 and 10 minutes and for any signs/symptoms of hypotension such as dizziness, nausea, lightheadedness. If any these signs usually pass within 10 minutes with subject supine. A commercially available software package (Vascular Analysis Tools 5.5, Medical Imaging Applications, LLC) will be used to acquire and analyze ECG-gated brachial artery diameters. Images will be digitalized and stored for later analysis on a personal computer. Brachial artery dilation will be determined as the % change and mm change from baseline. FMD is dependent upon the post-occlusion increase in hyperemic blood flow or shear stress (62, 63). However since blood viscosity will not be available, shear rate will be used and is a reasonable estimate of shear stress (63). Shear rate will be calculated using the following formula: shear rate= Ve/D, where Ve and D represent velocity (cm/s) and diameter (mm). The PI has ~8 years of experience performing the brachial artery FMD technique in human subjects (17, 19, 60, 64-67).

d) Retinal microvascular assessment. Subjects will undergo retinal color imaging using a non-mydriatic fundus camera (Topcon NW200, Topcon Corporation, Tokyo, Japan), by an experienced ophthalmic photographer. Two fields are obtained, a fovea centered and disc centered field. Subjects will also undergo Spectral Domain Optical Coherence Tomography (SD-OCT) imaging (Spectralis, Heidelberg, Heidelberg, Germany), by experienced an ophthalmic photographers. A single macular field of 6x6mm (101 slices of 1024 x 1024 voxels each), will be obtained. From each macular OCT volume, 11 intraretinal surfaces, and 10 retinal layers, will be automatically segmented by the Iowa Reference Algorithm, which uses an extensively validated, robust, three dimensional graph search. The layers will include nerve fiber layer and ganglion cell layer. From each fundus image, diabetic retinopathy presence will be determined according to the International Classification of Diabetic Retinopathy (Wilkinson 2004), by a retinal specialist (Dr. Abramoff). Arterial widths and venous widths will be automatically determined in all 4 images using our automated approach (Xu 2010, Xu 2011), and normalized to express central retinal arterial width and central retinal venous width equivalents.

e) Blood chemistries at baseline and after 4 weeks of the intervention: The following will be obtained under fasting conditions at baseline and after 4 weeks of the intervention including: basic metabolic panel, lipid panel, HbA1C.

f) Circulating oxidative stress/inflammation markers at baseline and after 4 weeks of the intervention. Plasma oxidized low-density lipoprotein, a marker of oxidant lipid modification will be assessed by enzyme-linked immunoassay (ELISA), and total F2 isoprostanes, will be assessed by MS/GS. Plasma c-reactive protein, tumor-necrosis factor-alpha and interleukin-6, will be measured by ELISA as described previously. Adiponectin and leptin will be measured by the ICTS core lab via radioimmunoassay.

g) NFkappaB DNA binding activity in peripheral blood mononuclear cells (PBMCs). NFkappaB DNA binding activity will be assessed at baseline and after 4 weeks of Salsalate in PBMCs isolated from whole blood to verify inhibition of NFkappaB in a second cell type in addition to endothelial cells as previously described (81). PBMCs will be lysed and DNA-bound NFkappaB will be detected using anti-p65 antibody and

quantified using HRP-conjugated anti-rabbit IgG by ELISA using neutra-avidin plates (Pierce Biotechnology) with NFkappaB binding oligonucleotides.

Experimental Methods for Visit 4.

a) Brachial artery catheterization and forearm blood flow studies.
Venous Occlusion Plethysmography: Venous occlusion plethysmography will be used to measure forearm blood flow (FBF) responses to local intra-brachial artery infusions of vasoactive drugs to test endothelium-dependent and independent dilation of forearm resistance arteries before and after the Salsalate or placebo intervention. All FBF studies will be performed in the Human Cardiovascular Physiology Laboratory staff in the ICTS Clinical Research Unit. Briefly, subjects lie supine and have blood pressure cuffs (venous occlusion) placed around upper arms and pediatric blood pressure cuffs around wrists. Forearm blood flow will be measured by placing a gallium-in-silastic strain gauge around the widest part of the forearm which measures small changes in forearm volume during periodic inflation (8 sec inflated:4 sec deflated) of upper arm cuffs to 40 mmHg (to temporarily prevent venous outflow and measure arterial inflow into forearm) and continuous wrist cuffs inflated to 240 mmHg (to exclude hand blood flow). VOP is well established and validated technique for measuring FBF responses to intra-brachial infusion of vasoactive drugs in human subjects.

Brachial artery cannulation: Using strict sterile technique, a physician in the Human Cardiovascular Physiology Laboratory will catheterize the brachial artery of the subject's non-dominant arm under local anesthesia (1% lidocaine) with a 27 gauge steel catheter attached to an 18 gauge epidural catheter. All drugs will be dissolved in 0.9% saline, which will be infused at 1.0 ml/min. Following catheter placement and other instrumentation the subjects will be allowed to rest quietly for 15 minutes before infusions are started. Blood pressure in the non-catheterized arm will be obtained in duplicate at baseline after 15 min, and again at the end of each dose of drug infusion.

Drug infusion protocol: First, baseline FBF responses to intra-brachial artery infusions of 0.9% saline will be measured for 20 min at rate of 1 ml/min. Next, FBF responses during intra-brachial artery infusions of 2 incremental doses of the endothelial agonist acetylcholine (Ach; doses 3 and 30 mcg/min) or the endothelium-independent agonist sodium nitroprusside (SNP; doses 1 and 10 mcg/min) at 1 ml/min will be performed for 6 min for each dose. Both drugs will be diluted in 0.9% saline and sequence of Ach and SNP will be randomly assigned. FBF from each drug infusion will be recorded during the 3rd to 6th minute of each dose. A washout period of 0.9% saline at 1 ml/min will be performed for 20 min or less if FBF returns to baseline sooner. Next, the effects of oxidative stress on EDD will be determined by measuring the effect of ascorbic acid (vitamin C) infusion (25 mg/min) at an infusion rate 1 ml/min for a 10 minute loading dose prior to ACh infusion, and then continuous infusion throughout incremental infusion of AcH (doses 3 and 30 mcg/min). Specifically, any difference in the FBF response to ACh and the FBF response to ACh + vitamin C can be attributed to a reduction in vascular oxidative stress. SNP is used as an NO donor to test endothelium-independent dilation to establish that any observed group differences in Ach-mediated vasodilation is due, at least in part, to stimulated endothelial NO release per se, and not impairments in smooth muscle vasodilator function to an NO donor. Note: The concentrations selected for each of these agents have been shown to elicit changes in FBF without affecting systemic hemodynamics (i.e., no changes in heart rate or blood pressure).

b) Microneurography. Direct intra-neural recordings of sympathetic activity to skeletal muscle will be obtained using microneurography, as previously described. A tungsten micro electrode (200 μm diameter shaft; 1-5 μm uninsulated tip) is inserted into the peroneal nerve posterior to the head of the fibula. Well-validated criteria are used to

determine that a neurogram represents sympathetic activity to muscle or skin: We have used this technique safely in over 2000 studies since February 1984 and it is well tolerated and reproducible. The mental stress test is a non-baroreflex mediated sympathoexcitatory stimulus used to test sympathetic responsiveness. This test requires that each subject to subtract continuously the number 7 from a 3-digit number as quickly and as accurately as possible for 3 minutes. During the test, the participants are intentionally frustrated by being asked to calculate faster and by being corrected in case of wrong answers. This stimulus has little effect on muscle SNA and increases forearm blood flow.

c) 24-hour ambulatory blood pressure variability and baroreflex sensitivity. Twenty-four hour systolic blood pressure will be recorded using standard ambulatory blood pressure assessment (90207-IQ, Spacelabs Healthcare, Inc)and 24 hour blood pressure variability determined from the standard deviation of systolic and mean blood pressure recordings. Baroreflex sensitivity will be determined by recording blood pressure and heart rate continuously for 15 minutes during visit 3 and 6 using via beat-to-to beat finger blood pressure (Finometer MIDI, Finopress Medical Systems) and calculated using the sequence technique.

Microneurography training controls:

We will recruit 20 additional healthy control volunteers who will have microneurography performed on them by Dr. Pierce. The training will be provided by Dr. William Haynes and Marcelo Correia, both of whom are highly trained in the technique and will provide close oversight in the training. The 20 volunteers will sign and separate consent form (see attached) and will be compensated $25.

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***
No

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

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

Obese prediabetic Subjects will be compensated for the entire study in the amount of $300. If subjects do not complete all study visits they will be compensated in pro-rated fashion on an hourly basis of $15 per hour for each experimental visit. They will also be reimbursed for 1 hour for visits 5.
Visit 1: $35
Visit 2: $30

Visit 3: $60
Visit 4: $45
Visit 5: $15
Visit 6: $75
Visit 7: $45

Obese non-prediabetic subjects will be paid a total of $170 for completing the entire study and $15 per hour on a pro-rated basis of visits 3 and 4 if they do not complete the entire study.
Visit 1: $35
Visit 2: $30
Visit 3: $60
Visit 4: $45

Microneurography training controls will be compensated a total of $25 by check mailed to them.
Visit 1: $25

VIII. Risks

VIII.1 ***What are the risks to subjects including***
- emotional or psychological
- financial
- legal or social
- physical?

Physical risks:

Fasting for 8 hours: The most common risk when fasting is dehydration, therefore the subject will be encouraged to drink plenty of water. Subjects may experience hunger and irritability and if they experience fainting, nausea, or vomiting they will be instructed to stop fasting.

Blood sample: 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 team member trained and certified in drawing blood or a nurse. There are no known risks associated with urine collection.

Endothelial cell collection. The risks related to the venous endothelial cell collection do not appear to be any greater than to those of placement of an intravenous catheter into antecubital vein. In the PIs experience with this technique at the University of Colorado Clinical and Translational Research Center, there were no adverse events encountered in >150 of these procedures performed between 2005-2009 in young, middle-aged and older healthy adults (17, 19, 52).

Pulse wave velocity: There are no known or foreseeable risks associated with the use of applanation tonometry for pulse wave analysis. ECG electrodes may cause minor irritation to the skin.

Carotid artery compliance and transcranial doppler of MCA via ultrasound. There are no known or foreseeable risks associated with the use of carotid and transcranial doppler echocardiography. ECG electrodes may cause minor irritation to the skin.

Brachial artery flow-mediated dilation (FMD): There is a risk of mild discomfort on the forearm when the blood pressure cuff is inflated and a mild temporary sensation of "pins and needles" in forearm and hand. This feeling is completely reversed within several minutes after the cuff is released with no permanent discomfort.

Cerebral artery blood flow velocity: There are no known risks associated with the use transcranial Doppler ultrasound of the middle cerebral artery.

Risks of breathing 2%, 4% and 6% carbon dioxide: Breathing in low concentrations of carbon dioxide at 2-4% for short period of time may be associated with possible symptoms such as drowsiness, rapid breathing, rapid heart rate or reduced hearing. At 6% carbon dioxide, this may be associated with headache, dizziness or shortness of breath, and feelings of being anxious. If these symptoms occur, they are reversed quickly upon breathing room air.

Forearm blood flow test: There is a risk of mild discomfort on the forearm and wrist when the blood pressure cuffs are inflated. This feeling is completely reversed within several minutes after the cuff is released with no permanent discomfort. There is a small risk of lightheaded or fainting in response to local lidocaine. There is a small risk of bruising at the sight of needle insertion a very small risk of permanent nerve damage or damage to the artery.

Microneurography: Approximately 7% of subjects experience minor tingling in the leg, foot or arm for a few days after the study, but these symptoms have been transient.

Retinal (eye) assessment: The flashes produced by the retinal imagining can be bright and the aftereffects can last several minutes at most.

Risks of drugs to be used in the study.

Salsalate. 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.

Several small trials and one large multicenter clinical trial have been conducted using Salsalate in the dose and duration similar to the proposed dose and duration in this study. In one study of 20 obese middle-aged adults treated for 4 weeks with 4 g/day of Salsalate, 3 subjects required dose reduction of 500 mg due to tinnitus, headache, or dizziness(35). Subjects subsequently had stepped reductions of 500 mg titrated to maximal tolerable dose without symptoms, two tolerated 3.5 g/day and the other tolerated 3.0 g/day and both subjects completed the study. Mean serum salicylate concentrations at 4 weeks were 17.1 mg/dl (in therapeutic range 10-30 mg/dl). Three subjects were withdrawn from the study due to rash, but none had changes in serum measures of renal, electrolytes, anion gap or liver function.

In a 2nd study comparing 4.5 g/day to 3.0 g/day for 2 weeks in patients with Type 2 diabetes, 6 of 7 subjects receiving 4.5 g/day experienced tinnitus but symptoms resolved with dose reduction (45). Serum salicylate concentrations were 28.4 and 19.0 mg/dl at 1 and 2 weeks respectively. At 3.0 g/day, no subjects experience tinnitus or any side effects, but serum salicylate concentrations were in the sub-therapeutic range (5.4 mg/dl) based on rheumatologic standards. In that same study, another 17 diabetic patients were randomly assigned to the maximal tolerable dose of 3-4 g/day for 4 weeks. Three of the 8 subjects randomized to 4 g/day, developed tinnitus but symptoms resolved with reduction of dose to 3.5 g/day for remainder of study (45). Serum salicylate concentration was 13.3 mg/dl after 4 weeks and there was no change in serum creatinine or anion gap.

A recent multicenter trial (Golfine A, et al., Ann Intern Med 2010) Targeting Inflammation Using Salsalate in Type 2 Diabetes (TINSAL-T2D; NCT00392678), 108 patients with type 2 diabetes were randomly assigned to placebo, 3.0, 3.5, or 4.0 g/day for 14 weeks with the primary endpoint being reduction in HbA1c (83). In this longer duration study (compared to our study), about 19-22% of subjects reported tinnitus, one with baseline tinnitus withdrew and 3 patients required dosage reduction but who completed the study. Although there were no serious adverse events, 22-30% of patients experienced hypoglycemia. However, these patients were also receiving concurrent anti-glycemic sulfonylurea therapy for glycemic control. There was also a small trend for an increase in urinary albumin and the 3.5 g/day group showed a small increase in serum creatinine concentration, but no changes in liver function or anion gap. Mild GI symptoms (heartburn, nausea, vomiting, diarrhea) were slightly more common in Salsalate than placebo groups, but there was no episodes of GI bleeding and no subjects required reductions in Salsalate dose. A follow-up multicenter trial (TINSAL-T2D II) is currently underway investigsting the effects to 3.5 g/day of Salsalate on HbA1c for 1 year in patients with type 2 diabetes (NCT00799643). Lastly, in previous study by the PI using high dose Salsalate (4.5 g/day) for 4 days in healthy obese middle-aged/older adults, 2 out of the 17 subjects during the Salsalate treatment withdrew from the study due to dizziness (n=1) and tinnitus (n=1), but neither subject had serum concentrations in the toxic range (19).

Metabolic acidosis: There is a very small risk of metabolic acidosis from salicylate toxicity although none of the above 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 f 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). Also tell the research team if you have or have ever had ulcers, bleeding in your stomach or intestines, or other bleeding disorders. 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.

Risk of Reye’s syndrome: If the subject has a recent history of a viral infection such as chickenpox or influenza they will not be allowed to participate because of the risk of developing Reye's syndrome (potentially fatal disease associated with viral illness and salicylate/aspirin use). If he subject has had recent flu-like symptoms you will be required to wait 2 weeks without any flu symptoms before participating.

• Forearm blood flow test drugs:
o Acetylcholine, Sodium nitroprusside, and Vitamin C. These drugs will be infused into the brachial artery and will cause changes only in the blood flow in the forearm. Because the doses used are very small, there should be little or no effect on systemic hemodynamics (e.g., blood pressure and heart rate).

o Lidocaine: 1% lidocaine will be used to numb the area on the arm for insertion of the brachial artery needle. Likely and expected effects include mild, temporary burning at the site of injection and a sensation of coolness or numbness at the site of the injection. Unlikely, but possible reactions include lightheadedness, dizziness, ringing in the ears, nervousness, and blurred vision. Individuals known to be allergic to anesthetics like lidocaine (e.g., novocaine- used for tooth fillings) should notify the investigator before participating and will not undergo the forearm blood flow test.

• Brachial artery FMD test drugs.

o Sublingual (under the tongue) nitroglycerin tablet: Nitroglycerin tablet may cause transient low blood pressure, dizziness, flushing, and headache but should only last for 5-10 minutes. If subject feels any of these symptoms we will keep him/her lying down until the symptoms pass and blood pressure is back to normal.

Psychological risks: The study only enrolls healthy subjects without psychiatric diagnoses and 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.

Confidentiality and financial risks: Subjects are at risk of breach of their confidentiality. All research team members have undergone confidentiality training, and are aware of potential consequences for breach of confidentiality. This will minimize this risk. Other than the cost of transportation there is no forseeable financial risk with this study.

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.)***

Plan for Monitoring for Risks:

During the study 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 in to the Research Nurse during business hours. If after business hours, weekends, or holiday, the subject will be instructed to call in the UIHC access number and ask for Dr. Pierce to be paged. The Research Nurse or Dr. Pierce (although he 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 at 500 mg less and continue at that dose. Dr. Pierce will call/page Dr. Haynes for

consultation if necessary and Dr. Haynes will serve as back up if Dr. Pierce is out of town or unavailable. If Dr. Haynes is out of town or unavailable then Dr. Jess Fiedorowitz, MD will be available to consult. 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. If side effects that are life threatening, the subject will be instructed to call 911. If serum salicylate concentrations are not in the toxic range (<30 mg/dl), the subject will be will be re-assessed in 12 hours to confirm that salicylate concentrations are decreasing and symptoms resolved. If symptoms resolve, the subject can take next am or pm dose at 500 mg lower or be withdrawn from the study as recommended by Dr. Haynes.

If blood plasma salicylate concentrations are in the toxic range (>30 mg/dl), the subject will be withdrawn from the study and Dr. Haynes and Pierce notified. Serum electrolytes (including anion gap), urinalysis and respirations may be obtained in the case of suspected metabolic acidosis due to Salsalate toxicity. If metabolic acidosis is suspected, then Dr. Haynes will be notified and determine appropriate medical management in the CRU or referral to the UIHC Emergency Department.

Scheduled safety visit. Subjects will come in at 2 weeks for Visit 5 (approximately Day 14 depending on scheduling) and to have serum salicylate concentrations measured and to confirm no toxicity. The Research Coordinator/Nurse or research staff will review their 2 week side effect log (see attached survey) and survey subjects for any symptoms/side effects and. Subjects will also have serum salicylate concentrations measured at experimental visits 6 and 7.

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 Data Safety Monitoring Plan will consist of a bi-annual independent review of the protocol by Dr. Roberto S. Kalil, Department of Internal Medicine, Division of Nephrology. The PI will provide a bi-annual report to the Dr. Kalil summarizing the following:
a) Data on progress of the protocol including subject recruitment, attrition, and minority involvement. Reasons for attrition or other recruiting issues
b) Data on safety of research participants including unblinded data of serum salicylate, creatinine, ALT/AST concentrations at baseline and from safety visits, data on reasons for any dosing reductions in Salsalate that occurred, and data collected from evening telephone questionnaires for subject symptoms;
c) Data on compliance in reporting of any adverse events
d) Data on protocol compliance and any amendments to the protocol
e) Data on any safety issues that occurred during the 6 month period.
f) He will confirm that any action that results in the temporary or permanent suspension of the protocol is reported to all the appropriate monitoring bodies such as the CRR protocol committee, IRB, FDA, NIH, or other sponsor, etc.

Every 12 months, the PI will summarize outcome data and provide to Dr. Kalil for review of the efficacy of treatment intervention on primary outcomes

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:***
Roberto Kalil, MD

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)?***
There may be no direct benefits to the subject, but subject may experience lowering of fasting blood glucose, triglycerides,HbA1C and blood pressure

IX.2 ***What are the potential benefits to society in terms of knowledge to be gained as a result of this project?***
The potential benefits to society include determining if a commonly used antiinflammatory drug used to treat chronic inflammatory diseases such as rheumatoid arthritis, will be effective for improving cardiovascular function in obese adults with prediabetes. This could have favorable clinical implications for older adults in possibly reducing risk of cardiovascular diseases most notably atherosclerosis.

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. During screening and experimental procedures will be conducted in private exam rooms in the CRU with the door closed. 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?***
Yes

X.3 ***Provide the intended usage of SSN:***
- To provide compensation to subjects

X.4 ***How will information/data be collected and stored for this study (check all that apply):***
- Paper/hard copy records (hard copy surveys, questionnaires, case report forms, pictures, etc.) - All hard copies of records will not contain any personal identifiers but only an individual subject code. Folders will be kept in a folder to keep out of public view when transported from CRU to the coordinagtors office. Data folders will contain hard copies of data capture forms such as surveys and data collected during experimental visits. All data folders will be kept in folder and locked in storage cabinet in the coordinators office (C-44 GH). The office is locked when the coordinator is not in the office. Signed informed consent documents and will be kept in a separate folder in a different locked file cabinet in the office.
- 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. This server is maintained by Paul Schroeder, IT Support Services, College of Liberal Arts and Sciences.
    - Name - Paul Schroeder
    - Title - IT Support Services II, College of Liberal Arts and Sciences
    - University/VA Job Classification - IT Support Services II
- Biologic samples (blood draws, check swabs, saliva samples, tissue samples, etc.) - Basic blood chemistries will be sent to the UIHC pathology lab for analysis. Remaining biological specimens such as blood, endothelial cells, and DNA will be labeled with subject code, date collected and IRB protocol number and transported from the CRU to the PIs laboratory (522 Field House) in a secure unbreakable biohazard container. Samples will be stored in the PIs laboratory in a -80C freezer in N400 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 his research staff will have access to the samples.
    - Name - Gary Pierce, PhD
    - Title - Assistant Professor
    - University/VA Job Classification - Assistant Professor

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

XI. Data Analysis

XI.1 ***Describe the analysis methods you will use, including, if applicable, the variables you will analyze***
Statistical analyses. To determine if differences in outcomes are present at baseline between obese pre-diabetic vs. non-pre-diabetic groups, an independent two-sample t-test will be performed. To determine if baseline (pre-intervention) differences exist between salsalate and placebo prediabetic groups after randomization, independent two-sample t-tests will be performed. To test the main hypotheses, a repeated measures ANOVA will be performed in which treatment (salsalate vs. placebo) is the between-subjects factor, and time (pre- vs. post-intervention outcomes) is the within-subjects factor for the primary dependent variables. The critical test for the dependent variable will be the interaction between the within-subjects and between-subjects factors. Tests for mean contrasts will be used to compare variables of interest pre vs. post Salsalate or placebo. Based on a previous study, we expect small reductions in fasting glucose, triglycerides, LDL cholesterol and possibly systolic blood pressure after Salsalate treatment. Covariates (e.g., variables that change after Salsalate) will be included into the mixed model. Bivariate pearson correlation analysis will be performed on the change in each dependent variable and aforementioned selected covariates after Salsalate.

XI.2 ***Provide the rationale or power analysis to support the number of subjects proposed to complete this study.***
Sample size calculations. Sample sizes were calculated based on 80% power at an alpha level of 0.05. Estimated effect sizes for Aim 1 were used for final sample size calculation for obese prediabetic subjects because carotid-femoral PWV is the primary outcome and has the smallest effect size (d) in pilot data of 4 days before vs. after Salsalate. A sample size of 16 subjects per group was determined based on the d of 1.06, based on mean difference in carotid-femoral PWV (-1.0 ± 1.0 m/sec) after Salsalate vs. Placebo.

With a conservative estimated attrition rate of 15-20% and the 2:1 randomization, a total of 20 subjects will be assigned to the Salsalate group, and 10 subjects to the placebo group was determined to be required for the study (total sample size=30). A

sample size of 12 for Aim 2 (FMD) was determined from d of 1.2 for a mean difference in FMD of +2.6 ± 2.2 after 4 days Salsalate vs placebo based on previously published work by the PI (19). Because the effect sizes for Aim 1 and 2 are based on preliminary data of 4 days of Salslalate treatment and our proposed study is 4 weeks, we predict that the actual effect sizes will be slight higher because of the longer duration of treatment. Therefore, the proposed study will help determine actual effect sizes for chronic Salsalate therapy for a future larger randomized clinical trial.

A total of 10 obese non-prediabetic control subjects will be recruited to established differences in main outcomes at baseline but will NOT undergo randomization or salsalate/placebo treatment period.

Therefore, total sample size to be enrolled is 40.

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:***
The telephone screening information including name, telephone number, address, and email address will be kept on file if the subject consents to be contacted for future studies. If the subject does not consent to be contacted for future studies, the telephone screening will be destroyed at the end of the study.

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.***
Language is added to the consent document informing subjects about the planned retention of data, tissue or specimens for future research use.
If the subject indicates on the informed consent that he/she does not consent to storing personal identifiable data, all personal identifiable data in the data base will be destroyed at the end of the study. The PI will confirm this and report it in the Data Saftey Monitoring Plan report. If the subject indicates on the informed consent that he/she does not consent to storing tissue or specimens for future research, these remaining samples (blood, cells, DNA) will be pulled from the -80C freezer and destroyed at the end of the study. The PI will confirm that the samples are disposed of and reported in the DSMP report. Data, tissue or samples will be stored only for members of the PIs research team and not other researchers.