Project Title: Anxiety-mediated impairments in large elastic artery function and the autonomic nervous system

NCT 03109795

February 13, 2020

ATLAS

PI: Seth Holwerda
IRB ID #: 201701762

Project Details

I. Project Introduction

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

I.2 ***Project Title:***
Anxiety-mediated impairments in large elastic artery function and the autonomic nervous system

I.3 ***Short Title (optional):***
ATLAS

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

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

The goal of this study is to evaluate the effectiveness of a short-term (4 weeks) pharmacological blockade of sympathetic nerve activity (clonidine) on anxiety symptoms, vascular function, inflammation, muscle sympathetic nerve activity, and oxidant stress in individuals with moderate-to-high anxiety. Individuals who are interested in the study will be identified by an online screening survey and will be contacted by the research team; advertisements and mass emails will direct individuals to the online screening survey. Those deemed eligible to participate will be randomized to either the clonidine intervention or hydrochlorothiazide as a blood-pressure lowering control condition. If eligible participants are currently being treated with blood pressure-lowering medications, they will be asked to go off these medications for 2 weeks prior to and during the course of the study. During the 2 week washout of blood pressure-lowering medications, participants will have safety visits (2 additional visits) that include measurements of blood pressure at 4 days and 7 days after the beginning of the washout period before the intervention. Assessments of anxiety symptoms via various surveys, vascular function (via non-invasive, well-established techniques), retinal vascular measurements, inflammation, muscle sympathetic nerve activity, and oxidant stress will be performed at baseline and at the post intervention session. Similar baseline measurements will be performed in control subjects with low or no anxiety for comparison, but these individuals will not undergo the intervention.

Participants with moderate-to-high anxiety will have a total of 6 visits to the laboratory, which includes the screening and consent (visit 1). Visit 2 (baseline measurements) and visit 6 (post-intervention measurements) will be more extensive (~4.5 hours) compared to the other visits (~30 min). Participants completing the washout will have an additional 2 visits (~30 min each) before "visit 2." Control subjects with low or no anxiety will only participate in visit 1 (screening and consent) and visit 2 (baseline measurements).

I.5 ***Specify your research question(s), study aims or hypotheses (do not indicate "see protocol")***
The purpose of this study is to 1) determine the extent to which measures of vascular and autonomic function (large elastic artery stiffness, retinal vascular measurements, vascular inflammation and baroreflex function) is impaired in subjects with moderate-to-high anxiety, and 2) test the magnitude by which short-term (4 weeks) sympathetic nerve activity blockade (clonidine) improves large elastic artery stiffness, vascular inflammation and baroreflex function in subjects with moderate-to-high levels of anxiety.

I.6 ***Background and significance and/or Preliminary studies related to this project.***
(do not indicate "see protocol")
Anxiety disorders are the most common mental health problems in the United States, occurring in about 18% of adults per year, and a lifetime prevalence of approximately 28% (25). Importantly, anxiety disorders are associated with increased risk for sudden cardiac death and non-fatal myocardial infarction (27, 46) independent of other mood disorders (13, 15). However, establishing a clear consensus on the mechanism(s) by which chronic anxiety confers cardiovascular disease (CVD) risk has proven difficult. Previous studies examining the potential role of vascular dysfunction in subjects with anxiety have been confounded by co-morbidities (e.g., hypertension, smoking, obesity) (31, 38) and added psychiatric disorders (63). Additionally, studies focusing on the relation between anxiety and robust predictors of CVD mortality, such as large elastic artery (e.g., aortic, carotid) stiffness, have been lacking.

Anxiety is experienced as negative feelings of threat, restlessness, tension and irritability, and somatic symptoms, such as palpitations, sweating, trembling, and dry mouth (57). Patients with clinically diagnosed anxiety disorder demonstrate more than 2-fold increase in future CVD events (23). Despite the strong association between anxiety and CVD risk, there is currently a gap in knowledge describing potential mechanisms by which anxiety leads to CVD. Evidence suggests that chronically high levels of anxiety may be associated with the progression of subclinical atherosclerosis such as carotid artery intima-media thickness (38) and elevated inflammation (5, 44). Symptoms of anxiety may also lead to impairment in resistance vessel dilator function (53). However, few studies have examined large elastic artery stiffness in subjects with high levels of anxiety. This is clinically important because large elastic artery stiffness (i.e., carotid and aortic) is a robust independent risk factor for CVD events such as stroke and myocardial infarction (6, 19, 59, 61). Interestingly, greater large elastic artery stiffness (aortic) is observed with higher resting muscle sympathetic nerve activity (MSNA) in healthy individuals even after adjusting for BP (9, 55). In this regard, numerous studies have shown that high MSNA independent of any increase in blood pressure can have deleterious vascular (7, 17, 32), metabolic (2, 20), cardiac (50, 52), and renal effects (1, 14, 54). Higher tonic MSNA is an independent determinant of aortic artery stiffness as assessed by the gold standard carotid-femoral pulse wave velocity (PWV) in healthy humans (55). Even acute increases in MSNA, such as during mental stress which is a potent stimulus for increases in MSNA (3), can lead to transiently greater large elastic artery stiffness (40). Furthermore, in healthy humans, acute mental stress induces transient endothelial dysfunction (16), an important modulator of arterial stiffness. Given findings from previous studies (22) showing that anxiety symptoms are associated with

indices of elevated sympathetic nerve activity (e.g., elevated circulating norepinephrine), lead us to our overall hypothesis that anxiety-induced sympathetic overactivity leads to increased large elastic artery (carotid and aortic) stiffness in subjects with chronic anxiety.

In healthy subjects, chronic anxiety has been associated with increased risk of cardiac events (13, 15, 23). Interestingly, evidence suggests baroreflex function is reduced in subjects with anxiety (48, 60), thus adding additional cardiac risk burden to this population. Consistent with this, impairment in cardiac baroreflex sensivity (BRS) is a significant predictor of cardiac arrhythmias and myocardial infarction mortality (29, 56). Moreover, reduced cardiac BRS is a sensitive predictor of mortality after myocardial infarction (28, 30), particularly in subjects with anxiety (47). Reduced baroreflex activation can be attributed, in part, to reduced distensibility of baroreceptor regions within the elastic carotid and aortic arteries as a result of increased arterial stiffness (8). Given the associated risk of cardiac BRS impairment with anxiety, and the increase in large elastic artery stiffness in this population, there is a critical need to examine whether reductions in SNA and large elastic artery stiffness ameliorates cardiac BRS impairment in subjects with anxiety, thus providing experimental support for the novel idea that anxiety leads to increased CVD risk at least in part through elevated large artery stiffness.

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

1. Amann K, Rump LC, Simonaviciene A et al. Effects of low dose sympathetic inhibition on glomerulosclerosis and albuminuria in subtotally nephrectomized rats. J Am Soc Nephrol. 2000;11(8):1469-78.
2. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. J Clin Invest. 1991;87(6):2246-52.
3. Anderson EA, Wallin BG, Mark AL. Dissociation of sympathetic nerve activity in arm and leg muscle during mental stress. Hypertension. 1987;9(6 Pt 2):III114-9.
4. Arch JJ, Wolitzky-Taylor KB, Eifert GH, Craske MG. Longitudinal treatment mediation of traditional cognitive behavioral therapy and acceptance and commitment therapy for anxiety disorders. Behav Res Ther. 2012;50(7-8):469-78.
5. Bankier B, Barajas J, Martinez-Rumayor A, Januzzi JL. Association between C-reactive protein and generalized anxiety disorder in stable coronary heart disease patients. Eur Heart J. 2008;29(18):2212-7.
6. Ben-Shlomo Y, Spears M, Boustred C et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. J Am Coll Cardiol. 2014;63(7):636-46.
7. Bevan RD. Trophic effects of peripheral adrenergic nerves on vascular structure. Hypertension. 1984;6(6 Pt 2):III19-26.
8. Bruno RM, Ghiadoni L, Seravalle G, Dell'oro R, Taddei S, Grassi G. Sympathetic regulation of vascular function in health and disease. Front Physiol. 2012;3:284.
9. Casey DP, Curry TB, Joyner MJ, Charkoudian N, Hart EC. Relationship between muscle sympathetic nerve activity and aortic wave reflection characteristics in young men and women. Hypertension. 2011;57(3):421-7.
10. Chapleau MW, Cunningham JT, Sullivan MJ, Wachtel RE, Abboud FM. Structural versus functional modulation of the arterial baroreflex. Hypertension. 1995;26(2):341-7.
11. Dindo L, Recober A, Marchman JN, Turvey C, O'Hara MW. One-day behavioral treatment for patients with comorbid depression and migraine: a pilot study. Behav Res Ther. 2012;50(9):537-43.
12. Fairfax ST, Padilla J, Vianna LC, Davis MJ, Fadel PJ. Spontaneous bursts of muscle sympathetic nerve activity decrease leg vascular conductance in resting humans. Am J Physiol Heart Circ Physiol. 2013;304(5):H759-66.
13. Fiedorowicz JG, He J, Merikangas KR. The association between mood and anxiety disorders with vascular diseases and risk factors in a nationally representative sample. J Psychosom Res. 2011;70(2):145-54.
14. Fisher JP, Young CN, Fadel PJ. Central sympathetic overactivity: maladies and mechanisms. Auton Neurosci. 2009;148(1-2):5-15.
15. Frasure-Smith N, Lesperance F. Depression and anxiety as predictors of 2-year cardiac events in patients with stable coronary artery disease. Arch Gen Psychiatry. 2008;65(1):62-71.
16. Ghiadoni L, Donald AE, Cropley M et al. Mental stress induces transient endothelial dysfunction in humans. Circulation. 2000;102(20):2473-8.
17. Grassi G, Giannattasio C, Failla M et al. Sympathetic modulation of radial artery compliance in congestive heart failure. Hypertension. 1995;26(2):348-54.
18. Grassi G, Turri C, Seravalle G, Bertinieri G, Pierini A, Mancia G. Effects of chronic clonidine administration on sympathetic nerve traffic and baroreflex function in heart failure. Hypertension. 2001;38(2):286-91.
19. Hansen TW, Staessen JA, Torp-Pedersen C et al. Ambulatory arterial stiffness index predicts stroke in a general population. J Hypertens. 2006;24(11):2247-53.
20. Hausberg M, Mark AL, Hoffman RP, Sinkey CA, Anderson EA. Dissociation of sympathoexcitatory and vasodilator actions of modestly elevated plasma insulin levels. J Hypertens. 1995;13(9):1015-21.
21. Holwerda SW, Reynolds LJ, Restaino RM et al. The influence of reduced insulin sensitivity via short-term reductions in physical activity on cardiac baroreflex sensitivity during acute hyperglycemia. J Appl Physiol (1985). 2015:jap 00584 2015.
22. Hughes JW, Watkins L, Blumenthal JA, Kuhn C, Sherwood A. Depression and anxiety symptoms are related to increased 24-hour urinary norepinephrine excretion among healthy middle-aged women. J Psychosom Res. 2004;57(4):353-8.
23. Janszky I, Ahnve S, Lundberg I, Hemmingsson T. Early-onset depression, anxiety, and risk of subsequent coronary heart disease: 37-year follow-up of 49,321 young Swedish men. J Am Coll Cardiol. 2010;56(1):31-7.
24. JG AT, Davis ML, Morina N, Powers MB, Smits JA, Emmelkamp PM. A meta-analysis of the efficacy of acceptance and commitment therapy for clinically relevant mental and physical health problems. Psychother Psychosom. 2015;84(1):30-6.
25. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 2005;62(6):593-602.
26. Knight RG, Waal-Manning HJ, Spears GF. Some norms and reliability data for the State--Trait Anxiety Inventory and the Zung Self-Rating Depression scale. Br J Clin Psychol. 1983;22 (Pt 4):245-9.
27. Kubzansky LD, Cole SR, Kawachi I, Vokonas P, Sparrow D. Shared and unique contributions of anger, anxiety, and depression to coronary heart disease: a prospective study in the normative aging study. Ann Behav Med. 2006;31(1):21-9.
28. La Rovere MT, Bigger JT, Jr., Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Lancet. 1998;351(9101):478-84.
29. La Rovere MT, Pinna GD, Maestri R, Sleight P. Clinical value of baroreflex sensitivity. Netherlands heart journal : monthly journal of the Netherlands Society of Cardiology and the Netherlands Heart Foundation. 2013;21(2):61-3.
30. La Rovere MT, Specchia G, Mortara A, Schwartz PJ. Baroreflex sensitivity, clinical correlates, and cardiovascular mortality among patients with a first myocardial infarction. A prospective study. Circulation. 1988;78(4):816-24.
31. Lambert E, Dawood T, Straznicky N et al. Association between the sympathetic firing pattern and anxiety level in patients with the metabolic syndrome and elevated blood pressure. J Hypertens. 2010;28(3):543-50.
32. Mancia G, Giannattasio C, Grassi G. Arterial distensibility in cardiovascular diseases. J Nephrol. 1998;11(6):284-8.
33. Muzi M, Goff DR, Kampine JP, Roerig DL, Ebert TJ. Clonidine reduces sympathetic activity but maintains baroreflex responses in normotensive humans. Anesthesiology. 1992;77(5):864-71.
34. Newsom SA, Richards JC, Johnson TK et al. Short-term sympathoadrenal inhibition augments the thermogenic response to beta-adrenergic receptor stimulation. J Endocrinol. 2010;206(3):307-15.
35. Padilla J, Young CN, Simmons GH et al. Increased muscle sympathetic nerve activity acutely alters conduit artery shear rate patterns. Am

J Physiol Heart Circ Physiol. 2010;298(4):H1128-35.
36. Parati G, Di Rienzo M, Bertinieri G et al. Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. Hypertension. 1988;12(2):214-22.
37. Parati G, Saul JP, Di Rienzo M, Mancia G. Spectral analysis of blood pressure and heart rate variability in evaluating cardiovascular regulation. A critical appraisal. Hypertension. 1995;25(6):1276-86.
38. Paterniti S, Zureik M, Ducimetiere P, Touboul PJ, Feve JM, Alperovitch A. Sustained anxiety and 4-year progression of carotid atherosclerosis. Arterioscler Thromb Vasc Biol. 2001;21(1):136-41.
39. Peltonen GL, Scalzo RL, Schweder MM et al. Sympathetic inhibition attenuates hypoxia induced insulin resistance in healthy adult humans. J Physiol. 2012;590(Pt 11):2801-9.
40. Phillips AA, Bredin SS, Cote AT, Drury CT, Warburton DE. Aortic distensibility is reduced during intense lower body negative pressure and is related to low frequency power of systolic blood pressure. Eur J Appl Physiol. 2013;113(3):785-92.
41. Pierce GL, Casey DP, Fiedorowicz JG et al. Aortic pulse wave velocity and reflecting distance estimation from peripheral waveforms in humans: detection of age- and exercise training-related differences. Am J Physiol Heart Circ Physiol. 2013;305(1):H135-42.
42. Pierce GL, Donato AJ, LaRocca TJ, Eskurza I, Silver AE, Seals DR. Habitually exercising older men do not demonstrate age-associated vascular endothelial oxidative stress. Aging Cell. 2011;10(6):1032-7.
43. Pierce GL, Lesniewski LA, Lawson BR, Beske SD, Seals DR. Nuclear factor-{kappa}B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. Circulation. 2009;119(9):1284-92.
44. Pitsavos C, Panagiotakos DB, Papageorgiou C, Tsetsekou E, Soldatos C, Stefanadis C. Anxiety in relation to inflammation and coagulation markers, among healthy adults: the ATTICA study. Atherosclerosis. 2006;185(2):320-6.
45. Powers MB, Zum Vorde Sive Vording MB, Emmelkamp PM. Acceptance and commitment therapy: a meta-analytic review. Psychother Psychosom. 2009;78(2):73-80.
46. Roest AM, Martens EJ, de Jonge P, Denollet J. Anxiety and risk of incident coronary heart disease: a meta-analysis. J Am Coll Cardiol. 2010;56(1):38-46.
47. Roest AM, Martens EJ, Denollet J, de Jonge P. Prognostic association of anxiety post myocardial infarction with mortality and new cardiac events: a meta-analysis. Psychosom Med. 2010;72(6):563-9.
48. Sanchez-Gonzalez MA, Guzik P, May RW et al. Trait anxiety mimics age-related cardiovascular autonomic modulation in young adults. J Hum Hypertens. 2015;29(4):274-80.
49. Saul JP, Rea RF, Eckberg DL, Berger RD, Cohen RJ. Heart rate and muscle sympathetic nerve variability during reflex changes of autonomic activity. Am J Physiol. 1990;258(3 Pt 2):H713-21.
50. Sen S, Young D. Effect of sodium deprivation on cardiac hypertrophy in spontaneously hypertensive rats: influence of aging. J Mol Cell Cardiol. 1991;23(6):695-704.
51. Silver AE, Christou DD, Donato AJ et al. Protein expression in vascular endothelial cells obtained from human peripheral arteries and veins. J Vasc Res. 2010;47(1):1-8.
52. Simpson P. Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an alpha 1 adrenergic response. J Clin Invest. 1983;72(2):732-8.
53. Stillman AN, Moser DJ, Fiedorowicz J, Robinson HM, Haynes WG. Association of anxiety with resistance vessel dysfunction in human atherosclerosis. Psychosom Med. 2013;75(6):537-44.
54. Strojek K, Grzeszczak W, Gorska J, Leschinger MI, Ritz E. Lowering of microalbuminuria in diabetic patients by a sympathicoplegic agent: novel approach to prevent progression of diabetic nephropathy? J Am Soc Nephrol. 2001;12(3):602-5.
55. Swierblewska E, Hering D, Kara T et al. An independent relationship between muscle sympathetic nerve activity and pulse wave velocity in normal humans. J Hypertens. 2010;28(5):979-84.
56. Thayer JF, Lane RD. The role of vagal function in the risk for cardiovascular disease and mortality. Biological psychology. 2007;74(2):224-42.
57. Tyrer P, Baldwin D. Generalised anxiety disorder. Lancet. 2006;368(9553):2156-66.
58. Vallbo AB, Hagbarth KE, Torebjork HE, Wallin BG. Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. Physiol Rev. 1979;59(4):919-57.
59. van Sloten TT, Sedaghat S, Laurent S et al. Carotid Stiffness Is Associated With Incident Stroke: A Systematic Review and Individual Participant Data Meta-Analysis. J Am Coll Cardiol. 2015;66(19):2116-25.
60. Victor RG, Leimbach WN, Jr. Effects of lower body negative pressure on sympathetic discharge to leg muscles in humans. J Appl Physiol (1985). 1987;63(6):2558-62.
61. Virtanen R, Jula A, Salminen JK et al. Anxiety and hostility are associated with reduced baroreflex sensitivity and increased beat-to-beat blood pressure variability. Psychosom Med. 2003;65(5):751-6.
62. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55(13):1318-27.
63. Westen D, Morrison K. A multidimensional meta-analysis of treatments for depression, panic, and generalized anxiety disorder: an empirical examination of the status of empirically supported therapies. J Consult Clin Psychol. 2001;69(6):875-99.
64. Yeragani VK, Tancer M, Seema KP, Josyula K, Desai N. Increased pulse-wave velocity in patients with anxiety: implications for autonomic dysfunction. J Psychosom Res. 2006;61(1):25-31.

II. Research Team

II.1 ***Principal Investigator***

Name	E-mail	College
Seth Holwerda	seth-holwerda@uiowa.edu	

II.2 ***Team Members***

UI Team Members

Name	E-mail	College	Contact	Key Prsn	UI COI	VAMC COI	Consent Process Involvement	Deactivated
Seth Holwerda, PHD	seth-holwerda@uiowa.edu		Yes	Yes	No		Yes	Yes
Michael Collins, High School	michael-t-collins@uiowa.edu		No	No	No		No	Yes
Lyndsey DuBose, MS	lyndsey-dubose@uiowa.edu		No	No	No		No	Yes
Nitsan Duvdevan-Strier, MD	nitsan-duvdevan-strier@uiowa.edu	Carver College of Medicine	No	No	No		No	Yes

Name	E-mail	College	Contact	Key Prsn	UI COI	VAMC COI	Consent Process Involvement	Deactivated
Jess Fiedorowicz, MD, PHD	jess-fiedorowicz@uiowa.edu	College of Public Health	No	Yes	No		Yes	No
Jan Full, BSN	jan-full@uiowa.edu	Carver College of Medicine	No	No	No		Yes	No
Ryuya Hashimoto, MD	ryuya-hashimoto@uiowa.edu	Carver College of Medicine	No	Yes	No		No	No
Talon Hoefer, BS	talon-hoefer@uiowa.edu	College of Liberal Arts and Sciences	No	No	No		No	Yes
Jared Hueser, High School	jared-hueser@uiowa.edu	University Hospitals	No	No	No		No	Yes
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		Yes	No
Rachel Luehrs, MS	rachel-luehrs@uiowa.edu	College Lib Arts and Sciences	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	College Lib Arts and Sciences	No	No	No		No	No
Gary Pierce, PHD, MS	gary-pierce@uiowa.edu	College Lib Arts and Sciences	No	Yes	No		Yes	No
Mark Santillan, MD	mark-santillan@uiowa.edu	Carver College of Medicine	No	Yes	No		No	No
Amy Marie Stroud, MSN	amy-stroud@uiowa.edu	Carver College of Medicine	No	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:***
Fellow or Research Scholar

II.3.a ***Select the mentor or faculty advisor:***
Gary Pierce

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
Seth Holwerda, PHD	Yes
Michael Collins, High School	No
Lyndsey DuBose, MS	No
Nitsan Duvdevan-Strier, MD	No
Jess Fiedorowicz, MD, PHD	Yes
Jan Full, BSN	No
Ryuya Hashimoto, MD	Yes
Talon Hoefer, BS	No
Jared Hueser, High School	No
Diana Jalal, MD	Yes
Randy Kardon, MD	Yes
Rachel Luehrs, MS	No
Julie Nellis, BSN	No
Virginia Nuckols, MS	No
Gary Pierce, PHD, MS	Yes
Mark Santillan, MD	Yes
Amy Marie Stroud, MSN	No

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

III. Funding/Other Support

III.1 ***Funding Sources***

Source Entered as Text	DSP Link	Type	Source	Pr
Source is entered as text no		Federal Agency	US Department of Health & Human Services, National Institutes of Health	Do cau vas dys

Source Entered as Text	DSP Link	Type	Source	Pr
Source is entered as text no		Private Foundation/Association	American Heart Association, Midwest Affiliate	thr inf and syr act An me im in ela fur the aut ner sys

* 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
Seth Holwerda, PHD	No
Michael Collins, High School	No
Lyndsey DuBose, MS	No
Nitsan Duvdevan-Strier, MD	No
Jess Fiedorowicz, MD, PHD	No
Jan Full, BSN	No
Ryuya Hashimoto, MD	No
Talon Hoefer, BS	No
Jared Hueser, High School	No
Diana Jalal, MD	No
Randy Kardon, MD	No
Rachel Luehrs, MS	No
Julie Nellis, BSN	No
Virginia Nuckols, MS	No
Gary Pierce, PHD, MS	No
Mark Santillan, MD	No
Amy Marie Stroud, MSN	No

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

Source	Status	Other Status Description
US Department of Health & Human Services, National Institutes of Health	Awarded	
American Heart Association, Midwest Affiliate	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")***
2/1/2017

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
Hydrochlorothiazide
Tropicamide
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***

Hydrochlorothiazide (Esidrix, Microzide, Oretic)

Name of Sponsor	
Investigator's Brochure Version	West-ward Pharmaceutica
Investigator's Brochure Date	Jan 2006
Planned Use in this Study	
Condition/Disease Indication(s)	Vascular function and blood flow
Subject Population	Anxiety, obesity and hypertension
Dose(s)	12.5 mg twice per day
Administration	Oral
Dosing Regimen	12.5 mg twice per day
FDA Approved Use	
Approved Condition/Disease Indication(s)	Hypertension
Approved Patient Population	Hypertension
Approved Dose(s)	25 mg/day up to 50 mg/day
Approved Administration	Oral
Approved Dosing Regimen	12.5 mg twice per 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 dose of hydrochlorothiazide is the standard FDA approved dose for hypertension in adults

Clonidine (Catapres)

Name of Sponsor	
Investigator's Brochure Version	Boehringer Ingelheim
Investigator's Brochure Date	Oct 2009
Planned Use in this Study	
Condition/Disease Indication(s)	Vascular function and blood flow
Subject Population	Anxiety and obesity
Dose(s)	0.1 mg (oral)
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 - 2.4 mg/day (oral)
Approved Administration	Oral
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 indicated (hypertension), therefore there is no increased risk.

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?***
72

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 36 participants ages 18-79 years (50% men, 50% women) with moderate-to-high anxiety to participate in the drug intervention aim of the study (clonidine or hydrochlorothiazide). The GAD7 questionnaire, which is completed by subjects during initial screening, will determine the presence of moderate-to-high anxiety (score lower than 5 = low anxiety; score 10 or higher = moderate-to-high anxiety).

Because obesity is linked with hypertension and type 2 diabetes, enrollment of subjects may include those with pre-hypertension or hypertension (systolic blood pressure >/= 120 <180 mmHg average of at least 3 measurements 2 min apart after 10 min seated resting position), and/or pre-diabetes (defined as fasting blood glucose between 100-126 mg/dl, fasting blood glucose of 140-199 mg/dl at 120 min of an oral glucose tolerance test, or HbA1C of 6-6.5%) or type 2 diabetes (defined as fasting blood glucose between >126 mg/dl, fasting blood glucose of >199 mg/dl at 120 min of an oral glucose tolerance test, or HbA1C > 6.5%). These subjects may be taking anti-hypertensive and/or diabetes (anti-hyperglycemic) medications. Subjects will be asked to refrain from medications the morning of the study visit, and to bring their medications with them to take immediately following their study visit. If currently taking anti-hypertensive medication(s), subjects will be asked to undergo a 2-week wash out of anti-hypertensive medications, but remain taking other medications such as anti-hyperglycemic medications.

Inclusion criteria:

Willing and able to provide written, signed consent after the nature of the study has been explained, and prior to any research-related procedures.

Age is > or = 18 and < or = 79 years

No history of cardiovascular disease (e.g., heart attack, stroke, heart failure, valvular heart disease, cardiomyopathy), 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 ventricular hypertrophy, left-bundle branch block, 2nd or 3rd degree AV block, atrial fibrillation/flutter, atherosclerosis).

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 stable thyroid medication with no dose change for 3 months.

Exclusion Criteria:

Current use of clonidine or beta-blockers

Current use of antihypertensive medications for reasons other than hypertension (e.g., hydrochlorothiazide for leg edema or kidney stone prevention, beta-blockers for tremor)

Difficult to control hypertension (e.g., on 2 or 3 antihypertensive medications)

Low blood pressure (e.g., systolic BP < 110 mmHg)

Hypertensive and have not been stable on their current antihypertensive medication regimen for at least 6 months

Blood pressure not controlled either on or off antihypertensive medications (e.g., BP > 150/100)

Current diagnosis or history of cancer, liver disease, HIV/AIDS

History of brain tumor, aneurysm or injury

Clinical diagnosis of mental health disorders such as bipolar disorder or schizophrenia

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, atherosclerosis.

Current tobacco user or history of tobacco use within the past 3 months (cigarettes, cigars, chewing tobacco, Hookah).

History of lung emphysema, chronic bronchitis or chronic obstructive pulmonary disease (COPD).

Abnormal resting 12-lead ECG (e.g., evidence of myocardial infarction, left ventricular hypertrophy, left-bundle branch block, 2nd or 3rd degree AV block, atrial fibrillation/flutter, atherosclerosis).

Serious neurologic disorders including seizures.

History of renal failure, dialysis or kidney transplant.

Use of any investigational products or investigational medical devices within 30 days prior to screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.

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.

History of rheumatoid arthritis, Grave's disease, systemic lupus erythamatosis, and Wegener's granulomatosis.

Taking anticoagulation, anti-seizure, or antipsychotic agents.

Start of or dose change to an antidepressant or anti-anxiety medication within the past 3 months (if no change in medication or dose in past 3 month, then subject will be eligible).

Immunodeficiency or systemic autoimmune disease.

History of bleeding disorders or conditions of the microcirculation (i.e. von Willebrand disease, Raynaud's disease).

History of co-morbid condition that would limit life expectancy to <1 year.

Taking chronic non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin, naproxen, acetaminophen (Tylenol®), ibuprofen (Advil®, Motrin®) and not able or willing to go off of for 2 weeks prior to each study visit.

Taking cox-2 inhibitors (Celebrex®, Vioxx®, etc) or allopurinol (Zyloprim®, Lopurin®, Aloprim®).

Taking steroids or biologics: corticosteroids (prednisone); methotrexate, infliximib (Remicade®), etaneracept (Enbrel®); anakinra (Kineret®).

Those who are currently donating blood, platelets, or plasma at the time of screening.

Vulnerable populations (prisoners, etc.) will not be eligible to participate in this study.

On weight loss drugs (i.e. orilistat (Xenical®), sibutramine (Meridia®), phenylpropanol-amine (Acutrim®)), 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

Any condition that, in the view of the PI or Co-I, places the subject at high risk or poor treatment and study compliance.

We will also enroll 36 participants ages 18-79 years (50% men, 50% women) with low or no anxiety as control subjects to participate in only baseline testing (not participate in the drug intervention). The control subjects will be matched to the drug intervention subjects by sex, age and BMI. These factors will be collected during screening (i.e, height, weight, BMI, age, sex).

Because obesity is linked with hypertension and type 2 diabetes, enrollment of subjects may include those with pre-hypertension or hypertension (systolic blood pressure >/= 120 <180 mmHg average of at least 3 measurements 2 min apart after 10 min seated resting position), and/or pre-diabetes (defined as fasting blood glucose between 100-126 mg/dl, fasting blood glucose of 140-199 mg/dl at 120 min of an oral glucose tolerance test, or HbA1C of 6-6.5%) or type 2 diabetes (defined as fasting blood glucose between >126 mg/dl, fasting blood glucose of >199 mg/dl at 120 min of an oral glucose tolerance test, or HbA1C > 6.5%). These subjects may be taking anti-hypertensive and/or diabetes (anti-hyperglycemic) medications. Subjects will be asked to refrain from medications the morning of the study visit, and to bring their medications with them to take immediately following their study visit.

Inclusion criteria:

Willing and able to provide written, signed consent after the nature of the study has been explained, and prior to any research-related procedures.

Age is > or = 18 and < or = 79 years

No history of cardiovascular disease (e.g., heart attack, stroke, heart failure, valvular heart disease, cardiomyopathy), 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 ventricular hypertrophy, left-bundle branch block, 2nd or 3rd degree AV block, atrial fibrillation/flutter, atherosclerosis).

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 stable thyroid medication with no dose change for 3 months.

Exclusion Criteria:

Current diagnosis or history of cancer, liver disease, HIV/AIDS

History of brain tumor, aneurysm or injury

Clinical diagnosis of mental health disorders such as bipolar disorder or schizophrenia

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, atherosclerosis.

Current tobacco user or history of tobacco use within the past 3 months (cigarettes, cigars, chewing tobacco, Hookah).

History of lung emphysema, chronic bronchitis or chronic obstructive pulmonary disease (COPD).

Abnormal resting 12-lead ECG (e.g., evidence of myocardial infarction, left ventricular hypertrophy, left-bundle branch block, 2nd or 3rd degree AV block, atrial fibrillation/flutter, atherosclerosis).

Serious neurologic disorders including seizures.

History of renal failure, dialysis or kidney transplant.

Use of any investigational products or investigational medical devices within 30 days prior to screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.

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.

History of rheumatoid arthritis, Grave's disease, systemic lupus erythamatosis, and Wegener's granulomatosis.

Taking anticoagulation, anti-seizure, or antipsychotic agents.

Start of or dose change to an antidepressant or anti-anxiety medication within the past 3 months (if no change in medication or dose in past 3 month, then subject will be eligible).

Immunodeficiency or systemic autoimmune disease.

History of bleeding disorders or conditions of the microcirculation (i.e. von Willebrand disease, Raynaud's disease).

History of co-morbid condition that would limit life expectancy to <1 year.

Taking chronic non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin, naproxen, acetaminophen (Tylenol®), ibuprofen (Advil®, Motrin®) and not able or willing to go off of for 2 weeks prior to each study visit.

Taking cox-2 inhibitors (Celebrex®, Vioxx®, etc) or allopurinol (Zyloprim®, Lopurin®, Aloprim®).

Taking steroids or biologics: corticosteroids (prednisone); methotrexate, infliximib (Remicade®), etaneracept (Enbrel®); anakinra (Kineret®).

Those who are currently donating blood, platelets, or plasma at the time of screening.

Vulnerable populations (prisoners, etc.) will not be eligible to participate in this study.

On weight loss drugs (i.e. orilistat (Xenical®), sibutramine (Meridia®), phenylpropanol-amine (Acutrim®)), 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

Any condition that, in the view of the PI or Co-I, places the subject at high risk or poor treatment and study compliance.

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 2013 U.S. Census indicates that there are 259,227 adults between the ages of25-64 in Johnson County and its 8 surrounding counties (Linn, Washington, Louisa, Benton, Iowa, Muscatine, Cedar, and Jones). Generalized anxiety disorder (GAD) has a prevalence of3.l% in the US adult population with 32.3% of these cases being classified as "severe" (12).
Therefore we have a sizable population to draw from.

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 lowa community, advertise in the Daily Iowan newspaper, the 'volunteer research' clinical trials website on UIHC website (http://www.uihealthcare.org/ClinicalTrials.aspx/) and in the 'Noon news' in UIHC.

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 about pregnant women?***
No

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

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

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

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

VII.A. Project Description (A)

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

- CRU
- Other UI campus site - 522 FH and 518 FH (laboratory)
- UIHC - C202; C202 BT

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

VII.B. Project Description (B)

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

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

VII.B.1.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.1.b ***Provide the NCT (National ClinicalTrials.gov Identifier) number***
NCT03109795

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 moderate-to-high anxiety subjects (intervention group) are currently taking blood pressure-lowering drugs, they may be in the study but will be asked, during the consenting process to go off of these drugs for 2 weeks before participating in experimental study visits. If subjects are unable or unwilling to go off these for 2 weeks prior to experimental study visits and/or 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 document and complete all screening measures at Visit 1 and experimental Visit 2 will be scheduled after the the 2 week washout is complete. If the subject is unclear if they can go off of their medications they will be required to contact their personal physician to confirm they can go off these compounds for 2 weeks and/or the study duration.

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

After the 2 week washout has been completed, subjects will be invited back to the CRU to complete experimental study Visit 2.

VII.B.5 ***Who (list names) will be available on a 24/7 basis for questions or emergencies during the washout period?***
Jess Fiedorowicz, MD, PhD; Gary Pierce, PhD

VII.B.6 ***Will any subjects receive a placebo in this study when, if they were not participating, they could be receiving an FDA-approved treatment for their condition?***
No

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

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

VII.C. Project Description (C)

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

VII.D. Project Description (D)

VII.D.1 ***Check all materials/methods that will be used in recruiting subjects (you will need to attach copies of all materials at the end of the application):***
- Website - We will list the study on the UIHC Clinical Research Website where volunteers are able to learn about different clinical trials being performed at the University (http://www.uihealthcare.org/Clinica1Research).
- E-mail -

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

VII.D.9 ***Describe the physical location where the consent process will take place:***
Research staff will discuss the study with the potential subject, answer questions and consent in an exam room 2112 BT in the ICTS Clinical Research Unit (CRU) or in the study lab C204.

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:***

After viewing an mass email, the potential subject will complete the online screening survey (either anxiety or control group, see attached: "ATLAS_ Online Pre-Screen Survey_3-3-17.pdf"; link: http://j.mp/2lpbGLW. If determined to be eligible from the online screening survey, he/she will be contacted by a research team member to complete a further health screening survey over the phone; see attached: "ATLAS_Pre-Consent Phone Screen_updated 3-3-17_anxiety.pdf" for anxiety subjects, and "ATLAS_Pre-Consent Phone Screen_updated 3-3-17_Controls.pdf" for control subjects. During the screening call, the research team member will answer any questions and describe the study to the participant as needed. If the participant continues to meet eligibility after screening, he/she will be told briefly about the study and will be told that he/she can review the consent in detail during an in-person meeting (Visit l).

Patients who complete an online screening survey will provide their address, phone number and email at the end of the screening. If they are eligible, a member of the research team will contact the patient by phone to complete further screening measures, discuss the study and answer any questions he/she may have. If the potential subject is still interested in participating, Visit 1 will be scheduled in which completion of the informed consent document will take place. Those who complete the online screening and are ineligible will be contacted by email to inform them that they are ineligible.

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

Name	Consent Process Involvement
Seth Holwerda, PHD	Yes
Michael Collins, High School	No
Lyndsey DuBose, MS	No
Nitsan Duvdevan-Strier, MD	No
Jess Fiedorowicz, MD, PHD	Yes
Jan Full, BSN	Yes
Ryuya Hashimoto, MD	No
Talon Hoefer, BS	No
Jared Hueser, High School	No
Diana Jalal, MD	No
Randy Kardon, MD	Yes
Rachel Luehrs, MS	No
Julie Nellis, BSN	Yes
Virginia Nuckols, MS	No
Gary Pierce, PHD, MS	Yes
Mark Santillan, MD	No
Amy Marie Stroud, MSN	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)?***
Yes

VII.D.17 ***Choose one of the following to indicate why you are requesting that the IRB waive the requirement to obtain a subject signature as documentation of consent:***
A. The research presents no more than minimal risk (minimal risk means the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests)

AND

The study involves no procedures for which consent is normally required ouside of a research context. *(This type of waiver is often permitted for a minimal risk mail-out survey that includes a cover letter with all elements of consent, and returning the survey indicates consent. You cannot request this waiver if the study also involves the use of any protected health information (PHI).)*

VII.D.18 ***Explain why this meets the chosen criteria in A. or B. above:***
The waiver of documentation of consent is only for the screening portion of the study. The probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests because the potential participants are only completing surveys and answering questions over the phone.

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

VII.D.20 ***List any screening questions you will directly ask the potential subject to determine eligibility.***
Below are questions listed on the online pre-screening survey that the potential participant will take if interested in the study that we advertise via email. Here is also a link to view the online pre-screening survey that is provided with the mass email for advertisement: http://j.mp/2lpbGLW

1. Where did you first hear about the study?
a. Poster
b. Brochure
c. Friend
d. Noon News
e. Ulowa Email
f. Other
2. Are you between the ages 18-79? Yes or No

3. With what gender do you identify?
a. Male
b. Female
c. Transgender
d. None of the above
4. Do you currently have or have a history of cardiovascular disease such as heart attack, stroke, congestive heart failure, heart angioplasty/stent or bypass surgery? Yes or No
5. Do you currently have or have a history of valvular heart disease, peripheral arterial disease or cardiomyopathy? Yes or No
6. Do you currently have or have a history of chronic obstructive pulmonary disease (COPD) such as lung emphysema or chronic bronchitis? Yes or No
7. Do you currently have or have a history of kidney or liver disease such as kidney failure, transplant or on dialysis? Yes or No
8. Do you currently have or have a history of serious neurologic disorders including seizures? Yes or No
9. Do you currently have or have a history of rheumatoid arthritis, Grave's disease, systemic lupus erythematosus, Wegener's granulomatosis or any other autoimmune disease? Yes or No
10. Have you ever been diagnosed with a serious medical illness such as cancer, brain tumor, aneurysm, injury or HIV/AIDS? Yes or No
11. Have you ever been clincally diagnosed with a psychiatric illness such as bipolar disorder or schizophrenia? Yes or No
12. Are you currently pregnant or breastfeeding, or plan to become pregnant within the next 12-14 weeks? Yes or No or Not Applicable
13. Have you recently donated whole blood, plasma, or platelets? Yes or No
14. Do you currently use any tobacco products (i.e. cigarettes, cigars, chewing tobacco, Hookah?? Yes or No
15. Have you used any tobacco-related products in the past? Yes or No If yes, when did you quit?
16. How often do you have a drink containing alcohol?
a. Never
b. Monthly or less
c. 2-4 times a month
d. 2-3 times a week
e. 4 or more times a week
17. How many standard drinks containing alcohol do you have on a typical day?
a. Never
b. 1 or2
c. 3 or4
d. 5 or6
e. 7 to 9
f. 10 or more
18. How often do you have six or more drinks on one occasion?
a. Never
b. Less than monthly
c. Monthly
d. Weekly
e. Daily or almost daily
19. Are you currently taking any medications? Yes or No If yes, please specify
20. Have you started any new medications or changed current medication dosage in the past 3 months? Yes or No If yes, please specify
21. Are you planning to start any new medications in the next 12-14 weeks? Yes or No If yes, please specify
22. Are you currently receiving any counseling or therapy? Yes or No If yes, please specify
23. Do you have any intentions to begin receiving counseling or therapy within the next 12-14 weeks? Yes or No If yes, please specify
24. Over the last 2 weeks, how often have you been bothered by any of the following problems?
- Little interest or pleasure in doing things
a. Not at all
b. Several days
c. Over half the days
d. Nearly every day
- Feeling down, depressed or hopeless
a. Not at all
b. Several days
c. Over half the days
d. Nearly every day
25. Over the last 2 weeks, how often have you been bothered by any of the following problems?
- Feeling nervous, anxious, or on edge
a. Not at all
b. Several days
c. Over half the days
d. Nearly every day
- Not being able to stop or control worrying
a. Not at all
b. Several days
c. Over half the days
d. Nearly every day
- Worrying too much about different things
a. Not at all
b. Several days
c. Over half the days
d. Nearly every day
- Trouble relaxing
a. Not at all
b. Several days
c. Over half the days
d. Nearly every day
- Being so restless that it's hard to sit still
a. Not at all
b. Several days

c. Over half the days
d. Nearly every day
- Becoming easily annoyed or irritable
a. Not at all
b. Several days
c. Over half the days
d. Nearly every day
- Feeling afraid as if something awful might happen
a. Not at all
b. Several days
c. Over half the days
d. Nearly every day
26. Weight (indicate in pounds)
27. Height (indicate in inches)
28. BMI

Below are questions that are asked on the pre-consent phone screening form that is given after the potential participant takes the online pre-screening survey questions listed above. The following questions will be asked via phone, after potential subjects have passed the online pre-screening survey and are contacted by the research team. Some questions are duplicates to the aforementioned online questionnaire and will be asked again to confirm the absence of major exclusion criteria.

1. Are you currently participating in another clinical research study at the University oflowa or elsewhere? Yes or No
- If yes, what study?
2. Are you on any prescription medications? Include frequency, dosage and reason for taking.
3. Are you on any over-the-counter medications, supplements, vitamins, minerals, etc.? Include frequency, dosage and reason for taking.
4. Have any of the medications listed above changed in dose or frequency within the past 3 months? Indicate Yes or No. If yes, please describe:
5. Do you have an allergy to latex or any other medications? Indicate Yes or No
6. Are you currently seeking or have intentions to start psychotherapy for anxiety and/or depression? Indicate Yes of No. If yes, please describe:
7. Do you have or have a history of any of the following conditions or diseases? Indicate Yes or No
Cancer?
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?
Brain tumor, aneurysm or injury?
Kidney disease or kidney failure?
Thyroid disease/disorder?
Liver disease?
HIV/AIDS?
Clincal diagnosis of mental health disorders such as bipolar disorder or schizophrenia?
Currently using tobacco related products in the past 3 months or less (Cigarettes, cigars, chewing tobacco, Hookah)
Quit using tobacco related products in the past 3 months or less?

8. Do you currently have or have a history of any of the following conditions or diseases? Indicate Yes or No
Heart attack?
Angina (chest discomfort/pain/pressure upon physical exertion)?
Congestive heart failure?
Cardiomyopathy?
Heart angioplasty, stent or bypass surgery?Heart valve surgery/replacement or valve disease (i.e. aortic stenosis, mitral stenosis, or regurgitation)?
Pacemaker or implantable defibrillator?
Peripheral artery or vascular disease in legs?
Atrial fibrillation or flutter?
9. Do you currently have or have a history of any of the following conditions or diseases? Indicate Yes or No
Grave's Disease or granulomatosis?
Fibromyalgia or lupus?
Lung emphysema or chronic bronchitis?
Rheumatoid arthritis?
Immunodeficiency or systemic autoimmune disease?
Vasculitis?
Bleeding disorders or conditions of the microcirculation (i.e. von Willebrand disease, Raynaud's disease?
Currently using an investigational or study medical device or drug?
Chicken pox, shingles or flue in the last 2 weeks?
11. *Women only: Indicate Yes or No or N/A
Are you pregnant?
Are you trying to get pregnant?
Are you postmenopausal?
If postmenopausal, how long since your last period?
If postmenopausal, have you been on any hormone replacement therapy within the past 6 months?
12. Subject Tolerance Questions: Are you willing to do the following: Indicate Yes or No
Fast overnight for 8 hours?
Hold medications on the morning of testing?
Willing to have your blood drawn?
13. Weight (indicate in pounds)

14. Height (indicate in inches)
15. BMI

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.***
We will keep two separate screening logs. One screening log will contain name and contact information only. This log will be kept in case the research staff needs to reschedule Visit 1 or for follow-up if the subject does not show for Visit 1. This screening log will also be destroyed/deleted when recruitment has ended. See attached ("Subject Screening Log_20141029" Sheet 2 titled "PHI")
1. Name
2. Phone number
3. Email address

The second screening log will contain other demographic information and information on whether or not the individual was screened, enrolled and/or consented. See attached ("Subject Screen Log_20141029", Sheet 1 titled "No PHI")
1. Age
2. Gender
3. Date contacted study
4. Mode of contact
5. How did they hear of the study?
6. Date of phone screen
7. Pass phone screen?
- If yes, date Visit 1 scheduled
- Date of signed informed consent
- If no, reason for phone screen fail

The purpose for collecting and keeping this information is to keep track of the number and demographics of subjects phone screened in order to determine the level of success of recruiting strategies. Contact information is required to contact the subjects after the phone screening in case the research staff needs to reschedule Visit 1 or for follow up if the subject does not show for Visit 1. As described above, the screening log containing name and other personal health information (PHI) will be deleted once recruitment has ended; the second screening log which contains no PHI will be kept for data management regarding recruitment strategies.

Information on how the subjects heard of the study will help the research team understand the most successful methods for advertising for the study. Information on reasons for failing the phone screen is kept to report to the NIH (if/when the study receives extramural funding) and to monitor our recruiting process.

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

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

VII.D.26 ***List and describe screening***
-Medical history and physical exam
-Health history survey
-Resting vitals (heart rate, blood pressure)
-Resting 12-lead ECG
-Blood chemistries (thyroid stimulating hormone, ALT, AST, creatinine)

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 consider participating in the study as long as the study remains 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 consent is completed (Visit 1). The subject will return within 1-2 weeks of Visit 1 for Visit 2 in which experimental procedures will take place.

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***

There is no difference in the recruitment, consent, or enrollment process for the intervention group with moderate-to-high anxiety and the control group with low or no anxiety (no intervention).

Potential subjects will be directed to the online screening survey by mass email advertisement through the University of Iowa. If individuals are interested in the study, they will be instructed to contact the research team to complete the brief telephone survey to ensure eligibility.

After the online screening survey has been completed, potentially eligible subjects will be contacted via phone by the research study coordinator. This phone call will consist of further screening measures including current medication use and past medical history. Duplicate questions will be asked to confirm the absence of major exclusion criteria. If the subject passes this second, pre-consent phone screening measure, Visit 1 will be scheduled to review the consent document with the PI and/or research study coordinator. The informed consent document will be reviewed in its entirety and the potential subject will be encouraged to ask questions. The potential subject will again be reminded that participation in the study is

voluntary, and even after signing the consent, they may drop out at any time. They may take as much time as they need to think about joining the study, and they may discuss it with friends/family.

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?***
No

VII.E.2 ***Describe randomization scheme/assignment including ratio such as 1:1, 2:1 etc.***
A statistician will develop a computer program in R that will generate a 1:1 randomization for the clonidine vs. HCTZ.

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)***
Information collected initially online when the potential participant clicks the link when interested in the study described on the mass email (online pre-screening survey): http://j.mp/2lpbGLW, or see attachment "ATLAS_ Online Pre-Screen Survey_3-3-17.pdf"
Information collected over the phone following the pre-screening survey:
1) Pre-Consent Phone Screen
Information collected following the subject signing the consent form during visit 1:
1) Contact and Demographics Information
2) Health History Questionnaire
3) Modifiable Activity Questionnaire
4) MINI International Neuropsychiatric Interview
The following surveys will be completed online through RedCap at their study visits (visits 1, 2, and 6). A link to view these questions online cannot be included here because the link that is created for the participant is created and attached to the participant record at the time of the visit. Attached are hard copies from which the electronic versions are created from.
1) State-Trait Anxiety Inventory
2) Positive and Negative Affect Schedule-Expanded Form
3) Anxiety Inventory
4) Depression Inventory
5) Early Experiences Questionnaire
6) Acceptance and Action Questionnaire II
7) Committed Action Questionnaire
8) Short Form Health Survey
9) Sleep Surveys (including the Berlin Questionnaire, Karolinska Sleep log and the Sleep Quality Assessment [PSQI]) (only visits 2 and 6)
All participants involved in this project will take every survey listed here for all visits.
The following surveys will be completed by the participant following visits 2 and 6:
1) Microneurography questionnaire (ATLAS_mSNA Questionnaire_Holwerda.pdf)

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***

Subjects with moderate-to high anxiety will undergo experimental testing at baseline and again after the intervention (4 weeks of clonidine or hydrochlorothiazide). 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 study procedures are as follows:

Visit 1 (1.5 hours): Consent and Screening

1. Explanation of the study; reading and signing of written informed consent document. If subject consents, but requires a 2 week washout for vitamins or supplements, aspirin, NSAIDs, PDE-5 or PDE-3 inhibitors, Visit 2 will not be scheduled for 2 weeks post-Visit 1 completion. If subject consents and no washout is needed, screening tests will be performed on the same day to determine further eligibility by the following:
2. Research staff will obtain resting vitals (heart rate, blood pressure) and resting 12 lead ECG.
3. Research staff will obtain anthropometrics including height, weight, waist and hip circumference with tape measure.
4. Subject will fill out the Demographics Document, Health History Survey (including current medications), and Modified Activity Questionnaire. The following questionnaires will be completed online: State-Trait Anxiety Inventory, Positive and Negative Affect Schedule-Expanded Form, Anxiety Inventory, Depression Inventory, Early Experience Questionnaire, Acceptance and Action Questionnaire II, Committed Action Questionnaire, Short Form Health Survey.
5. Research nurse or trained staff will obtain venous blood draw (~1 teaspoon) using butterfly needle for UIHC pathology labs: Lipid panel, TSH, metabolic panel, electrolytes, ALT, AST and FSH (women only to confirm >40 mIU/l) (4.5 ml blood 1 light green PST tube); HbA1C (3 ml blood 1 lavendar EDTA plasma tube).
6. Subject will meet with a physician or other trained interviewer who will ask detailed questions about their medical history, structured psychiatric history (MIN1) and medication use.
7. The subject may skip or refuse to discuss any questions/topics they wish not to answer. To minimize participant burden and accommodate participant schedules, visit procedures may be split across multiple days.
8. Subject will be instrumented with 24hour 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.

If the subject is completing a 2 week washout of antihypertensive medications, they will complete 2 safety visits (Washout Visit 1 and Washout Visit 2)

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): ~ 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".

Visit 2 (4.5 hours): Pre-Intervention Experimental Visit
1. Prior to arriving at the visit, the subject will complete (for a 2nd time) the following surveys/questionnaires via an emailed, secure REDCap link:
1.State-Trait Anxiety Inventory
2.Positive and Negative Affect Schedule-Expanded Form
3.Anxiety Inventory
4.Depression Inventory
5.Early Experience Questionnaire
6.Acceptance and Action Questionnaire II
7.Committed Action Questionnaire
8.Short Form Health Survey
2. Subject will arrive at the CRU between 7-9 am after an overnight 8 hour fast (water encouraged). Return 24hour blood pressure monitor
3. Prior to experimental testing, the subject will complete the following surveys/questionnaires:
1.Sleep Surveys (including the Berlin Questionnaire, Karolinska Sleep log and the Sleep Quality Assessment [PSQI])
4. CRU staff/nurse will obtain urine or serum sample for pregnancy test (women of childbearing age only).
5. IV catheter insertion. Subject will lie supine and research nurse will insert venous 18 G or 20 G catheter into antecubital vein.
6. Subject will be instrumented with ECG, finger blood pressure cuff, brachial blood pressure cuff, respiratory band
7. After 20 min, research nurse will then obtain blood samples through catheter:
•Lipid panel, insulin/glucose, hsCRP, basic metabolic panel (1 x 4.5 ml blood light green PST tube)
•Extra blood collected for specialized labs performed in PIs lab:
1. Serum: 4 x 5 ml red top SST tube: for interleukin6, tumor necrosis factoralpha, 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
3. Catecholamines (1 x 3 ml red top tube)
4. Copeptin (1 x 8.5 ml ACD-A yellow top tube)
5. Vasopressin (1 x 4.5 ml blood light green PST tube)

8. Ocular Coherence tomography (OCT) and intraocular pressure (IOP) will be performed (see 'Retinal Vascular Measurements' in the 'Experimental Methods' for details).
9. Pulse Wave Velocity (PWV) and Carotid Compliance (CC) (see 'Experimental Methods' for details).
10. A research staff member trained in microneurography will record from the peroneal nerve, which is located just below the knee on the outer part of the leg, with a microelectrode for measurement of muscle sympathetic nerve activity (See 'Experimental Methods' for details). Subject will be given to assess any follow up discomfort at the sight of the microneurography electrode over the next 7 days and mail it back to the study staff in 7 days.
11. The lower portion of the subject`s body below the waist will be enclosed in a box-like chamber to allow the application of negative pressure to the lower body (see 'Experimental Methods' for details for lower body negative pressure) for measures of microneurography, Pulse Wave Velocity (PWV), blood chemistries (catecholamines, copeptin, and vasopressin) and Carotid Compliance (CC) during elevated sympathetic nerve activity. During lower body negative pressure, retinal vascular measurements will also be performed using laser-speckle blood flow imaging (see 'Experimental Methods' for details).
12. A cold pressor test or mental math test (see 'Experimental Methods' for details) may also be performed to assess sympathetic activation and blood pressure responses.
13. Subject will receive snack or meal from CRU dining room.

Visit 3 (30 min): Randomization
1. Subjects randomized to oral clonidine (0.2 mg/day; 0.1 mg in the morning and 0.1 mg in the evening) or hydrochlorothiazide (25 mg/day 12.5 mg in the morning and 12.5 mg in the evening) 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).
2. Subjects sit quietly for a few minutes and undergo blood pressure measurement.

Visit 4, week 1 (30 minutes): ~Day 7 day Blood electrolytes, Compliance check/Pill Count
1. 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.

Visit 5, week 2 (30 minutes): ~Day 14 day day Blood electrolytes, Compliance check/Pill Count
1. 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.

Visit 6, week 4-5 (4.5 hours): Postintervention
Subject will take morning dose of study pills and return to CRU between Day 26 and 35 for venous blood sample, blood pressure, HR, pill count and survey of any side effects.
1. Prior to arriving at the visit, the subject will complete (for a 2nd time) the following surveys/questionnaires via an emailed, secure REDCap link:
1.State-Trait Anxiety Inventory
2.Positive and Negative Affect Schedule-Expanded Form
3.Anxiety Inventory
4.Depression Inventory
5.Early Experience Questionnaire
6.Acceptance and Action Questionnaire II
7.Committed Action Questionnaire
8.Short Form Health Survey
2. Subject will arrive at the CRU between 7-9 am after an overnight 8 hour fast (water encouraged).
3. Prior to experimental testing, the subject will complete (for a 3rd time) the following surveys/questionnaires:
1.Sleep Surveys (including the Berlin Questionnaire, Karolinska Sleep log and the Sleep Quality Assessment [PSQI])
4. CRU staff/nurse will obtain urine or serum sample for pregnancy test (women of childbearing age only).
5. IV catheter insertion. Subject will lie supine and research nurse will insert venous 18 G or 20 G catheter into antecubital vein.
6. Subject will be instrumented with ECG, finger blood pressure cuff, brachial blood pressure cuff, respiratory band
7. After 20 min, research nurse will then obtain blood samples through catheter:
•Lipid panel, insulin/glucose, hsCRP, basic metabolic panel (1 x 4.5 ml blood light green PST tube)
•Extra blood collected for specialized labs performed in PIs lab:
1. Serum: 4 x 5 ml red top SST tube: for interleukin6, tumor necrosis factoralpha, 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
3. Catecholamines (1 x 3 ml red top tube)
4. Copeptin (1 x 8.5 ml ACD-A yellow top tube)
5. Vasopressin (1 x 4.5 ml blood light green PST tube)
8. Pulse Wave Velocity (PWV) and Carotid Compliance (CC) (see 'Experimental Methods' for details).
9. A research staff member trained in microneurography will record from the peroneal nerve, which is located just below the knee on the outer part of the leg, with a microelectrode for measurement of muscle sympathetic nerve activity (See 'Experimental Methods' for details). Subject will be given to assess any follow up discomfort at the sight of the microneurography electrode over the next 7 days and mail it back to the study staff in 7 days.
10. The lower portion of the subject`s body below the waist will be enclosed in a box-like chamber to allow the application of negative pressure to the lower body (see 'Experimental Methods' for details for lower body negative pressure) for measures of microneurography, Pulse Wave Velocity (PWV), blood chemistries (catecholamines, copeptin, and vasopressin) and Carotid Compliance (CC) during elevated sympathetic nerve activity. During lower body negative pressure, retinal vascular measurements will also be performed using laser-speckle blood flow imaging (see 'Experimental Methods' for details).
11. A cold pressor test or mental math test(see 'Experimental Methods' for details) may also be performed to assess sympathetic activation and blood pressure responses.
12. Subject will be instrumented with 24 hour blood pressure monitor to wear home for 24 hours.
13. Subject will receive snack or meal from CRU dining room.

Subjects with low or no anxiety (control) will undergo only baseline testing to be compared to participants with moderate-to-high anxiety, and will not participate in the drug intervention. The study procedures are as follows:

Visit 1 (1.5 hours): Consent and Screening
1. Explanation of the study; reading and signing of written informed consent document. If subject consents, but requires a 2 week washout for vitamins or supplements, aspirin, NSAIDs, PDE-5 or PDE-3 inhibitors, Visit 2 will not be scheduled for 2 weeks post-Visit 1 completion. If subject consents and no washout is needed, screening tests will be performed on the same day to determine further eligibility by the following:
2. Research staff will obtain resting vitals (heart rate, blood pressure) and resting 12 lead ECG.
3. Research staff will obtain anthropometrics including height, weight, waist and hip circumference with tape measure.
4. Subject will fill out the Demographics Document, Health History Survey (including current medications), and Modified Activity Questionnaire. The following questionnaires will be completed online: State-Trait Anxiety Inventory, Positive and Negative Affect Schedule-Expanded Form, Anxiety Inventory, Depression Inventory, Early Experience Questionnaire, Acceptance and Action Questionnaire II, Committed Action Questionnaire, Short Form Health Survey.
5. Research nurse or trained staff will obtain venous blood draw (~1 teaspoon) using butterfly needle for UIHC pathology labs: Lipid panel, TSH, metabolic panel, electrolytes, ALT, AST and FSH (women only to confirm >40 mIU/l) (4.5 ml blood 1 light green PST tube); HbA1C (3 ml blood 1 lavendar EDTA plasma tube).
6. Subject will meet with a physician or other trained interviewer who will ask detailed questions about their medical history, structured psychiatric history (MIN1) and medication use.
7. The subject may skip or refuse to discuss any questions/topics they wish not to answer. To minimize participant burden and accommodate participant schedules, visit procedures may be split across multiple days.
8. Subject will be instrumented with 24hour 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.

Visit 2 (4.5 hours): Baseline Experimental Visit
1. Prior to arriving at the visit, the subject will complete (for a 2nd time) the following surveys/questionnaires via an emailed, secure REDCap link:
1.State-Trait Anxiety Inventory
2.Positive and Negative Affect Schedule-Expanded Form
3.Anxiety Inventory
4.Depression Inventory

5.Early Experience Questionnaire
6.Acceptance and Action Questionnaire II
7.Committed Action Questionnaire
8.Short Form Health Survey
2. Subject will arrive at the CRU between 7-9 am after an overnight 8 hour fast (water encouraged). Return 24hour blood pressure monitor
3. Prior to experimental testing, the subject will complete the following surveys/questionnaires:
1.Sleep Surveys (including the Berlin Questionnaire, Karolinska Sleep log and the Sleep Quality Assessment [PSQI])
4. CRU staff/nurse will obtain urine or serum sample for pregnancy test (women of childbearing age only).
5. IV catheter insertion. Subject will lie supine and research nurse will insert venous 18 G or 20 G catheter into antecubital vein.
6. Subject will be instrumented with ECG, finger blood pressure cuff, brachial blood pressure cuff, respiratory band
7. After 20 min, research nurse will then obtain blood samples through catheter:
•Lipid panel, insulin/glucose, hsCRP, basic metabolic panel (1 x 4.5 ml blood light green PST tube)
•Extra blood collected for specialized labs performed in PIs lab:
1. Serum: 4 x 5 ml red top SST tube: for interleukin6, tumor necrosis factoralpha, 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
3. Catecholamines (1 x 3 ml red top tube)
4. Copeptin (1 x 8.5 ml ACD-A yellow top tube)
5. Vasopressin (1 x 4.5 ml blood light green PST tube)

8. Pulse Wave Velocity (PWV) and Carotid Compliance (CC) (see 'Experimental Methods' for details).
9. A research staff member trained in microneurography will record from the peroneal nerve, which is located just below the knee on the outer part of the leg, with a microelectrode for measurement of muscle sympathetic nerve activity (See 'Experimental Methods' for details). Subject will be given to assess any follow up discomfort at the sight of the microneurography electrode over the next 7 days and mail it back to the study staff in 7 days.
10. The lower portion of the subject`s body below the waist will be enclosed in a box-like chamber to allow the application of negative pressure to the lower body (see 'Experimental Methods' for details for lower body negative pressure) for measures of microneurography, Pulse Wave Velocity (PWV), blood chemistries (catecholamines, copeptin, and vasopressin) and Carotid Compliance (CC) during elevated sympathetic nerve activity. During lower body negative pressure, retinal vascular measurements will also be performed using laser-speckle blood flow imaging (see 'Experimental Methods' for details).
11. A cold pressor test or mental math test (see 'Experimental Methods' for details) may also be performed to assess sympathetic activation and blood pressure responses.
12. Subject will receive snack or meal from CRU dining room.

Experimental Methods (Visits 2 and 6):

Microneurography: Direct intraneural recordings of multiunit muscle sympathetic nerve activity (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; 15 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 3lead ECG, beatbybeat 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 noninvasively measure mean arterial blood velocity and diameter. 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): Carotidfemoral, carotidbrachial, and carotidradial PWV will be measured noninvasively by recording carotid, femoral, brachial and radial artery pressure waveforms sequentially with an applanation tonometer (Noninvasive 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 carotidfemoral transit distance (CFTD) is estimated between the 2 anatomical sites as the difference between the suprasternal notch (SSN) to carotid (SSNC) and femoral (SSNF) sites. Thus, the CFTD is calculated as CFTD= (SSNF)(SSNC) 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). The intrasubject reproducibility of carotidfemoral PWV is excellent with a coefficient variation of 2.1% for triplicate measurements on non consecutive days in 7 young adults.

Carotid Artery Compliance (CC): Carotid artery compliance and Betastiffness index will be determined noninvasively by highresolution 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 offline 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.

Lower Body Negative Pressure (LBNP): During LBNP, the lower portion of the subject`s body below the waist will be enclosed in a box-like chamber. This allows the application of negative pressure to the lower body via the suction of a vacuum cleaner to temporarily pull blood toward the legs. The level of negative pressure applied decreases blood return to the heart to a similar degree as that which occurs when one assumes the upright position and gravity pulls blood towards the legs. The main purpose of LBNP is to cause a baroreflex-mediated increase in MSNA by unloading the cardiopulmonary baroreceptors (60). LBNP will be performed at low to moderate levels (-15mmHg, -30mmHg, and -45mmHg) such that no major changes in blood pressure occur (unlike the cold pressor test and mental math) and the subject does not experience symptoms such as feeling light-headed. This is particularly important for assessing the effects of increased MSNA on arterial stiffness using pulse wave velocity (PWV) and carotid compliance (CC) because blood pressure remains unchanged and does not become a confounding factor in the data analysis.

Retinal Vascular Measurements: To examine the influence of anxiety on sympathetic control of retinal blood flow (index of brain blood flow), laser-Speckle Blood Flow Imaging (LSFG) will be used in the eyes. LSFG is a camera that looks into the eyes and takes pictures of the microcirculation. This device uses a Class I laser diode. First, the subject will have baseline LSFG imaging in both eyes, which takes only moments to complete. Then LSFG will be used during the varying levels of LBNP. Two E4 wristbands (one on each wrist) will be worn by the participant during LSFG to non-invasively measure sympathetic activity (electrodermal response) and beat-to-beat heart rate variability continuously. Intraocular pressure (IOP) may be measured, and would 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. 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. This is called ocular coherence tomography (OCT). 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 and is only done once prior to lower body negative pressure.

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.

24hour ambulatory blood pressure variability and baroreflex sensitivity: Twenty-four hour systolic blood pressure will be recorded using standard ambulatory blood pressure assessment (90207IQ, 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 6 during microneurography using via beat-to-beat finger blood pressure (Nexfin, Edward Life Sciences, Inc.) and calculated using the sequence technique.

VII.E.7 ***Will you attempt to recontact subjects who are lost to follow-up?***
No - those lost to followup will not be recontacted

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 compensation for study participation is approximately $285. An additional $30 is provided if also undergoing an anti-hypertensive medication washout prior to participation. 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 (1.5 hours total)
Washout Visit 1 (If applicable): $15 (30 min total)
Washout Visit 2 (If applicable): $15 (30 min total)
Visit 2: $105 (4.5 hours total)
Visit 3: $15 (30 min total)
Visit 4: $15 (30 min total)
Visit 5: $15 (30 min total)
Visit 6: $105 (4.5 hours total)

Total compensation is $135 for control subjects with low or no anxiety because these participants will only be participating in baseline testing (Visit 1 and 2).

VIII. Risks

VIII.1 ***What are the risks to subjects including***
- emotional or psychological
- financial
- legal or social
- physical?
Fasting 8 hours: The most common risk when fasting is dehydration, therefore the subject will be encouraged to drink plenty of water. Subjects may experience hunger and irritability and if they experience fainting, nausea, or vomiting they will be instructed to stop fasting.

Blood Sample: Potential risks associated with obtaining blood samples are minimal but include slight bruising, pain, a temporary feeling of faintness, and/or a small risk of infection. All blood draws will be performed by CRU staff, nurse or research team member trained and certified in drawing blood. There are no known risks associated with urine collection.

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

Carotid Artery Compliance: There are no known or foreseeable risks associated with the use of carotid echocardiography. ECG electrodes may

cause minor irritation to the skin.

Non-invasive Blood Pressure Monitoring: There are no known risks associated with the use of a small cuff on your finger to monitor small changes in blood pressure.

Microneurography: Direct recording of nerve activity (microneurography) has a very small degree of risk. Over 2,000 microneurographic studies have been performed in our laboratories at the University oflowa since 1984. There have been no significant complications. Approximately 7% of subjects experience minor tingling in the leg, foot or arm for a few days after the study, but these symptoms have been transient. In the mental math test, the subject may become frustrated and in the cold pressor test, the subject may feel uncomfortable, however there are no known physical risks associated with these tests. The subject may experience discomfort at the site of microneurography following the study visit, and discomfort becomes more likely if they engage in high-intensity exercise with their legs (e.g. leg press, running, cycling, etc.), particularly within 24 hours of the procedure. In addition to this information being in the consent, study staff will verbally remind the subject before and after the procedure at the visit. If a subject does return the microneurography questionnaire indicating they have symptoms the study staff will call the participant and offer a physical exam by Dr. Fiedorowicz.

Lower body negative pressure (LBNP): Mildly reducing central blood volume during LBNP causes moderate increases in heart rate (~20 beats per min) similar to going from a sitting position to a standing position. Although no symptoms typically occur with low to moderate levels of LBNP, it is possible that this procedure can lead to light headedness, in which case the negative pressure in the LBNP tank will slowly be reduced to allow for recovery from this symptom.

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.

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.

Risks of drug intervention:

1. Clonidine (Catapres): Most systemic adverse effects during clonidine have been mild and have tended to diminish with continued therapy. In a 3-month multiclinic 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, blood dyscrasias, rash, photosensitivity, electrolyte imbalance, impotence, renal dysfunction/failure, interstitial nephritis.

3. 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.

4. 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. Kalil 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. Kalil or Dr. Fiedorowicz cannot be contacted, we will contact the oncall psychiatry resident or arrange for an evaluation in the UIHC Emergency Room or Adult Psychiatry Clinic.

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

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

7. 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.)***

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. Kalil to be paged. Dr. Kalil (although he 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. Kalil will notify Dr. Holwerda. If not life threatening, Dr. Kalil will instruct the appropriate medical course of action to the subject. If Dr. Kalil is out of town or unavailable then Dr. Jess Fiedorowicz, MD will serve as a backup and be available to consult. Dr. Kalil or Fiedorowicz will instruct Dr. Holwerda on 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. Kalil will prescribe 10 mg/day of KCl supplement for the subject. PI will pay this cost. 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. Kalil to be paged. Dr. Kalil will talk to the subject about their concerns. If side effects/symptoms are life threatening, the subject will be instructed to call 911. Dr. Kalil will notify Dr. Pierce. If not life threatening, Dr. Kalil will instruct the appropriate medical course of action to the subject. If Dr. Kalil is out of town or unavailable then Dr. Jess Fiedorowicz, MD will serve as a backup and be available to consult. Dr. Kalil or Fiedorowicz will instruct Dr. Holwerda 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.

All IV insertion, blood draws, and microneurography techniques will be performed by CRU staff, nurse or trained research team member.

Answers to questionnaires are confidential and the participant is able to skip any question they are not comfortable answering. To ensure confidentiality, a study number assigned at the beginning of the study will identify materials containing patient information. All study materials and consent forms will be kept in locked files stored in an office that will also be locked.

Some questions on the various health surveys or questionnaires may include questions about depressed mood or thoughts of suicide. To address this risk, we will provide the following information to all individuals who complete the online pre screen:

"Thank you for completing our survey, which included questions about depression and anxiety. If you are struggling with anxiety or depression, we encourage you to seek help. *Important note: The information below is provided as a courtesy and is NOT related to your responses on the screening survey you just completed.

+If you are a student, the University of Iowa offers confidential and professional mental health services through the University Counseling Services.
-University Counseling Service
(319) 335-7294 or e-mail ucs@uiowa.edu

+If you are an employee, The University oflowa offers services through the Employee Assistance program. We have provided the contact information for these and other mental health services available in the area.
-University oflowa Employee Assistance Program (EAP):
(319) 335-2085 or email eaphelp@uiowa.edu

+Other Resources:
-Mental Health Services Locator Website in Iowa:
Go to http://store.samhsa.gov/mhlocator, and click on Iowa on the map.
-Suicide Hotline:
Website: http://suicidehotlines.com 1-800-273-TALK (8255)
1-800-SUICIDE (784-2433)
Both hot lines offer immediate suicidal crisis counseling and information about crisis centers in your area as well as referrals to mental health centers in your area.
-2-1-1 Infoline:
Information, referral and crisis intervention service for the State of Iowa. Operates 24 hours a day. Can be reached by dialing 2-1-1 in IA."

Once the subject is enrolled in the study, if these or other questions on other health forms or during the MINI lead to a concern for the participant's safety or the safety of others, the PI will be notified immediately and will perform a risk assessment. The participant will be kept informed of the need for additional evaluation and will be encouraged to ask questions. If the participant is deemed a safety risk by the PI, the participant's primary psychiatrist will be notified. If the participant does not have a primary psychiatrist or if the primary psychiatrist cannot be contacted, we will arrange for an evaluation in the UIHC Emergency Room or Adult Psychiatry Clinic.

The questions related to harming self or others in the questionnaires and MINI will be reviewed before the subject leaves the study site. Concerning responses will be reviewed by Dr. Fiedorowicz or other qualified team member (can decide about and arrange emergency psychiatric care) before the subject leaves the study site.

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 biannual independent review of the protocol by Dr. Mark Santillan, Assistant Professor, Department of OB/GYN. The PI will provide a biannual 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 will be no direct benefits to the subject.

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 sympathetic nerve activity and improved endothelial function) in adults with anxiety and/or 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):***
- 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.
- Electronic records (computer files, electronic databases, etc.) - Data will be entered using subject ID code into the ICTS REDCap webbased 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 on the RDSS drive which is maintained by the University and is inaccessible to anyone without privilage. Only research staff on the IRB approved study will have access to the folder the study on the server.
  - Name - Laurie Hafner Dahms
  - Title - Title - Senior IT Support Consultant
  - University Job Classification - University Job Classification - PIC3
- Biologic samples (blood draws, check swabs, saliva samples, tissue samples, etc.) - 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 526 FH (Amy Sindler's lab). 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

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.

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.1 ***Describe the analysis methods you will use, including, if applicable, the variables you will analyze***
At baseline group differences in dependent variables between high anxiety and low or no anxiety subjects and among the 2 hypertensive treatment groups at baseline (after randomization) by an independent ttest. For the main analysis, a linear mixedmodel analysis for repeated measures will be used to test the effect of clonidine or HCTZ and placebo on sympathetic nerve activity, arterial stiffness, and blood flow. The fixed effects in the model include time (baseline vs. 4 weeks) which is the withinsubjects factor, and treatment (clonidine vs. HCTZ) which are the between subjects factors in the model. The model will also include a time x treatment interaction which corresponds to testing if the mean change in each of the dependent variables after compared with before the 4 week intervention significantly differs between the groups. Bivariate correlation analyses will be performed for relations of interest between change in the dependent variables and with change in secondary outcomes (e.g. blood pressure).

XI.2 ***Provide the rationale or power analysis to support the number of subjects proposed to complete this study.***
Assuming equal sample sizes, at total of 64 subjects (32 low anxiety vs. 32 moderate-to-high anxiety subjects) are needed to detect baseline differences in carotid-femoral PWV at >80% power at alpha=0.05 (one-sided). At a similar dose used in this study, clonidine has been shown to decrease plasma norepinephrine (-66%) and MSNA (-62%) in 16 young, sedentary adults after only 6 days (34). Only subjects with moderate-to-high anxiety will be randomized to either clonidine (n=16) or HCTZ (n=16). Because of multiple laboratory visits and need for quality repeat MSNA recordings in the same subjects, an approximate 10% attrition rate will be factored into the subject numbers, giving a total subject number of 36 with moderate-to-high anxiety to undergo the drug intervention. An equal number of subjects with low or no anxiety (n=36) will undergo only baseline testing and not participate in the drug intervention, creating an overall total of 72 subjects to participate in the study.

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.