Study Title: Vascular Dysfunction in Human Obesity Hypertension: Integrative Role of Sympathetic and Renin-Angiotensin Systems

NCT: NCT01983462

Date of IRB Approval/ Update: 03/04/21

Vascular Dysfunction in Human Obesity Hypertension

PI: Gary Pierce
IRB ID #: 201307779

Project Details

I. Project Introduction

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

I.2 ***Project Title:***
Vascular Dysfunction in Human Obesity Hypertension: Integrative Role of Sympathetic and Renin-Angiotensin Systems (VANISH study)

I.3 ***Short Title (optional):***
Vascular Dysfunction in Human Obesity Hypertension

I.4

The purpose of the study is to test the effects of: 1) blocking sympathetic nerve activity with a drug called clonidine, and 2) by blocking a kidney protein called renin, and on blood vessel function and muscle nerve activity in adults who are overweight or obese and have high blood pressure.

- Years 1 and 2: The 1st cohort of subjects will consist of 69 healthy young men and women age 18-79 years who are overweight or obese, defined as a body mass index > or = to 24 kg/m2, who have treated or untreated borderline systolic hypertension (i.e., prehypertension, systolic blood pressure > or = to 120 - <140 mmHg) or systolic hypertension (systolic blood pressure > or = to 140 - <180 mmHg) average of at least 3 measurements 2 min apart after 10 min seated resting position). These 69 subjects will then be randomized to 3 treatment arms: clonidine (0.1 mg/day), hydrochlorothiazide (25 mg/day) or placebo for 4 weeks in years 1 and 2. All tablets will be encapsulated by Nucara Pharmacy (Coralville, IA) to look identical. Subjects will randomly (1:1:1) receive one of the following combinations in a double-blind, placebo-controlled design:
  1) Oral clonidine (0.1 mg twice/day)
  2) Oral hydrochlorothiazide (12.5 mg twice/day)
  3) Oral placebo

I.5 ***Specify your research question(s), study aims or hypotheses (do not indicate "see protocol")***
Aim 1: Determine the extent to which central sympathetic nervous system (SNS) activity causes renin-angiotensin system (RAS) activation and impaired conduit and resistance artery endothelium-dependent (EDD) in obese hypertensive adults. Hypothesis 1: Pharmacological SNS inhibition will reduce muscle sympathetic nervous system activity (MSNA), circulating and vascular RAS activity and increase peripheral vascular EDD in overweight/obese hypertensive humans compared with placebo.

Aim 2: Determine the extent to which RAS activity causes impaired conduit and resistance artery EDD in overweight/obese hypertensive adults from vascular oxidative stress independent of SNS activity. Hypothesis 2: Pharmacological renin inhibition will increase peripheral vascular EDD to a similar degree as SNS inhibition in obese hypertensive humans and be associated with reductions in vascular endothelial oxidative stress in the absence of change in MSNA.

Aim 3: Determine the extent to which SNS or RAS activity causes impaired conduit and resistance artery endothelium-independent dilation (EID) in obese hypertensive adults in part from vascular RAS and oxidative stress. Hypothesis 3: Pharmacological SNS or renin inhibition will each increase peripheral vascular dilation to a nitric oxide donor in obese hypertensive humans and be associated with a reduction in vascular endothelial RAS and oxidative stress.

Aim 4: To measure circulating plasma (pro)renin receptor. Is it negatively associated with aging (lower in older vs. younger adults) and is it correlated with brachial FMD and carotid-femoral PWV?

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

Obesity has grown to epidemic proportions in the U.S. in the last two decades where by almost 2/3 of the population is overweight or obese (1). The significance of the obesity problem is underscored by the facts that obesity appears to lessen life expectancy by as much as 5-20 years in young adults (2), and children born today may have decreased life expectancy than that of their parents (3). These sobering statistics are attributed in large part to premature CVD-related morbidity and mortality in obese adults, of which obesity-related hypertension is the major contributor to this heightened cardiovascular disease(CVD) risk (4). Given that hypertension is present in ~50% of obese adults in the U.S. (5), determining the mechanisms that mediate hypertension in obesity and the resulting vascular complications is of high biomedical and public health importance.

Sympathetic neural activation and blood pressure in obesity. Several lines of evidence demonstrate that obesity is associated with an elevation in sympathetic neural activation in humans as a result of increases in circulating leptin, insulin or other unknown factors (6-11). Interestingly, sympathetic neural activation appears to be selectively increased to skeletal muscle vasculature (6-11) and kidney (12) and not heart or skin in obese compared with lean individuals (6). While our preliminary data do not clearly demonstrate elevated MSNA in obese adults with hypertension, this is largely a result of the high variability of MSNA among obese hypertensive individuals. In contrast to our data, Lambert et al. report a significantly higher MSNA in obese humans with, compared to without, hypertension (13). Therefore, it is possible that enhanced SNS activity mediates higher blood pressure in obese humans directly through enhanced alpha-adrenergic vascular tone. Consistent with this, local -adrenergic vasoconstrictor tone is elevated in obese hypertensive adults (14), and complete systemic alpha- and beta-adrenergic blockade (15) or autonomic inhibition with ganglionic blockade (16), decreases blood pressure more in obese hypertensive than obese normotensive adults supporting the idea of a greater sympathetic contribution of blood pressure when hypertension is present in obese humans. However, after complete autonomic blockade in obese hypertensive adults, blood pressure remains higher in obese hypertensive compared with obese normotensive adults, suggesting that additional non-autonomic/alpha-adrenergic mechanisms likely contribute to hypertension in obesity (16). We propose that the development of elevated blood pressure in select obese adults is mediated through a generalized vascular dysfunction (‘vasculopathy”) in part from hyperactivation of the local vascular RAS.

MSNA and vascular dysfunction in obesity hypertension. The mechanisms linking obesity to CVD in humans are complex, but impaired EDD has been advanced as a potential mechanism by which elevated MSNA contributes to increased CVD risk with obesity. Indeed, impaired EDD is present in obese adults with and without hypertension compared with normal-weight peers (17-22) and recent evidence links impaired EDD with higher MSNA in obese humans (23-25). Lambert et al. (2010) confirmed that elevated MSNA in obese young adults was associated with lower peripheral finger EDD (via EndoPat) even after adjustment for blood pressure, sex and body mass index (23) and was inversely related to EDD in young healthy adults (25). Moreover, central sympathetic blockade with moxonidine + dietary weight loss for 3 months improved brachial artery EDD (flow-mediated dilation) in obese insulin-resistant adults more than dietary weight loss alone, suggesting that central SNS activity was responsible for at least part of the impaired EDD in these obese subjects (24). Furthermore, our preliminary data confirm that both conduit and resistance artery EDD are reduced with obesity in young/middle-aged adults, and that brachial artery EDD is further reduced when hypertension is present in obesity. In addition to EDD, our preliminary data suggest that vascular EID (i.e., vascular smooth muscle dilation to a nitric oxide donor) is also impaired with obesity in both brachial conduit and forearm resistance arteries, and exacerbated in conduit arteries of obese subjects in the presence of hypertension. Interestingly, in agreement with several studies our data demonstrate reduced brachial artery dilation in response to nitroglycerin or EID, and strong inverse relations with measures of adiposity (26, 27). In contrast, our data differ with several other studies that demonstrate intact EID of forearm resistance arteries in obesity (19-22). The reasons for these conflicting findings are unclear, but may be explained by differences in age or co-morbidities of the populations studied, or technical differences in blood flow measurements. Nevertheless, our study will be the first to determine whether 4 weeks of central sympathetic inhibition with clonidine will improve both EDD and EID of resistance and conduit arteries in obese hypertensive humans.

Sympathetic nervous system (SNS) and RAS activity in obesity hypertension. Several lines of evidence suggest that activation of the RAS is a key mechanistic link between elevated SNS in obesity and CVD risk in humans (28). It is well established that the SNS system stimulates renin release from juxtaglomerular apparatus cells in renal afferent arterioles activating the classic RAS

axis resulting in production of the ang II and aldo (29). Indeed, multiple components of the RAS [i.e., plasma renin activity, angiotensinogen, anigiotensin converting enzyme (ACE) activity, aldodosterone (aldo)] are elevated in human obesity and decrease with weight loss (30). Our preliminary data demonstrate that select RAS components also are higher in primary vascular endothelial cells (i.e., local vascular RAS activation) from obese humans, and we predict that obesity hypertension will be associated with further increases in vascular RAS upregulation. This is important, because excessive production of downstream effector molecules of the RAS [e.g., angiotensin II (ang II), aldo] have multiple pathological effects including stimulation of reactive oxygen species in the vascular wall resulting in reduced nitric oxide-mediated EDD and vascular smooth muscle remodeling (29). Consistent with the idea that vascular oxidative stress likely is involved in vascular dysfunction in obesity, acute brachial artery infusions of vitamin c rescues resistance artery EDD in obese adults (18), and our preliminary data show that nitrotyrosine, a marker of oxidative damage to proteins, and p47phox, a subunit of the oxidant enzyme NADPH oxidase, are elevated in endothelial cells of obese compared with normal-weight (lean) humans. Additionally, we hypothesize that vascular oxidative stress (along with vascular RAS activation) will be further amplified in obese adults with hypertension and contributes to augmented vascular endothelial and smooth muscle dysfunction in obesity hypertension. However, there are currently no data on whether RAS inhibition improves vascular endothelial or smooth muscle dysfunction in obese hypertensive humans. As such, we will test whether 4 weeks of aliskiren therapy, a direct renin inhibitor, will improve EDD and EID in obese adults with hypertension independent of any alterations in MSNA.

Clinical impact of proposed study. Our proposal will have significant clinical impact related to obesity hypertension in at least 3 important ways: 1) it will provide direct evidence for the role of SNS activation in mediating vascular dysfunction in obesity hypertension in part through activation of the RAS 2) it will determine the degree by which the RAS activation mediates impaired vascular function independent of MSNA in obese adults with hypertension; and 3) it will provide initial support for the use central sympatholytics and/or renin inhibition as a potential clinical strategy to treat the vascular complications associated with obesity hypertension, although longer term studies will clearly be needed. Furthermore, future studies could also investigate combination SNS and renin inhibition and the effect of pharmacological blockade of other downstream RAS effectors such as aldo inhibition with eplerenone.

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

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II. Research Team

II.1 ***Principal Investigator***

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

II. ***Team Members***

2

UI Team Members

Name	E-mail	College	Contact	Key Prsn	UI COI	VAMC COI	Consent Process Involvement	Deactivated
Gary Pierce, PHD, MS	gary-pierce@uiowa.edu	College Lib Arts and Sciences	Yes	Yes	No		Yes	No
Zaidoon Al-Share, MD	zaidoon-alshare@uiowa.edu	Carver College of Medicine	No	Yes	No		No	No
Sanjana Dayal, PHD	sanjana-dayal@uiowa.edu	Carver College of Medicine	No	Yes	No		No	No
Kevin Doerschug, MD, MS	kevin-doerschug@uiowa.edu	Carver College of Medicine	No	Yes	No		No	No
Jess Fiedorowicz, MD, PHD	jess-fiedorowicz@uiowa.edu	College of Public Health	No	Yes	No		No	No
Jan Full, BSN	jan-full@uiowa.edu	Carver College of Medicine	No	No	No		No	No
Diana Jalal, MD	diana-jalal@uiowa.edu	Carver College of Medicine	No	Yes	No		No	No
Randy Kardon, MD	Randy-Kardon@uiowa.edu	Carver College of Medicine	No	Yes	No		No	No
Rachel Luehrs, MS	rachel-luehrs@uiowa.edu	College Lib Arts and Sciences	No	No	No		No	No
Ben Martin, High School	ben-martin@uiowa.edu	Graduate College	No	No	No		No	No
Julie Nellis, BSN	julie-nellis@uiowa.edu	Carver College of Medicine	No	No	No		Yes	No
Virginia Nuckols, MS	virginia-nuckols@uiowa.edu	Graduate College	No	No	No		No	No

Leah Reierson, High School	leah-reierson@uiowa.edu	Carver College of Medicine	No	No	No	No	No
Amy Marie Stroud, MSN	amy-stroud@uiowa.edu	Carver College of Medicine	No	No	No	Yes	No
Ryan Ward, MS	ryan-ward@uiowa.edu	Carver College of Medicine	Yes	No	No	Yes	No

Non-UI Team Members

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

Nothing found to display.

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

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

Name	Is Key Personnel
Gary Pierce, PHD, MS	Yes
Zaidoon Al-Share, MD	Yes
Sanjana Dayal, PHD	Yes
Kevin Doerschug, MD, MS	Yes
Jess Fiedorowicz, MD, PHD	Yes
Jan Full, BSN	No
Diana Jalal, MD	Yes
Randy Kardon, MD	Yes
Rachel Luehrs, MS	No
Ben Martin, High School	No
Julie Nellis, BSN	No
Virginia Nuckols, MS	No
Leah Reierson, High School	No
Amy Marie Stroud, MSN	No
Ryan Ward, MS	No

II.5 ***Select research team member who is the primary contact for study participants.***
Gary Pierce

III. Funding/Other Support

III.1 ***Funding Sources***

Type	Source	Grant Title	Name of PI on Grant
Private Foundation/Association	American Heart Association		Gary L. Pierce

* new source name

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

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

Name	Has Conflict of Interest
Gary Pierce, PHD, MS	No
Zaidoon Al-Share, MD	No
Sanjana Dayal, PHD	No
Kevin Doerschug, MD, MS	No
Jess Fiedorowicz, MD, PHD	No
Jan Full, BSN	No
Diana Jalal, MD	No
Randy Kardon, MD	No
Rachel Luehrs, MS	No
Ben Martin, High School	No
Julie Nellis, BSN	No
Virginia Nuckols, MS	No
Leah Reierson, High School	No
Amy Marie Stroud, MSN	No
Ryan Ward, MS	No

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

Source	Status	Other Status Description
American Heart Association	Awarded	

IV. Project Type

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

IV.2 ***Enter the date you will be ready to begin screening subjects/collecting data for this project. (If you do not have a specified date, add "upon IRB approval")***
August 1, 2013

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):***
Clonidine
Aliskiren
Hydrochlorothiazide
Sublingual nitroglycerin
.5% tropicamide
.5% proparacaine

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

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

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

V.5 ***Are 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 all drugs or substances in this study being used within the FDA approved route of administration?***

Yes

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

clonidine (Catapres)	
Name of Sponsor	
Investigator's Brochure Version	Boehringer Ingelheim
Investigator's Brochure Date	Oct 2009
Who is supplying the drug	
Who is dispensing the drug	
Planned Use in this Study	
Condition/Disease Indication(s)	Vascular function and blood flow
Subject Population	Obese Hypertensive
Dose(s)	0.1 mg bid
Administration	Oral
Dosing Regimen	0.1 mg twice daily by mouth
FDA Approved Use	
Approved Condition/Disease Indication(s)	Hypertension
Approved Patient Population	Hypertension
Approved Dose(s)	0.2 mg/day uo to 2.4 mg/day
Approved Administration	Oral, Cutaneous
Approved Dosing Regimen	0.2 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:	This is the FDA starting dose in the population (hypertension) indicated, therefore there is no increased risk.

aliskiren (Tekturna)	
Name of Sponsor	
Investigator's Brochure Version	Novartis
Investigator's Brochure Date	Sept 2012
Who is supplying the drug	
Who is dispensing the drug	
Planned Use in this Study	
Condition/Disease Indication(s)	Measure blood flow

Subject Population	Obese Hypertensive adults
Dose(s)	150 mg/day
Administration	Oral
Dosing Regimen	150 mg/day
FDA Approved Use	
Approved Condition/Disease Indication(s)	Hypertension
Approved Patient Population	Hypertensive adults
Approved Dose(s)	150 mg/day; 300 mg/day
Approved Administration	Oral
Approved Dosing Regimen	150 mg/day up to 300 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:	The dose and route of administration of aliskerin is the FDA approved dose and route of administration

hydrochlorothiazide (Esidrix, Microzide, Oretic)	
Name of Sponsor	
Investigator's Brochure Version	West-ward Pharmaceutica;
Investigator's Brochure Date	Jan 2006
Who is supplying the drug	
Who is dispensing the drug	
Planned Use in this Study	
Condition/Disease Indication(s)	Measure blood flow
Subject Population	Obese Hypertensive adults
Dose(s)	12.5 mg bid
Administration	Oral
Dosing Regimen	12.5 mg bid
FDA Approved Use	
Approved Condition/Disease Indication(s)	Hypertension
Approved Patient Population	Hypertensive adults
Approved Dose(s)	25 mg/day up to 50 mg/day
Approved Administration	Oral
Approved Dosing Regimen	12.5 mg bid
Is this study intended to be reported to the FDA as a well-controlled study in support of a new indication or a significant	No

change in the labeling for this product?	
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 of HCTZ used in this study is the standard FDA approved dose for hypertension in adults

Nitroglycerin sublingual tablet (Nitrostat)	
Name of Sponsor	
Investigator's Brochure Version	
Investigator's Brochure Date	
Who is supplying the drug	
Who is dispensing the drug	
Planned Use in this Study	
Condition/Disease Indication(s)	Vascular endothelial dysfunction
Subject Population	Obese hypertensive adults
Dose(s)	300 mg once
Administration	Other: sublingual
Dosing Regimen	300 mcg once
FDA Approved Use	
Approved Condition/Disease Indication(s)	Angina pectoris
Approved Patient Population	Cardiovascular disease
Approved Dose(s)	400 mcg; 300 mcg
Approved Administration	Other: sublingual
Approved Dosing Regimen	400 mcg; 300 mcg
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.

Tropocamide (Mydriacyl)	
Name of Sponsor	
Investigator's Brochure Version	
Investigator's Brochure Date	
Who is supplying the drug	
Who is dispensing the drug	
Planned Use in this Study	
Condition/Disease Indication(s)	Dilate the Pupil
Subject Population	Adult subjects between ages 18-79
Dose(s)	one drop of 0.5%
Administration	Ocular
Dosing Regimen	one drop of 0.5% in one eye
FDA Approved Use	
Approved Condition/Disease Indication(s)	Dilate the Pupil
Approved Patient Population	Pediatric and adult patients approved
Approved Dose(s)	one drop of 0.5%
Approved Administration	Ocular
Approved Dosing Regimen	one drop of 0.5% per eye
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 does not increase the risk because we are using the exact FDA-approved dosage.

Proparacaine (Alcaine)	
Name of Sponsor	
Investigator's Brochure Version	
Investigator's Brochure Date	
Who is supplying the drug	
Who is dispensing the drug	
Planned Use in this Study	
Condition/Disease Indication(s)	Procedures in which a topical ophthalmic anestheric is indicated
Subject Population	Adult subjects between ages 18-79
Dose(s)	one drop of 0.5%
Administration	Ocular
Dosing Regimen	one drop of 0.5% in one eye

FDA Approved Use	
Approved Condition/Disease Indication(s)	Procedures in which a topical ophthalmic anestheric is indicated
Approved Patient Population	Pediatric and adult patients approved
Approved Dose(s)	one drop of 0.5%
Approved Administration	Ocular
Approved Dosing Regimen	one drop of 0.5% per eye
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:	There is no increased risk of use as we are using the FDA-approved dosage.

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

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

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

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

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

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

- No

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

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

- Yes

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

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

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

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

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

VI. Subjects

VI.1 ***How many adult subjects do you expect to consent or enroll for this project?***
146

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

VI.3 ***What is the age of the oldest adult subject?***
79.0

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

VI.5 ***What is the percentage of adult female subjects?***
50

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***

We will enroll 111 healthy men and women age 18-79 years who are overweight or obese, defined as a body mass index > or = 24 kg/m2, and who have treated or untreated borderline systolic hypertension or systolic hypertension (systolic blood pressure >/= 120 - <180 mmHg- average of at least 3 measurements 2 min apart after 10 min seated resting position) per JNC7 guidelines; these subjects will be enrolled and randomized to one of the 3 treatment arms (pending screening). We will also enroll 35 overweight or obese adults body mass index > or = 24 kg/m2 with 'normal/optimal' systolic blood pressure (<120 mmHg) who will undergo baseline testing only (Visits 1, 2 and 3). Therefore, the grand total subjects enrolled will be n=146.

All subjects will be healthy, non-smokers, as described below under Inclusion and Exclusion criteria. All subjects will also be weight stable (+/- 5 lbs) for the previous 3 months.

- 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.
Systolic blood pressure >/= 120 mmHg and <180 mmHg
Age is > or = 18 and < or = 79 years of age
Weight stable (+/- 5 lbs) for the previous 3 months
Healthy, as determined by health history questionnaire, blood chemistries, 12-lead ECG
Blood chemistries indicative of normal renal (creatinine <2.0mg/dl), liver (<3 times upper limit for ALT, AST), and thyroid function (TSH between 0.4 - 5.0 mU/L) or on thyroid medication stable with no dose change for 3 months
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; or omega-3 fatty acids.
If currently taking one antihypertensive medication, be willing and able to discontinue taking it for 2 weeks prior and throughout the treatment period
If currently taking two antihypertensive medications (one of which must be a diuretic), be willing and able to discontinue taking them for 2 weeks prior and throughout the treatment period
No history of cardiovascular disease (e.g., heart attack, stroke, heart failure, valvular heart disease, cardiomyopathy), Type 1 or Type 2 diabetes, or peripheral arterial disease
Non-smokers, defined as no history of smoking or no smoking for at least the past 3 months
Normal resting 12-lead ECG (no evidence of myocardial infarction, left-bundle branch block, 2nd or 3rd degree AV block, atrial fibrillation/flutter)

Exclusion Criteria:
Systolic blood pressure >/=180 mmHg or diastolic blood pressure >110 mmHg
History of cardiovascular disease such as heart angioplasty/stent or bypass surgery, myocardial infarction, stroke, heart failure with or without LV ejection fraction <40%, cardiomyopathy, valvular heart disease, cardiomyopathy, heart transplantation, Type 2 and Type 1 diabetes
History of abdominal aortic aneurysm
Taking one or two antihypertensive medications and not able or willing to go off of for 2 weeks prior and during the study
Taking two antihypertensive medications in which one is not a diuretic
Taking more than two antihypertensive medications
Subjects currently being treated with Clonidine or Beta-Blockers
History of malignant hypertension
Subjects whose blood pressure is not controlled on current therapy (systolic blood pressure > or = to 140 mmHg or diastolic blood pressure > or = to 90 mmHg at Visit 1 and on 24 hour mean blood pressure based on monitor given to them by study staff)
Smoking or history of smoking within the past 3 months
History of gastric ulcers, bleeding disorders, dyspepsia, or metabolic acidosis
History of chronic obstructive pulmonary disease (COPD)
Abnormal resting 12-lead ECG (e.g., evidence of myocardial infarction, 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 > 2.0 mg/dL, or hepatic enzyme (ALT/AST) 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 lipid lowering (e.g., statins, niacin) for less than 3 months or plans to change dose or lipid lowering medication in the next month (if subject is on lipid lowering medication for at least 3 months at stable dose and no plans for change in dose or medication then subject is eligible)
Medications for glycemic control (e.g. metformin, insulin), anticoagulation, anti-seizure, or antipsychotic agents
Anti-depression or antianxiety started within the past 6 months or changed dose (if stable for 6 months with no change in medication or dose, then subject will be eligible)
History of co-morbid condition that would limit life expectancy to < 6 months.
Taking chronic non-steroidal anti-inflammatory drugs (NSAIDS) such asaspirin, indomethacin, naproxen, acetaminophen (Tylenol), ibuprofen (Advil, Motrin) and not able or willing to go off of for 2 weeks prior and during the study
Taking cox-2 inhibitors (Celebrex, Vioxx, etc) or allopurinol (Zyloprim, Lopurin, Alopurin;
Taking blood thinners such as coumadin (Wafarin), enoxaparin (Lovenox); clopidogrel (Plavix); dypyridamole (Persantine); heparin;
Taking diabetic medications (Metformin, glyburide, insulin, etc), TZDs (Avandia, Rezulin, Actos);

Taking steroids or biologics : corticosteroids (prednisone); methotrexate, infliximib (Remicade), etaneracept (Enbrel); anakinra;
Taking NEW thyroid medications in the past 3 months such as levothyroxine (Levoxyl, Synthroid, Levoxyl, Unithroid); Levodopa). After subject is on meds for 3 months with no medication or dose change then subject is eligible
Taking Phosphodiesterase (PDE) 5 inhibitors (e.g., Viagra®, Cialis®, Levitra®, or Revatio®); PDE 3 inhibitors (e.g., cilostazol, milrinone, or vesnarinone); lithium
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 (NSAIDS)
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.
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)
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
Those who currently donate blood, platelets, or plasma

If subjects that washout of antihypertensive medication(s) have systolic blood pressure >/= 160 mmHg or diastolic blood pressure >/= 110 mmHg at any point in the study they will be excluded

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 363,666 adults between the ages of 18-79 in Johnson County and its 8 surrounding counties (Linn, Washington, Louisa, Benton, Iowa, Muscatine, Cedar, and Jones). With a current obesity rate of 28.4% in Iowa, we expect that approximately a little less than 1/3 or 103,281 adults are obese (BMI > or = 30 kg/m2) in Johnson County and surrounding counties. Given that approximately 31.9% of US adults 20 years or older have hypertension, we estimate that ~32,947 adults between age 18-79 are both obese and have hypertension in Johnson County and its 8 surrounding counties.

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 and other local newspapers, the 'volunteer research' clinical trials website on UIHC website (http://www.uihealthcare.org/ClinicalTrials.aspx/), in the 'Noon news' in UIHC and on social media sites like Craigslist and Facebook (see attachments). We will contact registrants in the STAR registry at the University of Iowa Center on Aging. Brochures will also be handed out in the Healthy Weight Management Clinic located at Iowa River Landing to potentially eligible individuals (STAR brochures).

VI.16 ***Do you <u>plan</u> 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 <u>about</u> pregnant women?***

No

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

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

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

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

VII.A. Project Description (A)

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

- Other UI campus site - 522 FH and 518 FH- laboratory
- CRU
- UIHC - Pomerantz Family Pavilion, Ophthalmology Clinic, Rm 11279; UIHC-Iowa River Landing

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

VII.B. Project Description (B)

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

- ☐ **Interventional** – Includes **Clinical (or Treatment) trial**, **Physiology intervention/study**, **Behavioral intervention/study**, **Diagnostic Trial**.
- ☐ **Observational**
- ☐ **Expanded Access** – A process regulated by the Food and Drug Administration (FDA) that allows manufacturers to provide investigational new drugs to patients with serious diseases or conditions who cannot participate in a clinical trial. Examples of expanded access include non-protocol access to experimental treatments, including protocol exception, single-patient IND, treatment IND, compassionate use, emergency use, continued access to investigational drug, and parallel track (ClinicalTrials.gov & FDA).
- ☐ **Registry** – The collection and maintenance of data (not including biologic samples) in which: (1) the individuals in the registry have a common or related condition(s), and/or (2) the individuals in the registry are interested in being contacted for future studies by investigators other than those listed in Section II of this project.(UI Guide)
- ☐ **Repository** – The collection, storage, and distribution of human biologic samples and/or data materials for research purposes. Repository activities involve three components: (i) the collection of data and/or specimens such as blood, tissue, saliva, etc.; (ii) the storage of data or specimens, and data management function; and (iii) the sharing of data/specimens with recipient investigators other than the original investigators. (paraphrased from OHRP)
- ☐ **Other**

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

- ☐ **Phase I trials** – include initial studies to determine the metabolism and pharmacologic actions of drugs in humans, the side effects associated with increasing doses, and to gain early evidence of effectiveness; may include healthy participants and/or patients (ClinicalTrials.gov & FDA)
- ☐ **Phase II trials** – include controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks(ClinicalTrials.gov & FDA)
- ☐ **Phase III trials** – include expanded controlled and uncontrolled trials after preliminary evidence suggesting effectiveness of the drug has been obtained, and are intended to gather additional information to evaluate the overall benefit-risk relationship of the drug and provide an adequate basis for physician labeling(ClinicalTrials.gov & FDA)
- ☑ **Phase IV trials** – studies of FDA-approved drugs to delineate additional information including the drug's risks, benefits, and optimal use(ClinicalTrials.gov & FDA)

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 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 over-the-counter compounds for 2 weeks before participating. If subjects are unwilling to go off these 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 any over the counter compounds they will be required to contact their personal physician to confirm they can go off any of the compounds listed above for the study duration.

If subjects are being treated with monotherapy or dual therapy (a diuretic along with one other hypertensive medication), 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 to go off these medications for the 2 week washout 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 these medications, they will be required to contact their personal physician to confirm they can go off them for the study duration.

During the 2 week washout period, these subjects will have safety visits scheduled 4 days and 7 days after the beginning of the washout period. If, at either of these safety visits, their systolic blood pressure is > or = 160 mmHg or their diastolic blood pressure is > or = 110 mmHg, we will end their participation.

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 in person and agrees to go off of the aforementioned drugs for 2 weeks, they will be called by the study coordinator via phone after 2 weeks of the washout and invited back to the CRU for Visit #2.

After the subject sign the informed consent document in person and agrees to go off of the

antihypertensive medication(s) they will have 2 in person safety visits scheduled at the CRU 4 days and 7 days after the beginning of the washout period.

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; Jess Fiedorowicz, 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?***
Yes

VII.B.7 ***Why is a placebo justified in lieu of an approved drug in this study (check all that apply)?***
- Minimal harm may result from the use of placebo (e.g. ongoing disease has little adverse effect on the patient during the course of the trial and is reversible

VII.B.8 ***Describe the management plan, including when you would stop the subject's participation in the event the subject worsens during the placebo phase of the study.***
If subjects that washout of antihypertensive medication(s) have an average (o 3 seated resting measurements) systolic blood pressure >/= 160 mmHg or diastolic blood pressure >/= 110 mmHg at any point in the study they will be excluded and will be advised to see their physician.

VII.B.9 ***Describe the method (phone/in person) frequency of contact with the subject during the placebo phase of the study.***
After beginning the study medication (which may potentially be a placebo) the subject will have two safety visits (7 and 14 days from the start date of the medication). However the subject is encouraged to contact the study coordinator or Dr. Jalal with any questions, concerns, comments, or to report side effects at any time.

VII.B.10 ***Who (list names) will be available on a 24/7 basis for questions or emergencies during the placebo phase of the study?***
Gary Pierce, PhD; Diana Jalal, MD; Jess Fiedorowicz, MD, PhD

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 clonidine, aliskiren or hydrochlorothiazide 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 ID codes, date collected and protocol number.

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 (518 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 522 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?***
Yes

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):***

- Other - Set up display in UIHC lobby and provide flyer and/or consent to employees and visitors interested in learning more about our study. Give presentations or hand out information pertaining to study at pharmacies, medical clinics, public events, community bulletin boards within a 100 mile radius of UIHC. Only using IRB approved materials.
- Website - UIHC Clinical Trials and Research Website; http://www.uihealthcare.org/clinicalresearch/Default.aspx; https://iowacity.craigslist.org/; https://www.facebook.com/
- Advertisements -
- Existing Registry/database - STAR (Seniors Together in Aging Research) is a registry of volunteers age 50 and older, living within a two-hour drive of the University of Iowa. STAR is maintained by The University of Iowa Center on Aging and is a confidential and secure database restricting access to only IRB-approved staff. Currently over 1500 people are registered with the STAR database.
- Posters -
- Brochures -
- 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 - family practice clinic in the University of Iowa

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***
Data Elements Access through the use of electronic medical records (EPIC)
1. Adult patients 18-79
2. Normal systolic blood pressure of <120 mmHg and a body mass index of >24 kg/m2 or greater.
3. Systolic high blood pressure or systolic high blood pressure defined as systolic blood pressure greater than or equal to 120 and less than 180 mmHg and a body mass index great than or equal to 24 kg/m2
4. Medication use

VII.D.3 ***Describe why you could not practicably recruit subjects without access to and use of the information described above***
The screening process involves reviewing medical histories focusing on body mass index and blood pressure, based on clinical criteria; age and medication use. The use of electronic medical records would make preliminary screening as efficient as possible and would avoid errors in the screening process. We only want to 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***
For recruitment purposes each patient will receive a unique ID# and all medical data will be identified with the unique ID#. Screening logs will be retained with information about why the patient was not approached, consented or enrolled. The screening log will be kept in a lock cabinet behind a locked office door. Only study team members will have access to the completed logs.

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

The research team plans to store records beyond the point where individuals decline to participate or are determined to be ineligible. A screening log will be maintained by the research team, identifying information on people who do not enroll will be destroyed once enrollment closes to avoid screening subjects more than once.

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:***

Staff will discuss the study with potential subject and answer questions in a private exam room in the ICTS Clinical Research Unit (CRU).

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 potential subjects will fill out our online screening form (see attachment) or call/email the research staff in response to seeing one of our research advertisements, or after being contacted by the STAR registry. The potential subject will leave a contact telephone number for the 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. If the potential subject did not fill out the online screening form, then staff will 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 Visit 1 in which the informed consent document will be reviewed; 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, PHD, MS	Yes
Zaidoon Al-Share, MD	No
Sanjana Dayal, PHD	No
Kevin Doerschug, MD, MS	No
Jess Fiedorowicz, MD, PHD	No
Jan Full, BSN	No
Diana Jalal, MD	No
Randy Kardon, MD	No
Rachel Luehrs, MS	No
Ben Martin, High School	No
Julie Nellis, BSN	Yes
Virginia Nuckols, MS	No
Leah Reierson, High School	No
Amy Marie Stroud, MSN	Yes
Ryan Ward, MS	Yes

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 ***<u>Before</u> 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 address?
3. What is your phone number and email address?
4. What is your current age and date of birth?
5. What is your current height and weight?
6. Do you take any prescription medications? If so, which ones, how frequent, and what is the dose?
7. Do you take any over-the-counter medications, supplements, vitamins, minerals? If so, which ones?
8. Do you have any allergies to medications? (answer yes or no); If yes, which ones?
9. Do you have any allergies to latex? (answer yes or no)
10. Do you currently have or have a history of any of the following conditions or diseases (answer yes or no):

-Hypertension (high blood pressure)?
-Borderline high blood pressure (aka, prehypertension)?
-Cancer?
-Kidney disease or failure?
-Thyroid disease/disorder?
-Diabetes type I?
-Diabetes type II?
-Severe GI/gastric reflux/GERD?
-Quit using tobacco products in the past 3 months or less
-Currently using tobacco products?
-Liver disease?
-HIV/AIDS?
-Graves disease/Granulomatosis?

11. Do you currently have or have a history of any of the following conditions or diseases (answer yes or no):

-Brain tumor?
-Seizures?
-Brain injury?

12. Do you currently have or have a history of 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 disease in legs?
-Atrial fibrillation/flutter?
-Abdominal aortic aneurysm?

13. Do you currently have or have a history of any of the following conditions or diseases (answer yes or no):

-Fibromyalgia/lupus?
-Organ transplant?
-Lung disease-emphysema or chronic bronchitis?
-Rheumatoid arthritis?
-Vasculitis?
-Currently using investigational medical device or drug?
-Chicken pox, shingles or flu in last 2 weeks?

14. 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?

15. Do you currently have or have a history of any of the following conditions or diseases (answer

yes or no):
a. Stomach, esophageal, or GI bleeding or ulcer?
b. Any other bleeding disorders?
c. Any other GI disorders (e.g., colitis, Chrohn's, etc.)?
d. Currently taking any blood thinning medication (e.g., warfarin, coumadin)?
e. Drink more than 10 alcoholic drinks per week?
16. Do you currently donate blood, platelets, or plasma?
17. Are you willing to do the following (answer yes or no):
a. Fast overnight for 8 hours?
b. Hold medications on the morning of testing?
c. Take a study medication for 4 weeks?
c. Willing to have your blood drawn?

Link to online screening form: http://j.mp/1pJNnKx

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 and DOB
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 the number and demographics of subject's 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 AHA 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***
Health history questionnaire
Heart rate
Height and weight
Resting blood pressure
Resting 12-lead ECG
Standard blood chemistries- lipid panel, basic metabolic panel, thyroid stim hormone (TSH), foll stim hormone (FSH)- FSH for women only), serum electrolytes
Urine pregnancy test for women of childbearing age
24 Hour Blood pressure monitoring

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 being in the study as long as the study is actively recruiting subjects. Subjects are allowed to discuss the study with family/friends before deciding on participation.

VII.D.28 ***How long after the subject agrees to participate do study procedures begin?***
Screening tests can begin on the same day as consent (Visit 1). If subject is required a 2 week washout period from aforementioned substances, the subject will complete the second half of Visit 1 (blood sample) 2 weeks after signing the consent. The experimental procedures, beginning on Visit 2, will be completed within 2-3 weeks or less of Visit 1.

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

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

Subjects on an antihypertensive medication at the time of screening and visit 1 will sign the washout consents ("VANISH_ICD_Clonidine_Washout" and VANISH_ICD_Aliskiren_Washout), depending on the phase of the study. Subjects not on an antihypertensive medication at the time of screening and visit 1 will sign "VANISH_ICD_Clonidine" and "VANISH_ICD_Aliskiren" consents, depending on the phase of the study. Control subjects will sign the "VANISH_ICD_Controls" consent, regardless of the phase of the study.

The PI and his research staff will recruit subjects from the community via flyers posted on campus, emails to UI community, advertisements and "Noon News", posted on UIHC clinical trials and research website for research volunteers, the U Iowa Cambus, subject recruitment letter, and social media sites like Craigslist and Facebook. Social media site ads will use previously approved flyer or e-mail text in advertisement with link to REDCAP screening survey and/or Iowa Translational Physiology Lab contact information. We will also place advertisements in local area newspapers including, but not limited to, the Iowa City Press Citizen, The Gazette, and The Daily Iowan. Additionally, subject recruitment letters will be sent out to potential subjects identified through electronic medical record. Located at the bottom of the letter there are two options to participate. The subject may reply "yes" if interested and would like more information and provide a phone number and/or "no" if not interested, study coordinator phone number and email is also to communicate their option as well. They will then send the letter back in a business reply envelope provided. If no response is communicated study coordinator will follow up after two weeks with most recent telephone number obtained through electronic medical record. The subject will then have the option of completing an online screening form or calling or emailing the laboratory to express interest in the study (see attached online screening form). If they contact the laboratory via phone or email, reply "yes" to the recruitment letter, or do not respond to the recruitment letter after two weeks, a study team member will contact the subject via 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 document with the potential subject. The study coordinator or research staff will answer all questions asked by the potential subject and the subject will be informed of all potential risks before 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 and for any reason.

With permission from the business/clinic administrator(s) the PI and research staff will also recruit subjects at pharmacies, medical clinics, public events, and community bulletin boards by giving presentations or handing out information pertaining to study within a 100 mile radius of UIHC. Only using IRB approved materials which includes; IRB approved flyer and/or IRB approved consent.

STAR (Seniors Together in Aging Research) is a registry of research volunteers age 50 and older, living predominantly within a two-hour drive of the University of Iowa. STAR is maintained by The University of Iowa Center of Iowa Center on Aging and is a confidential and secure database restricting access to only IRB-approved staff at the Center of Aging. Currently over 1500 people are registered with the STAR

database. In order to maintain confidentiality, STAR makes the initial contact with all potential volunteers, providing them information about the study and contact information for study staff. This contact will be through brochures giving participants basic information about the study (see attached STAR brochure). Interested volunteers will contact the study staff to complete the phone screen or will complete the online screening form. If eligible based on the phone screen, potential subjects will schedule their first visit with study staff in which the Informed Consent Document will be discussed and signed.

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.***
The 111 healthy men and women who have treated or untreated borderline or systolic hypertension will be told what study arm they were assigned at the end of the final experimental visit or after subject is withdrawn from the study for any reason.

VII.E.2 ***Describe randomization scheme/assignment including ratio such as 1:1, 2:1 etc.***
In years 1 and 2, overweight or obese subjects (n=69) with systolic blood pressure >/= 120 mmHg will be randomized to 1 of 3 conditions for 4 weeks: clonidine (0.1 mg/day), hydrochlorothiazide (25 mg/day) or placebo for 4 weeks.

In years 3 and 4, a new cohort of overweight or obese subjects (n=42) with systolic blood pressure >/= 120 mmHg will be randomized to oral aliskiren (150 mg/day), oral hydrochlorothiazide (12.5 mg/day) or oral placebo for 4 weeks.

The 35 overweight or obese adults with 'normal/optimal' systolic blood pressure <120mmHg will undergo baseline testing (Visits 1, 2 and 3) only and will therefore not receive any randomization.

Blood pressure measurements from Visit 1, Visit 2 and 24-hour ambulatory blood pressure monitoring will be used to determine average blood pressure and resulting group placement (control or study medication randomization).

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 survey
Online screening suvey (REDCap)
Demographics survey
Health History survey
Modifiable Activity Questionnaire (MAQ)
Patient Health Questionnaire (PHQ-9)
State-Trait Anxiety Inventory Self-Evaluation Questionnaire (STAI)
Anxiety Inventory
Depression Inventory
Side effect log
Side effect survey
mSNA Questionnaire
24 hour AMBP activity log
Subject medication diary
Karolinska Sleep Log
Sleep Quality Assessment (PSQI)
Berlin Questionnaire

VII.E.5 ***Does this project involve creating any audiotapes, videotapes, or photographs?***

No

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

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

DESCRIBE:

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

Experimental design: A total of 111 healthy adults who are overweight or obese and have treated or untreated borderline systolic hypertension or systolic hypertension (systolic blood pressure >/= to 120 – < 180 mmHg on two separate office visits) will be enrolled in 2 separate studies over 4 years.

In Years 1-2, overweight/obese adults with borderline hypertension or with hypertension (n=69) will be randomized to 4 weeks of clonidine(0.2 mg/day), hydrochlorothiazide(25 mg/day) or placebo.

In years 3-4, a new cohort of overweight/obese subjects with borderline hypertension or with hypertension (n=42) will be randomized to aliskiren (150 mg/day), hydrochlorothiazide (25 mg/day) or placebo for 4 weeks.

This will be a randomized, double-blind, placebo controlled, parallel study design. A total of 35 healthy overweight or obese adults with optimal systolic blood pressure of <120 mmHg will be enrolled to establish age-associated differences in primary outcomes at baseline. These control subjects will undergo screening and baseline experimental visits only (Visits 1-3), but will NOT undergo the drug intervention or visits 4, 5, 6 or 7.

Order of visits: Subjects will undergo experimental testing at baseline and again after the 4 weeks of clonidine (if year 1 and 2), aliskiren (if year 3 and 4) hydrochlorothiazide or placebo. Subjects will be studied between 7:00am and 10:00am 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 is as follows:

Visit 1 (2 hours): Informed 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 antihypertensive medications, vitamins, or supplements, they will be scheduled to complete the remainder of Visit 1 (blood sample) in 2 weeks. If

subject consents and no washout period is needed, screening tests will be performed on the same day to determine eligibility via the following:
i. Research staff will obtain resting vitals (blood pressure, heart rate and resting 12 lead ECG).
ii. Research staff will obtain anthropometrics including height, weight, waist and hip circumference with tape measure.
iii. Subject will fill out the following documents: Demographics, Health History survey, the Modified Activity Questionnaire (MAQ), The Self-Evaluation Questionnaire (STAI), Patient
Health Questionnaire (PHQ-9), Anxiety Inventory and Depression Inventory (anxiety and mood questionnaires), the Berlin Questionnaire, the Karolinska Sleep Log, and the Sleep Quality Assessment (PSQI).
iv. Research nurse or trained research staff will obtain venous blood draw using butterfly needle to be sent to UIHC pathology labs:
•Lipid panel, TSH, metabolic panel, electrolytes, and FSH (women only to confirm >40 mIU/l) (4.5 ml blood- 1 light green PST tube)
c) Subject will be instrumented with 24-hour blood pressure monitor to wear home for 24 hours. Subject will be given an activity log to record any activities such as exercise, showering, or sleep over the 24 hrs. Subject will return monitor to the investigators when they come in for Visit 2. There is no long term follow up.

If the subject is completing a 1 week washout of antihypertensive medications, Visit 1 will be broken up into 3 visits (Prewashout visit, Washout Visit 1 and Washout Visit 2)

Prewashout visit (30 minutes): Initial visit with participant
a) Subject will come to CRU for initial visit, be consented, and have baseline blood pressures taken if agrees to participate. Visit 1 surveys will also be done at this visit (see above).

Washout Visit 1 (30 minutes): ~ 4 Days after Beginning of Washout, Safety Visit 1
a) Subject will return to CRU to measure blood pressure, HR, and survey of any side effects. If any side-effects/symptoms present, research nurse or coordinator will follow instructions as outlined in section VIII.2 "Plan for Managing Risks".

Washout Visit 2 (30 minutes to 1.5 hours): ~ 7 Days after Beginning of Washout, Safety Visit 2
a) Subject will return to CRU to measure blood pressure, HR, and survey of any side effects. If any side-effects/symptoms present, research nurse or coordinator will follow instructions as outlined in section VIII.2 "Plan for Managing Risks". If no side effects and participant still wishes to participate, the remainder of Visit 1 procedures will be done including any remaining surveys, blood draw, 24-hour blood pressure monitoring, anthropometric measures, and EKG (see above sections b.i. through b.iv.)

Visit 2 (4.5 hours): Baseline Measurements (Microneurography, PWV, Carotid Compliance Brachial artery flow-mediated dilation, Endothelial Cell Collection)
a) Subject arrives at the CRU between 7-10 am after overnight 8 hour fast
b) Return 24-hour blood pressure monitor.
c) Subject escorted to the CRU and a physician or research staff trained in microneurography will insert needle into peroneal nerve for measurement of muscle sympathetic nerve activity (See ‘Methods’ for details). Subject will be given "mSNA Questionnaire" to assess any follow-up discomfort at the sight of microneurography electrode over the next 7 days and mail it back to the study staff in 7 days. Participants will wear two E4 wristbands (one on each wrist) during the procedure. Measures of brachial artery blood flow using Doppler ultrasound, a mental math test, and a cold pressor test may also be performed during microneurography.
d) CRU staff/nurse perform urine pregnancy test (women).
e) IV catheter insertion. Subject will lie supine and research nurse will insert venous 18 G catheter into antecubital vein.
f) Endothelial cell collection: PI, nurse or physician will perform J wire endothelial cell collection (3 wires) through the 18 G IV catheter (see ‘Methods’ for details).
g) After 20 min, research nurse will then obtain blood samples through catheter:
• Lipid panel, insulin/glucose, hs-CRP, basic metabolic panel (1 x 4.5 ml blood- light green PST tube)
• Catecholamines (1 x 10 ml Na+ Hep green top tubes)
• Whole blood (2 x 10 ml citrate tubes) for platelet aggregation assay
• Extra blood collected for specialized labs performed in PIs lab:
1. Serum: 4 x 5 ml red top SST tube: for interleukin-6, tumor necrosis factor-alpha, ang II, aldosterone, and RAS and oxidative
stress proteins

2. Whole blood (17 ml blood- 3 x 8.5 ml CPT) for mononuclear cell DNA isolation and plasma
h) Pulse Wave Velocity (PWV) and Carotid Compliance (CC) (see 'Experimental Methods' for details). The Pulse Wave Velocity measure will be performed two times.
i) Forearm Blood Flow: Subject will be instrumented with upper arm venous occlusion cuffs and wrist cuffs for measurements of forearm blood flow using venous occlusion plethysmography (see 'Methods' for details).
j) Brachial artery flow-mediated dilation (FMD) and 0.3 mg sublingual nitroglycerin with non-invasive ultrasonography (See 'Methods' for details)
k) Subject receives meal/snack from CRU metabolic kitchen and is then free to leave.

Visit 3 (2 hours): Baseline Measurements
a) Subject arrives at the CRU between 7-10 am after overnight 8 hour fast. Women will be given a urine pregnancy test.
b) Subject will undergo visual acuity, intraocular pressure test as well as a blood pressure measurement. The subject will then undergo ocular coherence tomography and laser-speckle blood flow imaging. As with microneurography (Visit 2), two E4 wristbands (one on each wrist) will be worn and a mental math test and a cold pressor test may also be performed during laser-speckle blood flow imaging.
c) Randomization and medication dispensing:
i) Years 1 and 2: Subjects randomized to oral clonidine (0.2 mg/day- 0.1 mg in the morning and 0.1 mg in the evening), hydrochlorothiazide (25 mg/day- 12.5 mg in the morning and 12.5 mg in the evening) or placebo for 4-5 weeks will be given 5 week supply of pills (blinded). Subject will receive instructions on taking pills by research nurse or study coordinator, recognizing side effects, and review procedures for reporting side effects. Research nurse or study coordinator will administer first dose of study drug to subject in CRU and observe for 15 min. Subject will given side effect log survey (for subject to record any daily symptom/side effects), subject medication diary (to record daily medication intake) and Carenote on study drug (to provide take home educational information on potential side effects of study drugs).
ii) Years 3 and 4: Subjects randomized to oral aliskiren (150 mg/day- 150 mg in morning and placebo in the evening), HCTZ (25mg/day- 12.5 mg in the morning and 12.5 mg in the evening) or placebo for 4-5 weeks will be given 5 week supply of pills (blinded). Subject will receive instructions by research nurse or study coordinator on taking aliskiren or hydrochlorothiazide and take first dose in CRU. Research nurse or coordinator will review potential side effects of aliskiren and HCTZ and methods for reporting side effects. (Because the aliskiren is taken qd, and HCTZ dose is bid, this requires that the aliskiren evening dose be placebo). Subject will given side effect log survey (for subject to record any daily symptom/side effects), subject medication diary (to record daily medication intake) and Carenote on study drugs (to provide take home educational information on potential side effects of study drugs).
h) Subject will be given snack or lunch in CRU and is then free to leave.

Visit 4, week 1 (30 minutes): ~Day 7 day Blood electrolytes, Compliance check/Pill Count
a) Subject will return to CRU for blood sample to measure electrolytes, blood pressure, HR, pill count and survey of any side effects. If any side-effects/symptoms present, research nurse or coordinator will follow instructions as outlined in section VIII.2 "Plan for Managing Risks". Blood sample will be sent to UIDL stat, and if potassium is 3.5 or lower, oral 10 mg KCL will be prescribed to subject by Dr. Jalal.

Visit 5, week 2 (30 minutes): ~Day 14 day day Blood electrolytes, Compliance check/Pill Count
a) Subject will return to CRU for blood sample, blood pressure, HR, pill count and survey of any side effects. If any side-effects/symptoms present, research nurse or coordinator will follow instructions as outlined in section VIII.2 "Plan for Managing Risks". Blood sample will be sent to UIDL stat, and if potassium is 3.5 or lower, oral 10 mg KCL will be prescribed to subject by Dr. Jalal.

**Unplanned safety checks: Additional, "unplanned" safety checks may be scheduled at the discretion of Dr. Jalal or other advising investigator to monitor blood levels, etc.

Visit 6, week 4-5 (4.5 hours): Post-intervention (Anthropometrics, microneurography, FMD, Endothelial cells)
a) Subject will take morning dose of study pills and return to CRU between Day 26 -35 for venous blood sample, blood pressure, HR, pill count and survey of any side effects
b) Subject escorted to the CRU Human Cardiovascular Physiology Research Lab and a physician or research staff trained in microneurography will insert needle into peroneal nerve for measurement of muscle sympathetic nerve activity (See 'Methods' for details). Participants will wear two E4 wristbands (one on each wrist) during the procedure. Measures of brachial artery blood flow using Doppler ultrasound, a mental math test, and a cold pressor test may also be performed during microneurography.

c) IV catheter and blood sample: Subject will lie supine and research nurse will insert venous 18 G catheter into antecubital vein.
d) Endothelial cell collection: PI, nurse or physician will perform J wire endothelial cell collection (3 wires) through the 18 G IV catheter (see 'Methods' for details)
e) After 20 min, research nurse will then obtain blood samples through catheter:
• Lipid panel, insulin/glucose, hs-CRP, basic metabolic panel (1 x 4.5 ml blood- light green PST tube)
• Catecholamines (1 x 10 ml Na+ Hep green top tubes)
Whole blood (2 x 10 ml citrate tubes) for platelet aggregation assay
• Extra blood collected for specialized labs performed in PIs lab:
1. Serum: 4 x 5 ml red top SST tube: for interleukin-6, tumor necrosis factor-alpha, ang II, aldosterone, and RAS and oxidative
stress proteins
2. Whole blood (17 ml blood- 3 x 8.5 ml CPT) for mononuclear cell DNA isolation and plasma
f) Pulse Wave Velocity (PWV) and Carotid Compliance (CC) (see 'Experimental Methods' for details).
g) Forearm Blood Flow: Subject will be instrumented with upper arm venous occlusion cuffs and wrist cuffs for measurements of forearm blood flow using venous occlusion plethysmography (see 'Methods' for details).
h) Brachial artery flow-mediated dilation (FMD) and 0.3 mg sublingual nitroglycerin with non-invasive ultrasonography (See 'Methods' for details)
i) Subject will be instrumented with 24 hour blood pressure monitor to wear home for 24 hours. Subject will return it to the investigators when they come in for Visit 7. There is no long term follow up
j) Subject will be given snack or lunch in CRU and is then free to leave.

Visit 7, week 4-5 (1.5 hours): Post-intervention- 4 weeks
a) Subject arrives at the CRU between 7-10 am after overnight 8 hour fast
b) Return 24-hour blood pressure monitor
c) Subject will undergo visual acuity, intraocular pressure test as well as a blood pressure measurement. The subject will then undergo ocular coherence tomography and laser-speckle blood flow imaging. As with microneurography (Visit 2), two E4 wristbands (one on each wrist) will be worn and a mental math test and a cold pressor test may also be performed during laser-speckle blood flow imaging.
d) At end of visit, subject will be given snack or lunch in CRU and is then free to leave.

Screening Methods:
a) BP, HR 12-lead ECG. Subject will undergo resting BP and HR 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) Blood chemistries: Standard blood chemistry analysis will be obtained under fasting conditions including a basic metabolic panel, lipid panel, TSH and FSH (for women only).
c) 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).
d) 24 hour ambulatory blood pressure monitoring will be used to help determine which study group subjects will be placed in (either control or randomization to study medication group) as well as to see if subjects currently on monotherapy or dual therapy for hypertension have controlled blood pressures.

Experimental Methods (Visits 2 and 6):

Microneurography:
Direct intra-neural recordings of multiunit MSNA will be obtained from the right leg peroneal nerve using the microneurography technique as previously described. Briefly, a tungsten micro electrode (200 μm diameter shaft; 1-5 μm uninsulated tip) will be inserted into the peroneal nerve posterior to the head of the fibula by a physician or research staff trained in microneurography. Well-validated criteria are used to determine that a neurogram represents sympathetic activity to muscle or skin. The technique has been used safely in over 2000 studies since 1984 and it is well tolerated and reproducible. MSNA will be recorded for 30 minutes while subject is supine and quantified as burst frequency (bursts/minute) and bursts incidence (bursts/100 heartbeats). During MSNA recordings a 3-lead ECG, beat-by-beat blood pressure by finger plethysmography, and respiration will be monitored with a pneumobelt. Blood flow to the arm (brachial artery) will be measured using Doppler ultrasound to non-invasively measure mean arterial blood velocity and diameter. Two E4 wristbands (one on each wrist) will be worn by the participant to non-invasively measure sympathetic activity (electrodermal response) and beat-to-beat heart rate variability continuously. Also during the procedure subjects will undergo a mental math test and a cold pressor test. In the mental

math test, the subject will be asked to subtract continuously the number 7 (or another random number) from a 3-digit number as quickly and as accurately as possible for 3 minutes. In the cold pressor test, the subject will be asked to place their hand in ice water for 2 minutes. This procedure will be used to cause transient changes in heart rate and blood pressure.

Pulse Wave Velocity (PWV):
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 (*CITE 1, 2). 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 (i.e. bifurcation site of aortic arch and brachiocephalic artery) (13, 14). The intra-subject reproducibility of carotid-femoral PWV is excellent with a coefficient variation of 2.1% for triplicate measurements on non-consecutive days in 7 young adults (15).

Carotid Artery Compliance (CC):
Carotid artery compliance and Beta-stiffness index will be determined noninvasively by high-resolution ultrasonography (Logiq 7, GE Healthcare) of the right common carotid artery and contralateral assessment of carotid artery blood pressure via non-invasive carotid artery applanation tonometry respectively. Carotid artery diameters are measured ~2 cm proximal to the carotid bulb with the transducer placed at a 90° angle to the vessel by off-line analysis of DICOM images with image analysis software (Medical Imaging Applications, LLC). Maximal diameters (i.e. systolic expansion) and minimal diameters (i.e. diastolic relaxation) are measured in sync with carotid artery blood pressure waveforms. Carotid blood pressure waveforms are calibrated using diastolic and mean brachial artery blood pressure obtained from standard brachial artery cuff blood pressure.

Venous Occlusion Plethysmography (VOP):
Venous occlusion plethysmography (VOP) will be used to measure forearm blood flow (FBF) responses to local ischemia to test endothelium-dependent and independent dilation of forearm resistance arteries. Briefly, subjects lie supine and have blood pressure cuffs (venous occlusion) placed around upper arms and pediatric blood pressure cuffs around wrists. FBF 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 (which temporarily prevents venous outflow and measures arterial inflow into forearm) and continuous wrist inflation of a blood pressure cuff to 250 mmHg. VOP is a well-established and validated technique for measuring FBF response to ischemia in human subjects.

Brachial artery endothelium-dependent dilation (EDD) and endothelium-independent dilation (EID):
EDD will be determined non-invasively by measuring brachial artery flow-mediated dilation (FMD) and dilation to 0.3 mg sublingual nitroglycerin using a 8-14 MHz linear transducer and ultrasound (Logiq 7, GE Healthcare). Briefly, 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. 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 6.0, 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. However since blood viscosity will not be available, shear rate will be used and is a reasonable estimate of shear stress. 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 ~10 years of experience performing the brachial artery FMD technique in human subjects. Our within-subject reproducibility of flow-mediated dilation is excellent with a coefficient of variation of 7.3%, for duplicate measurements 8 weeks apart.

Primary Endothelial Cell Protein Expression:
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 ACE (Abcam), AT1 receptor (Abcam), ang II (Novus), aldo (Pierce), nitrotyrosine (Abcam), NADPH oxidase p47phox (Abcam); nox4, nox 2 (Abcam). Briefly, under sterile conditions a CRU nurse will insert an 18G catheter into an antecubital vein. The PI with nurse assisting will insert a 0.018- or 0.021-inch mesh St. Jude 3 mm flexible guide J wire (Daig Corp., Minnetonka, MN) 3-4 cm into and retracted 2-3 times through the catheter. The distal portion of the wire is clipped off and then transferred to a 50-ml conical tube containing a buffer solution. Cells are then 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 20 individual endothelial cell images are digitally captured by a digital camera (Weiss). 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 Image J(NIH, Bethesday, MD) 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 one control cultured human aortic endothelial cells (HAEC: passage 3-6 processed identically to the sample cells) slides are selected for each staining batch. Values are reported as a ratio of sample endothelial cells to HAEC average pixel fluorescence intensity to reduce variability between staining batches.

Circulating RAS, oxidative stress and standard chemistries:
Plasma renin activity (DiaSorin), angiotensin II (ALPCO) and aldosterone (DiaSorin) will be measured via radioimmunoassay by the ICTS core lab. ACE activity will be measured by colormetric assay; oxidized LDL, a marker of lipoprotein oxidation65 will be measured by ELISA (ALPCO) by PIs lab. Standard blood chemistries will be determined by the hospital Pathology lab.

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 2 and 5 during microneurography using via beat-to-to beat finger blood pressure (Nexfin, Edward Life Sciences, Inc.) and calculated using the sequence technique.

Experimental Methods for Visits (3 and 7):

Retinal Vascular Measurements:
1. Visual acuity will be measured by having the subject read the smallest letters on an eye chart with their glasses or best correction. This takes about 5 minutes or less.
2. Intraocular pressure (IOP) may be done using a tonopen. A drop of a topical anesthetic will be placed in the eye as a numbing agent before IOP is checked. The tonopen touches the surface of the cornea of the eye very briefly. This will take a few minutes.
3. Blood pressure will be checked using a portable blood pressure cuff. We will have the subjects sit quietly for a few minutes. This will take a few minutes.
4. Ocular Coherence Tomography (OCT): The thickness of the optic nerve and macula will also be measured inside of the eye using a special camera that forms an image of the layers of the retina The imaging is harmless and measures the thickness or structural health of retinal layers and optic nerve. This image will be compared to the LSFG for blood vessel comparison and identification. This test takes approximately 10 minutes.
5. Laser-Speckle Blood Flow Imaging (LSFG) with varying light stimuli. This device uses a Class I laser

diode. Most subjects do not need dilation for this test, but those with smaller pupils may be dilated. Subjects will be seated at the instrument and we will adjust the chinrest and then let the subject rest with the lights dimmed for about 5 minutes before doing the test. Next, they will place their chin in the chinrest and look at a fixation target. This baseline test will take about 5-10 minutes. This test will be repeated following a light stimulus given to one eye using a handheld calibrated instrument in order to measure the blood flow response to activation of the retina by light. This will take another 5-10 minutes. This will be repeated several times followed by LSFG with no light stimulus at the end of testing. Two E4 wristbands (one on each wrist) will be worn by the participant to non-invasively measure sympathetic activity (electrodermal response) and beat-to-beat heart rate variability continuously.

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***

The total possible compensation for the study is $305. For subjects that are not washing out of antihypertensive medications and thus do not complete Washout Visits 1 and 2, the total compensation for the study is $275. For control subjects, total compensation is $135. Subjects will receive separate reimbursement for parking expenses but not for gas. If subject does not complete all study visits because they are withdrawn from the study, or are withdrawn by the investigators, they will be compensated for the visits completed as follows:

Visit 1: $30*
Washout Visit 1 (If applicable): $15*
Washout Visit 2(If applicable): $15*
Visit 2: $75
- Visit 3: $30
Visit 4: $20**
Visit 5: $20**
Visit 6: $75
Visit 7: $25

*If washout visits occur, payments will be as follows (Prewashout-$15, Washout 1-$15, Washout 2-$15-30)
**Additional "Unplanned safety visits" may be scheduled at the discretion of Dr. Jalal or other advising investigator (to monitor potassium levels, etc). Compensation for these visits will coincide with that of normal safety visits ($20) unless significantly longer in duration (will be compensated $20/hour rate after the first half hour if this occurs).
All payments will be in the form of a check that will be sent to the subject after each study visit.

VIII. Risks

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

- legal or social
- physical?

1. Clonidine (Catapres): Most systemic adverse effects during clonidine have been mild and have tended to diminish with continued therapy (such as loss of appetite). In a 3-month multi-clinic trial of clonidibe in 101 hypertensive patients, the systemic adverse reactions were, dry mouth (25 patients) and drowsiness (12 patients), fatigue (6 patients), headache (5 patients), lethargy and sedation (3 patients each), insomnia, dizziness, impotence/sexual dysfunction, dry throat (2 each) and constipation, nausea, change in taste and nervousness (1 each).

2. Hydrochlorothiazide (HCTZ): The most common signs and symptoms observed are those caused by electrolyte depletion (hypokalemia, hypochloremia, hyponatremia) and dehydration resulting from excessive diuresis. If digitalis has also been administered, hypokalemia may accentuate cardiac arrhythmias. In the event of overdosage, symptomatic and supportive measures should be employed. Emesis should be induced or gastric lavage performed. Correct dehydration, electrolyte imbalance, hepatic coma and hypotension by established procedures. If required, give oxygen or artificial respiration for respiratory impairment. The degree to which hydrochlorothiazide is removed by hemodialysis has not been established. The oral LD50 of hydrochlorothiazide is greater than 10 g/kg in the mouse and rat.P ossible adverse effects include weakness, hypotension (including orthostatic hypotension), pancreatitis, jaundice, diarrhea, vomiting, loss of appetite, blood dyscrasias, rash, photosensitivity, electrolyte imbalance, impotence, renal dysfunction/failure, interstitial nephritis.

3. Aliskiren (Tekturna): Possible side effects are angioedema, diarrhea, cough, hypotension, hyperkalemia, increased serum creatinine. Other adverse effects with increased rates for Tekturna compared to placebo included rash (1% vs. 0.3%), elevated uric acid (0.4% vs. 0.1%), gout (0.2% vs. 0.1%) and renal stones (0.2% vs. 0%). Limited data are available related to overdosage in humans. The most likely manifestation of overdosage would be hypotension. If symptomatic hypotension occurs, supportive treatment should be initiated. Aliskiren is poorly dialyzed. Therefore, hemodialysis is not adequate to treat aliskiren overexposure. There is a teratognic risk for a fetus exposed to aliskiren in the second and third trimester of pregnancy.

4. Brachial artery FMD test drugs.
a) Sublingual 0.3mg (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.

5. Antihypertensive Medication Withdrawal: There is an increase in cardiovascular diseases such as strokes, heart failure, aortic aneurysms, and pulmonary embolism when these medications are stopped for longer periods of time than in this study, therefore the risk of these events as a result of discontinuation of antihypertensive medications in the current study is low. We do encourage subjects to discuss holding medication dosages with their personal physician before doing so.

6. Retinal Vascular Measurements: Since the subjects may be dilated with 0.5% tropicamide and there can be a risk in patients with angle closure glaucoma if they have not had a procedure to correct this. Patients at the UIHC eye clinic usually have a corrective procedure done as a preventative measure. If there is a question about angle closure glaucoma, we will do a slit lamp exam to rule this out before proceeding. Dilating drops may also cause an initial burning or stinging sensation that goes away after installation. There is a small risk of sensitivity or an allergic reaction that would cause redness or irritation. The dilating drops will temporarily affect close vision (reading distance) for 2 to 6 hours,if they do not normally use a reading correction. Some people may be uncomfortable driving during this time. Also, the sun and bright lights may cause some discomfort, but sun shades are provided if desired. Occasional temporary stinging, burning and conjunctival redness may occur with the use of 0.5% proparacaine.

7. 24-hour Ambulatory Blood Pressure: Participants may experience abrasions, petechiae, or bruising from the pressure exerted when the cuff inflates, particularly if s/he is taking anticoagulants. Cuff inflation may cause mild discomfort and/or may be disruptive to sleep.

8. Psychological risks: The study only enrolls healthy subjects without psychiatric diagnoses and there are no foreseeable psychological risks with this study. Some of the questionnaires pertaining to mood will be scored. If the research team comes to believe that the subject is at significant risk for harming himself/herself or others based on responses to these questionnaires, Dr. Jalal or Dr. Fiedorowicz (Department of Psychiatry) would be notified leading to a loss of confidentiality. Additionally, if it appears

that clinical treatment (i.e. for depression) is possibly needed, we may suggest appropriate referrals. If Dr. Jalal or Dr. Fiedorowicz cannot be contacted, we will contact the on-call psychiatry resident or arrange for an evaluation in the UIHC Emergency Room or Adult Psychiatry Clinic.

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

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

11. 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 foreseeable 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 Minimizing Risks:

1) Reporting of side effects: During the study if the subject experiences any expected side effects such as angioedema, diarrhea, cough, hypotension, rash 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 UIHC access line and have Dr. Pierce paged during business hours. If after business hours, weekends, or holiday, the subject will be instructed to also call in the UIHC access number and ask for Dr. Jalal to be paged. Dr. Jalal (although she will be blinded) will talk to the subject about their concerns. If side effects/symptoms are life threatening, the subject will be instructed to call 911. Dr. Jalal will notify Dr. Pierce. If not life threatening, Dr. Jalal will instruct the appropriate medical course of action to the subject. If Dr. Jalal is out of town or unavailable then Dr. Jess Fiedorowicz, MD will serve as a back-up and be available to consult. Dr. Jalal or Fiedorowicz will instruct Dr. Pierce on to any course of action which could be continue drug, discontinue drug, reduce dose or to come in to the CRU for consult and appropriate medical course of action will be taken.

2) Scheduled safety visits: Subjects will come in at 1 week (Visit 4- approximately Day 7) and 2 weeks (Visit 5- approximately Day 14 depending on scheduling) and have blood pressure, heart rate assessed and research staff will review their side effect log (see attached survey) and survey for any symptoms/side effects. Subjects will have a venous blood draw for measurement of serum electrolytes. If serum potassium is 3.5 or lower, Dr. Jalal will prescribe 10 mg/day of KCl supplement for the subject. PI will pay this cost. Additional, "unplanned" safety checks may be scheduled at the discretion of Dr. Jalal or other advising investigator to monitor blood levels (ie, potassium), etc. Additionally, if the subject was required to washout of antihypertensive medication(s), they will come in at 4 days (Washout Visit 1) and 7 days (Washout Visit 2) after beginning the washout period and have blood pressure and heart rate assessed and research staff will review their side effect log for any symptoms/side effects.

3) Reporting of side effects due to washing out of antihypertensive medication(s): During the study if the subject experiences any expected side effects associated with going off antihypertensive medication (such as headache, dizziness, blurry vision) 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 UIHC access line and have Dr. Pierce paged during business hours. If after business hours, weekends, or holiday, the subject will be instructed to also call in the UIHC access number and ask for Dr. Jalal to be paged. Dr. Jalal will talk to the subject about their concerns. If side effects/symptoms are life threatening, the subject will be instructed to call 911. Dr. Jalal will notify Dr. Pierce. If not life threatening, Dr. Jalal will instruct the appropriate medical course of action to the subject. If Dr. Jalal is out of town or unavailable then Dr. Jess Fiedorowicz, MD will serve as a back-up and be available to consult. Dr. Jalal or Fiedorowicz will instruct Dr. Pierce on to any course of action which could be continue their washout/study participation, discontinue their

washout/study participation, or to come in to the CRU for consult and appropriate medical course of action will be taken.

4) Retinal Vascular Measurements: Patients at risk for angle closure glaucoma who are seen at the UIHC eye clinic routinely have a corrective procedure done to prevent episodes of high intraocular pressure (high IOP) that can result when dilated. If there is a question about angle closure glaucoma, we will do a slit lamp exam to rule this out before proceeding. We will tell subjects before we put the drops in that they may cause a stinging or burning sensation that will go away. We will ask subjects if they have had a reaction to dilating drops in the past. Side effects of the tropicamide drops resolve after discontinuation of the drops. We will ask subjects if they have had any prior reaction to proparacaine. The burning or redness usually subside in a few minutes. All eye drops used in the study are routine eye drops used in the eye clinic at UIHCS. We will provide subjects with disposable sunglasses to add additional comfort from the light.

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 safety monitoring plan will consist of a bi-annual independent review of the protocol by Dr. Mark Santillan, Assistant Professor, Department of OB/GYN. The PI will provide a bi-annual report to the Dr. Santillan 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 blood pressure and side effects from safety visits, data on reasons for any dosing changes that occurred

- 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. Santillan 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:***
Mark Santillan, MD, Department of OB/GYN, Univ of Iowa

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 the subject may experience lowering of 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 commonly used antihypertensives and diuretics used to treat hypertension will be effective for improving cardiovascular function (via decreased blood pressure, decreased mSNA and improved endothelial function) in obese adults with hypertension. This could have favorable clinical implications for adults in possibly reducing risk of cardiovascular diseases such as 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. All 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):***

- 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 518 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 Job Classification - Assistant Professor
- 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 Job Classification - IT Support Services II
- 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 PI's 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 PI's office 412 FH. The office is locked when the PI is not in the office. Signed informed consent documents will be kept in a separate folder in a different locked file cabinet in the office.

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

No

X.6 ***Describe***

Any data in the form of a progress report sent to the American Heart Association funding agency will be summary (mean) data and not include any personal identifiable information of study subjects.

Deidentified blood samples will be sent to Frederique Yiannikouris, PhD at the University of Kentucky to measure plasma (pro)renin receptor. This is a very specialized assay we cannot do here at UIHC. The samples will not include any personal identifiable information of the study subjects.

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

No

XI. Data Analysis

XI.2 ***Provide the rationale or power analysis to support the number of subjects proposed to complete this study.***

Sample sizes were calculated based on 80% power at an alpha level of 0.05 assuming a correlation of r=0.5 between baseline and post-intervention outcomes. For Specific Aim #1, a sample size of 19 subjects was determined from the outcome (i.e., MSNA) with the smallest effect size (0.77) base on two studies of 8 weeks of clonidine that demonstrated a mean reduction in MSNA of 11 and 14 bursts/min, and a 3rd study of 6 days of clonidine that reduced MSNA 14 bursts/min. There have been no studies on the effects of clonidine on brachial artery FMD, but a recent study demonstrated a 1.1 mean change in brachial FMD(effect size=0.73) of moxonidine (similar central sympatholytic).

For Specific Aim #2, a sample size of 5, 11 and 11 were determined from effect sizes of 2.02, 1.14 and 1.14 for studies on the effects of aliskiren therapy on plasma renin activity (-0.7 ng/ml/hr), brachial artery flow-mediated dilation (+3.7%).

For Specific Aim #3, there are no studies on the effects of clonidine or aliskiren on EID. Therefore, based on preliminary data, we estimated that a change of +5 (%chngFMD) in brachial artery EID in overweight/obese hypertensive adults would be required for EID to approach EID of obese normotensive adults.

Therefore, based on a sample size of 19 for Aim 1 and a conservative estimated dropout rate of 20%, a total of 69 (n=23 per group) obese adults with untreated borderline hypertension (i.e., prehypertension) or hypertension (systolic blood pressure >/= 130 mmHg and <180 mmHg) will be enrolled and randomized in the first study(Years 1-2). For Aim 2, based on a sample size of 11 and a conservative drop out rate of 20%, a total of 42 (n=14 per group) overweight/obese adults with untreated borderline hypertension (i.e., prehypertension) or hypertension (systolic blood pressure >/= 130 mmHg and <180 mmHg) will be enrolled and randomized in the 2nd study(Years 3-4). We will also enroll 35 obese adults with 'normal/optimal' systolic blood pressure (systolic blood pressure <120 mmHg) who will undergo baseline testing only. This group will allow baseline comparisons between obese adults with and without hypertension. Therefore, our grand total sample size will be n=146.

Table 1: Sample Size Calculations

Outcomes	Diff in means	Effect size	Sample size	Source
Aim 1: after central SNS inhibition				
Change MSNA after 8 weeks clonidine (bursts/min)	-14.3	1.18	10	Grassi et al.35
Change MSNA after 8 weeks clonidine (bursts/min)	-11.0	0.77	19	Furlan et al.33
Change FMD after (moxonidine + diet) - diet alone (%chng)	+1.1	0.73	17	Topal et al.24
Aim 2: after renin inhibition				
Change Renin activity after 12 weeks aliskiren (ng/ml/hr)	-0.7	2.02	5	Virdis et al.47
Change Brachial FMD after 30 days aliskiren (%chng)	+3.7	1.14	11	Cherney et al. 50
Change FBFAch after 12 wks aliskiren (ml/100 ml FAV/min)	+4.0	1.14	11	Virdis et al.47
Aim 3: after SNS or renin inhibition				
Brachial artery dilation to sublingual nitroglycerin after clonidine or aliskiren (%chng)	-5.0	1.33	7	Preliminary data

We will add 20 additional healthy overweight controls with normal blood pressure (systolic BP <120 mmHg; and diastolic BP <90 mmHg) who will have baseline measurements (Visit 1, 2, 3 only) but will not be randomized. The reason is that when subjects are consented, they then have blood pressure assessed in Visit 1 to determine eligibility to be assigned to randomization (if systolic BP is >/= 130 mmHg or >/= 85 mmHg). If BP is normal they are assigned to non-randomized control group. Thus, multiple subjects end up in the control group for every one subject who have high BP and randomized. Therefore in order to meet our enrollment goals for randomized subjects we need to increase the number of control subjects.

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 Safety 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 safety monitoring report. Data, tissue or samples will be stored only for members of the PIs research team and no other researchers.