Does Treating Anxiety Symptoms With ACT Improve Vascular Inflammation and Function?
(ACT on Anxiety)

NCT# NCT02915874

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Contents

Introduction

Purpose

The goal of this study is to evaluate the effectiveness of a brief, intensive 1-day psychotherapy group intervention (Acceptance and Commitment Therapy, ACT), compared to a 12 week time control group on anxiety symptoms, vascular function, inflammation, muscle sympathetic nerve activity (mSNA), and oxidant stress. Individuals who are interested in the study will be identified by an online screening survey and will be contacted by the research team; advertisements, flyers and mass emails will direct individuals to the online screening survey. Those deemed eligible to participate will be randomized to the ACT intervention or the control group. Assessments of anxiety symptoms (via various surveys) and vascular function (via non-invasive, well-established techniques) will be performed at baseline and 12 weeks post-ACT group intervention session. In addition, reassessment of anxiety symptoms via aforementioned surveys will take place 6 weeks post-ACT group session. After 12 weeks, anxiety and vascular assessments will be repeated to re-evaluate severity of anxiety symptoms, vascular function, inflammation, and oxidant stress.

Research Question and Hypothesis

We hypothesize that reducing the burden of anxiety symptoms using Acceptance and Commitment Therapy (ACT) will improve vascular function, inflammation, mSNA, and oxidant stress. If our hypothesis is correct, then we predict that: 1) Vascular function, inflammation, muscle sympathetic nerve activity (mSNA), and oxidant stress will be elevated in individuals with anxiety compared to those with low or no anxiety, 2) ACT will improve forearm blood flow during reactive hyperemia; 3) ACT will decrease mSNA; 4) ACT will improve microvascular, including retinal vascular, and large elastic artery function per pulse wave velocity (PWV) measurements and 5) ACT will improve autonomic function measured by 24-hour blood pressure variability and heart rate variability measures. We will also be able to explore other secondary endpoints related to oxidant stress and inflammation in vascular endothelial cells. If anxiety increases inflammation, then we predict that ACT will reduce circulating pro-inflammatory cytokines, and produce a phenotype of endothelial cell proteins reflecting decreased inflammation compared to pre-treatment. And if anxiety increases oxidative stress, then we predict that ACT will produce a phenotype of endothelial cell proteins reflecting decreased oxidant stress and increased nitric oxide synthase activity.

Location

Project procedures will take place at

- Other University of Iowa campus site - 522 Field House and 518 Field House (laboratory)
- Clinical Research Unit
- University of Iowa Hospitals and Clinics - C202; C204 Boyd Tower; W240 General Hospital; Pomerantz Family Pavilion, Ophthalmology Clinic, Rm 11279

Funding

Type	**Source**	**Grant Title**	**Name of PI on Grant**
Federal Agency	Department of Health & Human Services, National Institutes of Health	Does Anxiety Cause Vascular Dysfunction Through Inflammation and	Francois M. Abboud

		Sympathetic Activation?	
Private Foundation/Association	American Heart Association, Midwest Affiliate	Anxiety-mediated impairments in large elastic artery function and the autonomic nervous system	Seth Holwerda

Key Study Personnel

Principal Investigator:	Jess Fiedorowicz, MD, PHD
Co-Investigators:	Francois Abboud, MD Seth Holwerda, PHD Randy Kardon, MD Gary Pierce, PHD, MS

Description of Study Population

We will enroll 150 healthy men and women age 25-65 years who are experiencing feelings of anxiety based on the online pre-screen survey.

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 = 25 and < or = 65 years of age.
- Healthy, as determined by health history questionnaire, blood chemistries, and 12-lead ECG.
- Blood chemistries indicative of normal renal (creatinine <2.0mg/dl), liver (<3 times upper limit for ALT, AST), and thyroid function (TSH between 0.4 - 5.0 mU/L) or on stable thyroid medication with no dose change for 3 months.
- If currently receiving treatment with or taking any of the following supplements, must be willing and able to discontinue taking for 2 weeks prior to each study visit and/or throughout the treatment period: Vitamin C, E or other multivitamins containing vitamin C or E; omega-3 fatty acids; Phosphodiesterase (PDE) 5 inhibitors (i.e. Viagra®, Cialis®, Levitra®, or Revatio®); PDE 3 inhibitors (e.g., cilostazol (Pletal®),milrinone, or vesnarinone).
- 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).

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, my lure 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).
- Intention to start or current psychotherapy for anxiety and/or depression while enrolled in study.
- 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.
- Current alcohol abuse.
- 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 condition that, in the view of the PI or Co-I, places the subject at high risk or poor treatment and study compliance.

Strategies for Recruitment and Retention

We will advertise via mass email to University of Iowa community, post flyers on buildings on University of Iowa and UIHC campus, advertise in the Daily Iowan newspaper, the 'volunteer research' clinical trials website on UIHC website (http://www.uihealthcare.org/ClinicalTrials.aspx/) and in the 'Noon News' in UIHC. We will contact registrants in the STAR registry at the University of Iowa Center on Aging.
Subjects will be provided compensation via check based on the following schedule:
Visit 1: $20 per hour (~3 hours total)
Visit 2: $20 per hour (~4 hours total)
6 week follow-up Surveys: $20
6 week follow-up visit: $20 per hour (~2 hours total)
Visit 4: $30 per hour (~4 hours total)
Total Compensation (expected): $320

Study Intervention

Acceptance and Commitment Therapy (ACT) held as a brief, intensive 1-day psychotherapy group intervention.

Randomization

A statistician will develop a computer program in R that will generate a 2:1 randomization for the ACT intervention group versus the control group, respectively.

Assessment Schedule

Intervention Group

1) Visit 1: Consent and Screening
    a) Explanation of the study; reading and signing of written informed consent document
    b) 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.
    c) If subject consents and no washout is needed, screening tests will be performed on the same day to determine further eligibility by the following:
        i) Research staff will obtain resting vitals (heart rate, blood pressure) and resting 12 lead ECG.
        ii) -Subject will fill out the Demographics Document, Health History Survey (including current medications), Sleep Surveys (including the Berlin Questionnaire, Karolinska Sleep log and the Sleep Quality Assessment [PSQI]) 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.
        iii) -Research nurse or trained staff will obtain venous blood draw (1/2 teaspoon) using butterfly needle for UIHC pathology labs: TSH only.
    d) Subject will meet with a physician or other trained interviewer who will ask detailed questions about their medical history, structured psychiatric history (MINI) and

medication use. 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.

e) Subject will undergo visual acuity, intraocular pressure test as well as a blood pressure measurement. The subject will then undergo ocular coherence tomography and laser-speckle blood flow imaging. As with microneurography (Visit 2), two E4 wristbands (one on each wrist) will be worn and a mental math test and a cold pressor test may also be performed during laser-speckle blood flow imaging.

2) Visit 2: Pre-Intervention Visit
   a) Subject will arrive at the CRU between 7-9 am after an overnight 8 hour fast (water encouraged).
      i) Subjects on vasoactive medications will be asked to hold medications on the morning of experimental testing, but will bring medications with them to be taken after the visit.
   b) CRU staff/nurse will obtain urine or serum sample for pregnancy test (women of childbearing age only).
   c) Prior to experimental testing, the subject will complete (for a 2nd time) the following surveys/questionnaires: 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; Sleep Surveys (including the Berlin Questionnaire, Karolinska Sleep log and the Sleep Quality Assessment [PSQI])
   d) A research staff member trained in microneurography will record from the peroneal nerve with a microelectrode for measurement of muscle sympathetic nerve activity (See 'Methods' for details). Subject will be given an mSNA Questionnaire to assess any follow-up discomfort at the sight of microneurography electrode over the next 7 days and mail it back to the study staff in 7 days. Participants will wear two E4 wristbands (one on each wrist) during the procedure. Measures of brachial artery blood flow using Doppler ultrasound, a mental math test, and a cold pressor test may also be performed during microneurography.
   e) Primary Endothelial Cell Protein Collection and blood sample: Subject will lie supine and a CRU nurse or physician will insert venous 18 G or 20 G catheter into antecubital vein.
      - Dr. Gary Pierce, nurse or physician will perform J wire endothelial cell collection (3 wires) through the 18 G IV catheter (see 'Experimental Methods' for details).
   f) After 15-20 minutes, CRU nurse will obtain blood samples through catheter:
      - 1 light green PST tube (4.5 mL) for lipid panel, insulin, glucose, hs-CRP; sent to UIDL
      - 1 dark green Na+Hep tube (10 mL) for catecholamines; sent to UIDL on ice
      - Extra blood collected for specialized labs performed in Co-I's lab:
        (1) 1 light green PST tube (4.5 mL each) for Pierce Research lab; extra plasma
        (2) 1 lavender EDTA tube (4 mL) for Pierce Research lab
        (3) 4 red top tubes (5 mL each) for Pierce Research lab; to test interleukin-6, tumor necrosis factor-alpha, and oxidative stress proteins)
   g) Venous Occlusion Plethysmography (VOP) will be performed (see 'Experimental Methods' for details).

h) Pulse Wave Velocity (PWV) and Carotid Compliance (CC) (see 'Experimental Methods' for details). Pulse wave velocity will be performed two times.
i) Subject will be instrumented with 24-hour ambulatory blood pressure monitor to wear home for 24 hours. Subject will return monitor to research coordinator the following day.
j) Subject will receive snack or meal from CRU dining room.

3) Visit 3: Group Intervention Workshop

Subjects randomized to the ACT Intervention group will attend a 1-day group workshop. Each ACT group will be held at the University of Iowa Hospitals and Clinics, include a total of 8-10 subjects, and last ~4-6 hours. At the start of the workshop, participants will be asked to fill out the STAI and PANAS-X questionnaires (hard-copy). The ACT group will be conducted by either 1 or 2 psychologists. Participants will be given a booklet in which two broad areas will be covered:

a) Behavioral Change training will involve
   i) teaching subjects how to recognize ineffective patterns of behavior and habits
   ii) exploring and setting life goals and those related to mental and physical health
   iii) promoting effective and committed actions to achieve these goals despite the urge to do otherwise
b) Mindfulness and Acceptance Training will emphasize new ways of managing troubling thoughts, feelings, and physical sensations (i.e. learning how to recognize, and develop cognitive distances from unhelpful thoughts such as "I can't take this anymore" and learning how to willingly face experiences that cannot be changed). In-session exercises and practice will be heavily emphasized during the group intervention and handouts will be distributed for home use. Lunch will be provided and breaks will be offered to minimize fatigue.

4) 6-Week Post Intervention Follow-up:

Subjects will be contacted via phone and email 6 weeks +/- 10 days after they have completed the ACT intervention group workshop.

a) Survey Portion

Subjects will be emailed a link to complete surveys listed below online via REDCap or have the option of completing via phone: State-Trait Anxiety Inventory; Positive and Negative Affect Schedule-Expanded Form; Anxiety Inventory; Depression Inventory; Early Experience Questionnaire; Acceptance and Action Questionnre II; Committed Action Questionnaire; Short Form Health Survey

b) Vascular portion of visit:
   i) Subject will arrive at the CRU between 7-9 am after an overnight 8 hour fast (water encouraged).
      (1) Subjects on vasoactive medications will be asked to hold medications on the morning of experimental testing, but will bring medications with them to be taken after the visit.
   ii) A research staff member will administer Sleep Surveys (including the Berlin Questionnaire, Karolinska Sleep log and the Sleep Quality Assessment [PSQI])
   iii) A research staff member trained in microneurography will record from the peroneal nerve with a microelectrode for measurement of muscle sympathetic nerve activity (See 'Methods' for details). Subject will be given an mSNA Questionnaire to assess any follow-up discomfort at the sight of microneurography electrode over the next 7 days and mail it back to the study staff in 7 days. Participants will wear two E4

wristbands (one on each wrist) during the procedure. Measures of brachial artery blood flow using Doppler ultrasound, a mental math test, and a cold pressor test may also be performed during microneurographay

iv) Venous Occlusion Plethysmography (VOP) will be performed (see 'Experimental Methods' for details).

v) Subject will receive snack or meal from CRU dining room.

5) Visit 4: 2nd Experimental Visit (12-weeks post-intervention)

All experimental procedures described in Visit 2 will be repeated.

a) Subject will arrive at the CRU between 7-9 am after an overnight 8 hour fast (water encouraged).
   i) Subjects on vasoactive medications will be asked to hold medications on the morning of experimental testing, but will bring medications with them to be taken after the visit.

b) CRU staff/nurse will obtain urine or serum sample for pregnancy test (women of childbearing age only).

c) Prior to experimental testing, the subject will complete (for a 2nd time) the following surveys/questionnaires:
   i) State-Trait Anxiety Inventory
   ii) Positive and Negative Affect Schedule-Expanded Form
   iii) Anxiety Inventory
   iv) Depression Inventory
   v) Early Experience Questionnaire
   vi) Acceptance and Action Questionnaire II
   vii) Committed Action Questionnaire
   viii) Short Form Health Survey
   ix) Sleep Surveys (including the Berlin Questionnaire, Karolinska Sleep log and the Sleep Quality Assessment [PSQI])

d) A research staff member trained in microneurography will record from the peroneal nerve with a microelectrode for measurement of muscle sympathetic nerve activity (See 'Methods' for details). Subject will be given an mSNA Questionnaire to assess any follow-up discomfort at the sight of microneurography electrode over the next 7 days and mail it back to the study staff in 7 days. Participants will wear two E4 wristbands (one on each wrist) during the procedure. Measures of brachial artery blood flow using Doppler ultrasound, a mental math test, and a cold pressor test may also be performed during microneurography.

e) Primary Endothelial Cell Protein Collection and blood sample: Subject will lie supine and a CRU nurse or physician will insert venous 18 G or 20 G catheter into antecubital vein.
   i) Dr. Gary Pierce, nurse or physician will perform J wire endothelial cell collection (3 wires) through the 18 G IV catheter (see 'Experimental Methods' for details)

f) After 15-20 minutes, CRU nurse will obtain blood samples through catheter:
   i) 1 light green PST tube (4.5 mL) for lipid panel, insulin, glucose, hs-CRP; sent to UIDL
   ii) 1 dark green Na+Hep tube (10 mL) for catecholamines; sent to UIDL on ice
   (1) Extra blood collected for specialized labs performed in Co-I's lab:
   iii) 1 light green PST tube (4.5 mL each) for Pierce Research lab; extra plasma
   iv) 1 lavender EDTA tube (4 mL) for Pierce Research lab

v) 4 red top tubes (5 mL each) for Pierce Research lab; to test interleukin-6, tumor necrosis factor-alpha, and oxidative stress proteins)

g) Venous Occlusion Plethysmography (VOP) will be performed (see 'Experimental Methods' for details).
h) Pulse Wave Velocity (PWV) and Carotid Compliance (CC) (see 'Experimental Methods' for details).
i) Subject will be instrumented with 24-hour ambulatory blood pressure monitor to wear home for 24 hours. Subject will return monitor to research coordinator the following day.
j) Subject will receive snack or meal from CRU dining room.

Control Group

If randomized to the 12-week time control group, study procedures are the same as the Intervention Group with the exception of Visit 3, the Group Intervention Workshop. However, the control subjects will be emailed to complete the STAI and PANAS-X surveys prior to the ACT Group Workshop Date. The surveys will be completed online via REDCap (with individualized link as indicated).

Safety Assessment

Physical Risks

- 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.
- Endothelial Cell Collection: The risks related to the venous endothelial cell collection do not appear to be any greater than those associated with the placement of an intravenous catheter into antecubital vein. In the Co-Investigator's experience (Dr. Gary Pierce) with this technique at the University of Colorado Clinical and Translational Research Center, there were no adverse events encountered in >150 of these procedures performed between 2005-2009 in young, middle-aged and older healthy adults.
- Venous Occlusion Plethysmography (VOP): There are no known risks associated with the strain gauge device or with temporary changes in blood flow using a blood pressure cuff. Minor discomfort in the forearm and/or hand may occur when the blood pressure cuffs are inflated. This feeling is completely reversed within several minutes after the cuff is released with no permanent discomfort.
- 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.

- 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. Subjects will be asked to bring medications with them to experimental Visits 2 and 4 to resume medication use once experimental testing is complete. We do encourage subjects to discuss holding medication dosages with their personal physician before doing so.
- 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.
- 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 of Iowa 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.
- 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.
- Fatigue may set in during the 6 hour group workshop.

Minimizing Risks

- All IV insertion, blood draws, and microneurography techniques will be performed by CRU staff, nurse or trained research team member.
- During experimental testing (VOP) the subject will relay any discomfort to research staff and will be reminded they can elect to stop testing if discomfort becomes intolerable.
- 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. Confidentiality among subjects will also emphasized at the group workshop.

- 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 of Iowa 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 of Iowa 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://sui cidehotlines.com
1-800-273-TALK (8255)
1-800-SUICIDE (784-243 3)
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.
- Regular breaks are given during the workshop to reduce fatigue.
- Retinal Vascular Measurements: Patients at risk for angle closure glaucoma who are seen at the UIHC eye clinic routinely have a corrective procedure done to prevent episodes of high intraocular pressure (high IOP) that can result when dilated. If there is a question about angle closure glaucoma, we will do a slit lamp exam to rule this out before proceeding. We will tell subjects before we put the drops in that they may cause a stinging or burning sensation that will go away. We will ask subjects if they have had a reaction to dilating drops in the past. Side effects of the tropicamide drops resolve after

discontinuation of the drops. We will ask subjects if they have had any prior reaction to proparacaine. The burning or redness usually subside in a few minutes. All eye drops used in the study are routine eye drops used in the eye clinic at UIHCS. We will provide subjects with disposable sunglasses to add additional comfort from the light.

Experimental Methods

1) Mini International Neuropsychiatric Interview (MINI):
   The MINI was designed as a brief structured interview for the major Axis I psychiatric disorders in DSM-IV and ICD-10. Validation and reliability studies have been done comparing the MINI to the SCID-P for DSM-III-R and the CIDI (a structured interview developed by the World Health Organization). The results of these studies show that the MINI has similar reliability and validity properties, but can be administered in a much shorter period of time (mean 18.7 +/- 11.6 minutes, median 15 minutes) than the above referenced instruments. It can be used by clinicians, after a brief training session. At Visit 1, Dr. Fiedorowicz or another trained interviewer will perform the interview with the subject. This interview will be more structured than usual, with very precise questions about psychological problems which require a yes or no answer by the subject.
2) Primary Endothelial Cell Protein Expression:
   Endothelial cells will be collected from an antecubital vein, washed, isolated to slides, and stained with primary and secondary (immunofluorescence) antibodies for quantification of: ACE (Abcam), AT1 receptor (Abcam), Ang II (Novus), Aldo (Pierce), nitrotyrosine (Abcam), NADPH oxidase p47phox (Abcam), Nox4 and Nox2 (Abcam). Briefly, under sterile conditions, a CRU nurse will insert an 18G catheter into an antecubital vein. Dr. Gary Pierce (with CRU nurse assisting) will insert a 0.018- or 0.021-inch mesh St. Jude 3 mm flexible guide J-wire (Daig Corp., Minnetonka, MN) 3-4 cm into catheter, then retracted 2-3 times from catheter. The distal portion of the wire is clipped off and then transferred to a 50 ml conical tube containing a buffer solution. Cells are then taken to the PIs lab (522 Fieldhouse) and cells are recovered by centrifugation, fixed to poly-lysine slides with formaldehyde, and then frozen at -80C until analysis. After blocking non-specific binding sites with 5% donkey serum (Jackson Immunoresearch), cells will be incubated with monoclonal antibodies for proteins of interest and a specific AlexaFlour488-conjugated secondary antibody (Research Diagnostics). Slides are then cover slipped with a VECTASHIELD DAPI (4', 6' diamidino-2-phenylindol hydrochloride) fluorescent mounting medium (Vector Labs) and stored at 4C overnight. Slides are viewed using a fluorescence microscope (Eclipse 600, Nikon) and 20 individual endothelial cell images are digitally captured by a digital camera (Weiss). These endothelial cells are documented by cell staining vWF and nuclear integrity is confirmed using DAPI staining. Once endothelial cells with intact nuclei are identified, they were analyzed using Image J (NIH, Bethesda, MD) to quantify the intensity of primary antibody-dependent AlexaFlour488 staining (i.e. average pixel intensity). The number of cells typically recovered from each guide wire results in approximately 50-100 cells per slide. Eight slides and one control cultured human aortic endothelial cell (HAEC: passage 3-6 processed identically to the sample cells) slides are selected for each staining batch. Values are reported as a ratio of sample endothelial cells to HAEC average pixel fluorescence intensity to reduce variability between staining batches.
3) Blood Sample:

Oxidized LDL (a marker of lipoprotein oxidation 65), TNF-alpha and IL-6 will be measured by ELISA (ALPCO) by Co-I's lab. Standard blood chemistries will be determined by the hospital Pathology lab.

4) Venous Occlusion Plethysmography (VOP):
Venous occlusion plethysmography (VOP) will be used to measure forearm blood flow (FBF) responses to local ischemia to test endothelium-dependent and independent dilation of forearm resistance arteries. Briefly, subjects lie supine and have blood pressure cuffs (venous occlusion) placed around upper arms and pediatric blood pressure cuffs around wrists. FBF will be measured by placing a gallium-in-silastic strain gauge around the widest part of the forearm which measures small changes in forearm volume during periodic inflation (8 sec inflated, 4 sec deflated) of upper arm cuffs to 40 mmHg (which temporarily prevents venous outflow and measures arterial inflow into forearm) and continuous wrist inflation of a blood pressure cuff to 250 mmHg. VOP is a well-established and validated technique for measuring FBF response to ischemia in human subjects.
5) Pulse Wave Velocity (PWV):
Carotid-femoral, carotid-brachial, and carotid-radial PWV will be measured non-invasively by recording carotid, femoral, brachial and radial artery pressure waveforms sequentially with an applanation tonometer (Non-invasive Hemodynamics Workstation, Cardiovascular Engineering, Inc.). Pressure waveforms are gated to the ECG R wave in order to calculate the transit time (t) between the foot of the carotid and the respective peripheral (femoral, brachial, radial) waveforms. The carotid-femoral transit distance (CFTD) is estimated between the 2 anatomical sites as the difference between the suprasternal notch (SSN) to carotid (SSN-C) and femoral (SSN-F) sites. Thus, the CFTD is calculated as CFTD= (SSN-F) - (SSN-C) and PWV calculated as CFTD/t (*CITE 1, 2). This approach accounts for parallel transmission of the pulse wave up the brachiocephalic and carotid arteries, and simultaneously along the aortic arch using the SSN as a fiducial point where parallel transmission begins (i.e. bifurcation site of aortic arch and brachiocephalic artery). The intra-subject reproducibility of carotid-femoral PWV is excellent with a coefficient variation of 2.1% for triplicate measurements on non-consecutive days in 7 young adults.
6) Carotid Artery Compliance (CC):
Carotid artery compliance and Beta-stiffness index will be determined noninvasively by high-resolution ultrasonography (Logiq 7, GE Healthcare) of the right common carotid artery and contralateral assessment of carotid artery blood pressure via non-invasive carotid artery applanation tonometry respectively. Carotid artery diameters are measured ~2 cm proximal to the carotid bulb with the transducer placed at a 90° angle to the vessel by off-line analysis of DICOM images with image analysis software (Medical Imaging Applications, LLC). Maximal diameters (i.e. systolic expansion) and minimal diameters (i.e. diastolic relaxation) are measured in sync with carotid artery blood pressure waveforms. Carotid blood pressure waveforms are calibrated using diastolic and mean brachial artery blood pressure obtained from standard brachial artery cuff blood pressure.
7) 24-hour Ambulatory Blood Pressure Variability and Baroreflex Sensitivity:
Twenty-four hour systolic blood pressure variability will be recorded from 24-hour blood pressure recordings using standard ambulatory blood pressure assessment (90207-IQ, Spacelabs Healthcare, Inc.). Baroreflex sensitivity will be determined by recording blood pressure and heart rate continuously for 15 minutes using beat-to-beat finger blood pressure (Finometer MIDI, Finopress Medical Systems). Blood pressure variability will be calculated

as the standard deviation of the systolic blood pressure over 24 hours, and baroreflex sensitivity will be calculated using the sequence technique as previously described.

8) Microneurography:
Direct intra-neural recordings of multiunit MSNA will be obtained from the right leg peroneal nerve using the microneurography technique as previously described. Briefly, a tungsten micro electrode (200 μm diameter shaft; 1-5 μm uninsulated tip) will be inserted into the peroneal nerve posterior to the head of the fibula by a physician or research staff trained in microneurography. Well-validated criteria are used to determine that a neurogram represents sympathetic activity to muscle or skin. The technique has been used safely in over 2000 studies since 1984 and it is well tolerated and reproducible. MSNA will be recorded for 30 minutes while subject is supine and quantified as burst frequency (bursts/minute) and bursts incidence (bursts/100 heartbeats). During MSNA recordings a 3-lead ECG, beat-by-beat blood pressure by finger plethysmography, and respiration will be monitored with a pneumobelt. Blood flow to the arm (brachial artery) will be measured using Doppler ultrasound to non-invasively measure mean arterial blood velocity and diameter. Two E4 wristbands (one on each wrist) will be worn by the participant to non-invasively measure sympathetic activity (electrodermal response) and beat-to-beat heart rate variability continuously. Also during the procedure subjects will undergo a mental math test and a cold pressor test. In the mental math test, the subject will be asked to subtract continuously the number 7 (or another random number) from a 3-digit number as quickly and as accurately as possible for 3 minutes. In the cold pressor test, the subject will be asked to place their hand in ice water for 2 minutes. This procedure will be used to cause transient changes in heart rate and blood pressure.

9) Retinal Vascular Measurements:
   - Visual acuity will be measured by having the subject read the smallest letters on an eye chart with their glasses or best correction. This takes about 5 minutes or less.
   - Intraocular pressure (IOP) may be done using a tonopen. A drop of a topical anesthetic will be placed in the eye as a numbing agent before IOP is checked. The tonopen touches the surface of the cornea of the eye very briefly. This will take a few minutes.
   - Beat-by-beat blood pressure will be measured by finger plethysmography. We will have the subjects sit quietly for a few minutes. This will take a few minutes.
   - Ocular Coherence Tomography (OCT): The thickness of the optic nerve and macula will also be measured inside of the eye using a special camera that forms an image of the layers of the retina The imaging is harmless and measures the thickness or structural health of retinal layers and optic nerve. This image will be compared to the LSFG for blood vessel comparison and identification. This test takes approximately 10 minutes.
   - Laser-Speckle Blood Flow Imaging (LSFG) with varying light stimuli. This device uses a Class I laser diode. Most subjects do not need dilation for this test, but those with smaller pupils may be dilated. Subjects will be seated at the instrument and we will adjust the chinrest and then let the subject rest with the lights dimmed for about 5 minutes before doing the test. Next, they will place their chin in the chinrest and look at a fixation target. This baseline test will take about 5-10 minutes. This test will be repeated following a light stimulus given to one eye using a handheld calibrated instrument in order to measure the blood flow response to activation of the retina by light. This will take another 5-10 minutes. This will be repeated several times followed by LSFG with no light stimulus at the end of testing. Two E4 wristbands (one on each wrist) will be

worn by the participant during LSFG to non-invasively measure sympathetic activity (electrodermal response) and beat-to-beat heart rate variability continuously.

Statistical Analysis Methods

Descriptive Analyses

Descriptive summary statistics will be provided for demographic and important baseline characteristics. For continuous variables, the number of patients, mean, standard deviation, median, minimum and maximum will be provided. For categorical variables, the number and percent of patients in each treatment arm will be summarized.

Primary Endpoint Analysis

Changes in our primary outcome of interest, anxiety as measured by the Beck anxiety inventory (BAI), will be assessed using a linear mixed effect model with intervention group as the fixed effect between-subjects factor of interest and repeated measurements (baseline, 6 weeks, 12 weeks) within-subjects of the anxiety inventory as the primary dependent variable. Individual participants will be initially modeled as a random effect including both an intercept term and, if necessary, slope. The random effect for slope will be removed for an intercept only model if the random slope does not substantively improve the model or if there are issues with model fitting. Fixed effects covariates for any sociodemographic or clinical variables that differ between groups despite randomization can be added.

Secondary Endpoint Analysis

State and Trait subscales of the STAI will be secondarily modeled as the dependent variable to assess changes in the domains of trait and state anxiety.

The novel physiological variables (vascular function, mSNA, inflammation) will serve as secondary outcomes and will be explored in analogous to the above. We will also assess for any relationship between these variables and changes in anxiety across groups in linear regression models (since these physiological variables are only measured at two points, there is no need for linear mixed models). For these latter analyses, variables for relevant sociodemographic and clinical confounds will need to be included as change in anxiety is not assigned by randomization akin to group assignment. Comparisons between the groups of higher anxiety and low anxiety will be made using one-way analysis of variance to determine differences in vascular function, mSNA, and inflammation. Baseline values for the higher anxiety group will be compared to the values obtained from the low anxiety group during their one-time experimental visit.

Sample Size

Using the estimating equations of Diggle et al. (2002) and based on three assessments for primary and secondary outcomes and a correlation of outcome measures over the duration of the study within individuals of 0.4, a total of 38 participants are needed per group to detect an effect size of 0.5 SD with 80% power at an alpha=0.05 (as calculated in SAS 9.3 using relevant formula). Assuming an 10% dropout rate our sample size would need to be adjusted to 38/(1-0.1)=43 per group using the formula from Friedman et al. (2010). A 0.5 SD difference on the anxiety inventory should represent a clinically significant effect. A corresponding change of similar magnitude on the better studied Hamilton Depression Rating Scale (not used in this study) has been considered clinically significant (Hegerl et al. 2012). Similarly, we are powered to detect clinically significant effects for the secondary outcomes of vascular function. We will

assess large vessel measures (e.g. pulse wave velocity), resistance vessel measures (forearm blood flow), and microvascular measures in the retina. In a study of brachial artery flow-mediated dilation (FMD), a reduction in FMD of 0.66 SD was associated with a 30% increased risk of cardiovascular events (Shimbo D et al. 2007). Similar results were seen in another population-based study wherein each 0.66 standard deviation in FMD was associated with an 18% increased risk of composite cardiovascular events and 36% increase risk of myocardial infarction, resuscitated cardiac arrest or coronary heart disease death (Yeboah J et al. 2009). Thus, we should be powered to detect clinically significant changes in vascular function.

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