Inorganic Nitrate Supplementation on Cerebrovascular Aging and Arterial Stiffness Study (INCA)

NCT03617302

6/23/23

INCA Study

PI: Gary Pierce
IRB ID #: 201805720

Project Details

I. Project Introduction

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

I.2 ***Project Title:***
Effects of Inorganic Nitrate Supplementation on Cerebrovascular Aging and Arterial Stiffness (INCA) Study

I.3 ***Short Title (optional):***
INCA Study

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

- The purpose of this study is to evaluate the effects of beetroot juice on 1) large elastic central artery stiffness and pulsatile pressure and flow hemodynamics; 2) cerebral blood flow (CBF) and cerebral pulsatility in middle-aged and older adults. Fifty-seven (57) middle aged and older adults (aged 50-79 years) will be recruited using mass email containing an online screening survey, advertisements to the University of Iowa community (noon news and 'volunteer research' clinical trials website on the UIHC websiteor from the studies Aging, Vascular Disease and Cognition (PI: David J. Moser, R03 AG047306-01, IRB#200301057), Targeting Inflammation in CV Disease study (PI: Gary L. Pierce, 1R21 AG043722, IRB# 201201739) and from the Comparison of Cerebral Blood Flow (CBF) Measured by MRI and Cognitive Function in Preeclampsia (COVA, PI: Gary Pierce, IRB#201808755) with follow-up contact by the research team. Individuals from these studies will only be contacted if they have agreed to be contacted regarding future research. Eligible participants will be invited to lab to undergo baseline vascular, neuroimaging and cognitive testing. Participants will complete vascular and neuroimaging testing following acute (single dose) dietary supplementation with nitrate-containing or nitrate-depleted beetroot juice. Blood draws will be performed to assess plasma nitrate levels. Deidentified blood samples will be sent to Jennifer Pollock, PhD, at the University of Alabama Birmingham to assess plasma nitrate levels.

I.5 ***Specify your research question(s), study aims or hypotheses (do not indicate "see protocol")***
Aim 1: Demonstrate the degree to which acute beetroot juice supplementation changes carotid artery stiffness and pressure and blood flow pulsatile hemodynamics in middle-aged and older adults.
Hypothesis: Acute beetroot juice supplementation will improve carotid artery stiffness index, pressure wave reflection and blood flow pulsatility index measured by carotid Doppler ultrasound and applanation tonometry in middle-aged and older adults.

Aim 2: Determine the extent to which acute inorganic nitrate supplementation alters endothelium-dependent flow-mediated dilation of the internal common carotid (ICA) in response to acute hypercapnia in middle-aged/older adults.
Hypothesis: Acute beetroot juice supplementation will improve the cerebrovascular response in the MCA to hypercapnia and augment endothelium-dependent ICA dilation measured by Doppler ultrasound in middle-aged and older adults compared with placebo.

Aim 3: Establish the degree to which acute inorganic nitrate changes basal CBF and pulsatility. We hypothesize that acute beetroot juice supplementation will augment basal CBF and reduced brain pulsatility in middle-aged and older adults.

Aim 4: Demonstrate the degree to which chronic (4 weeks) beetroot juice supplementation reduces carotid artery stiffness and pulsatile pressure hemodynamics, increases in basal and reactive global cerebral blood flow, and improves processing speed in middle-aged and older adults. Hypothesis 4A. Chronic (4-week) supplementation of beetroot juice will result in improvements in carotid artery stiffness, pressure wave reflection and blood flow pulsatility measured by carotid artery

Doppler ultrasound and applanation tonometry beyond a single acute dose. Hypothesis 4B. We hypothesize that chronic beetroot juice supplementation for 4 weeks will improve select cognitive domains such as processing speed, working memory and executive function.

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

Healthcare costs associated with dementia are estimated to be 60% greater than costs related to heart disease and cancer and are expected to rise as the prevalence of dementia increases dramatically from 5 to 14 million by 2050 (NIH). Unfortunately, prior clinical trials in the treatment or prevention of dementia have failed because studies have focused on targeting amyloid deposition, a downstream consequence in the pathogenesis of dementia. In contrast, support for a vascular contribution in the development of dementia is growing as the overlap between dementia phenotypes narrows between Alzheimer's disease and vascular dementia [1]. In support of this, cardiovascular and cerebrovascular risk factors such as age, hypertension and elevated large artery stiffness, including the aorta and carotid arteries [2, 3], are recognized as important risk factors for the development of dementia. Importantly our lab [4] and others [2] have demonstrated that carotid artery stiffness is a more sensitive predictor of select domains of cognitive aging (e.g., processing speed) compared with aortic stiffness. This is significant given the carotid arteries act to promote the coupling of cerebral blood flow (CBF) supply with metabolic demand as well as buffer the transmission of excessive pulsatile pressure and flow to the vulnerable cerebral microvasculature.

Age-related stiffening of the carotid arteries is mediated in part by a loss of vascular protective nitric oxide (NO) bioavailability and alters 'downstream' cerebrovascular hemodynamics and endothelial shear stress. Reduced NO bioavailability is hypothesized to mediate age-related declines in basal CBF regulation and CVR, however the degree to which carotid artery stiffness mediates these alterations remains unknown. This is mechanistically significant because elevated carotid artery stiffness and flow pulsatility are the strongest vascular correlates of cognitive decline in older adults. Taken together, the purpose of this project is to study the effects of beetroot juice on extracerebral and intracerebral hemodynamics.

Aim 1: Demonstrate the degree to which acute inorganic nitrate supplementation changes carotid artery stiffness and pressure and blood flow pulsatile hemodynamics in middle-aged and older adults.

Age-related increases in large elastic artery stiffness are hypothesized to contribute in part to the reductions in cognitive performance by increasing the transmission of pulsatile energy to the high flow, low resistance brain [3]. In support of this, carotid-femoral artery pulse wave velocity (cfPWV), the 'reference-standard' measure of aortic stiffness, is an independent predictor of longitudinal changes in cognitive performance [5] with one standard deviation increase in cfPWV equating to 1.2 years of cognitive aging [6]. Carotid artery stiffening with age occurs in part due to the loss of vascular protective nitric oxide (NO) bioavailability and contributes to 'downstream' cerebrovascular hemodynamics and endothelial shear stress. Therefore, maintaining NO bioavailability is critical because NO is synthesized by vascular endothelial cells and has important vasodilatory, anti-atherogenic and anti-thrombotic properties [7]. In addition to endogenous pathways, NO bioavailability can also be enhanced via the Nitrate-Nitrite-Nitric Oxide pathway through dietary supplementation of inorganic nitrate naturally found in leafy green vegetables such as spinach or beets [8, 9]. In this pathway, dietary consumption of nitrate is reduced to nitrite by commensal bacteria in the salivary glands and further reduced to NO by various endogenous nitrite reductases in the blood and other tissues [10]. In previous studies, inorganic nitrate supplementation reduced carotid pressure wave reflection in heart failure patients [11], with select studies demonstrating either reductions [12] or no statistical change in arterial stiffness [13]. However, of the studies that used the 'reference-standard' carotid-femoral PWV to quantify aortic stiffness, these studies did not statistically adjust for reductions in blood pressure, an important covariate of carotid-femoral PWV. This is mechanistically significant given blood pressure has been reduced by beetroot juice supplementation in some studies [12-15]. Furthermore, the degree to which inorganic nitrate supplementation improves carotid artery stiffness, pressure wave reflection hemodynamics and blood flow pulsatility in middle-aged and older adults is unknown. Therefore, the purpose of Aim 1 is to investigate the degree to which acute and chronic inorganic nitrate supplementation improves carotid artery stiffness and pressure and flow pulsatility in middle-aged and older adults. We hypothesize acute beetroot juice supplementation will improve carotid artery stiffness index (a blood pressure independent measure of carotid stiffness), pressure wave reflection, and flow pulsatility index(a result of stiffer artery and correlate of cognitive impairment) in middle-aged and older adults.

Aim 2: Determine the extent to which acute inorganic nitrate supplementation alters endothelium-dependent flow-mediated dilation of the internal common carotid (ICA) and middle cerebral artery (MCA) in response to acute hypercapnia in middle-aged/older adults.

Cerebral blood flow is regulated both by the vascular endothelium of the intracerebral and extracerebral vessels including the internal common carotid artery (ICA) [16]. The ICAs conduct 70% of blood flow to the brain. Assessment of peripheral endothelial function has been measured using flow-mediated dilation of the brachial artery for several decades however, until very recently there has been no validated measure of cerebrovascular endothelial function available [17]. In this method, carotid artery dilation is proportional to the upstream cerebrovascular response in the MCA following a hypercapnic stimulus and therefore represents a measure of cerebrovascular endothelial function. Previously, peripheral endothelial function was improved in middle-aged and older adults with increased CVD risk following 4 weeks of chronic inorganic nitrate supplementation [14]. However, the degree to which acute and chronic beetroot juice can improve cerebrovascular endothelium-dependent flow dilation of the ICA remains unknown. Therefore, we hypothesize that acute beetroot juice supplementation will improve the cerebrovascular response in the MCA to hypercapnia and augment endothelium-dependent ICA dilation in middle-aged and older adults compared with placebo.

Aim 3: Establish the degree to which acute inorganic nitrate changes basal CBF and pulsatility.

Vascular pulsatility is a strong vascular correlate of CBF [18] and cognitive performance [19,20]. Age-related increases in large elastic artery stiffness augment the transmission of pulsatile pressure and flow [18] to the high flow, low resistance brain. Vascular dysfunction, including cerebral pulsatility, is hypothesized to precede the development of 'downstream' amyloid-beta deposition, dysregulation of glucose metabolism and cognitive impairments. [21] The large cerebral arteries (i.e. Circle of Willis) propagate CBF to the microvasculature as well as buffers pulsatile pressure and flow transmitted by the heart during ventricular contraction. [22] Chronic exposure to pulsatile pressure and flow induces cerebrovascular remodeling via alterations in shear stress and the loss of vascular endothelium-dependent NO. [18,23] This is mechanistically important because NO is an important mediator in the age-related declines in basal CBF in the large arteries. [16, 24-26] Furthermore, reductions in the buffering capacity of the large cerebral vessels increases the penetration of pulsatile energy to the cerebral microvasculature and promotes cognitive impairment. [18] Therefore, beetroot juice supplementation may be a novel therapy for the attenuation of age-related increases in cerebrovascular pulsatility and reductions in CBF in part by augmenting the bioavailability of NO.

In support of this, inorganic nitrate supplementation improves peripheral artery endothelial function [27] and peripheral hypoxic microvascular blood flow [28] in adults, however, few studies have investigated the effects of inorganic nitrate supplementation on CBF or cerebrovascular pulsatility. These studies have demonstrated select regional [29] changes or no statistical change in basal CBF [30, 31]. However, these studies have considerable limitations in their respective study design including 1) young male participants only [30]; 2) non-gold standard neuroimaging modality used [30,31] that measured 3) microvascular CBF only [30,31]. Furthermore, no study has investigated the effects of inorganic nitrate supplementation on pulsatility. Therefore, we hypothesize that acute beetroot juice will improve basal CBF and pulsatility in the large cerebral vessels in middle-aged and older adults measured via 4D MRI.

Ami 4: Demonstrate the degree to which chronic (4 weeks) beetroot juice supplementation reduces carotid artery stiffness and pulsatile pressure hemodynamics, increases in basal and reactive global cerebral blood flow, and improves processing speed in middle-aged and older adults.
Processing speed is an early predictor of cognitive decline and is hypothesized to contribute to the declines of other domains of cognition such as working memory and executive function [32,33]. Maintenance of cognitive performance is dependent on the tight coupling between CBF supply with metabolic demand and is dependent in part on NO [34]. Therefore, targeted interventions aimed at improving the coupling of CBF supply and demand or improving cerebrovascular reserve may also lead to improvements in processing speed with chronic use. In this regard, we aim to assess the degree to which chronic beetroot juice supplementation improves processing speed in middle-aged and older adults. We hypothesize that chronic beetroot juice supplementation for four weeks will improve processing speed, which is hypothesized to mediate subsequent improvements in other

cognitive domains such as working memory and executive function, in middle-aged and older adults.

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

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II. Research Team

II.1 ***Principal Investigator***

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

II.2 ***Team Members***

UI Team Members

Name	E-mail	College	Contact	Key Prsn	UI COI	VAMC COI	Consent Process Involvement	Deactivated
Gary Pierce, PHD, MS	gary-pierce@uiowa.edu	College Lib Arts and Sciences	Yes	Yes	No		Yes	No

Non-UI Team Members

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

Nothing found to display.

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

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

Name	Is Key Personnel
Gary Pierce, PHD, MS	Yes

II.5 ***Select research team member who is the primary contact for study participants.***
Amy Marie Stroud

III. Funding/Other Support

III. ***Funding Sources***
1

Source Entered as Text	DSP Link	Type	Source	Grant Title	Name of PI on Grant
Source is entered as text no		Private Foundation/Association	American Heart Association	Inorganic Nitrate, Large Artery Stiffness and Cerebral Blood Flow in Aged Humans	Gary Pierce

* new source name

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

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

Name	Has Conflict of Interest
Gary Pierce, PHD, MS	No

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

Source	Status	Other Status Description
American Heart Association	Awarded	

IV. Project Type

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

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

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

V. Other Committee Review

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

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

- Yes

V.1.a ***What is/are the substance(s):***
SuperBeets concentrate will be used for the experimental beverage: 20g of active beetroot powder (Superbeet, HumanN) will be mixed in 120-180mL of water. Each dose will contain 500 mg (~8.06 mmol) of nitrate and 40 mg (~0.58 mmol) of nitrite.

The nitrate-depleted control beverage will be made identical to above only Beet Essence, a nitrate-depleted powder, will be substituted.

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)?***
No

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?***
No

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

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

Nitrate-depleted beetroot juice (Beet Essence Beetroot concentrate)	
Name of Sponsor	
Investigator's Brochure Version	Waiver
Investigator's Brochure Date	
Who is supplying the drug	
Who is dispensing the drug	
Planned Use in this Study	
Condition/Disease Indication(s)	Aging
Subject Population	Middle-aged and older adults
Dose(s)	20 grams of powder diluted in 120-180 mL of water
Administration	Oral
Dosing Regimen	1 dose per day
FDA Approved Use	
No approved use	
Is this study intended to be reported to the FDA as a well-controlled study in support of a new indication or a significant change in the labeling for this product?	No
Is this study intended to support a significant change in the advertising for this product?	No

Does this planned use of the product in this study, taking into consideration the route of administration, the dosage level, and the subject population, significantly increase the risk (or decrease the acceptability of the risk) associated with the use of this product?	No
Rationale:	This dietary supplement will be used as a control because it is depleted of nitrate/nitrite. 10 grams of BeetEssence (2 servings) will contain 40 kcal, 10 mg of sodium, 9.4g of carbohydrate, 4.6g of sugar and 5.6g of calcium. BeetEssence is made from organic beet juice powder (beta vulgaris) and organic maltodextrin. See 'Beet Essence Nutrition Label' for the nutrition label provided by the manufacturer or the manufacturer website (https://greenfoods.com/collections/greens/products/beet-essence. Additionally, we submitted an IND application to the FDA. The FDA decided to waive the IND requirement, using their regulatory enforcement discretion, and the absence of safety concerns. See the miscellaneous category attachment 'FDA Response_ IND 140893 Advice-Information Request (Final) (2).pdf'

Nitrate containing beetroot juice (SuperBeets Beetroot concentrate)	
Name of Sponsor	
Investigator's Brochure Version	Waiver
Investigator's Brochure Date	
Who is supplying the drug	
Who is dispensing the drug	
Planned Use in this Study	
Condition/Disease Indication(s)	Aging
Subject Population	Middle-aged and older adults
Dose(s)	20 grams of powder diluted in 120-180 mL of water
Administration	Oral
Dosing Regimen	1 dose per day
FDA Approved Use	
No approved use	
Is this study intended to be reported to the FDA as a well-controlled study in support of a new indication or a significant change in the labeling for this product?	No
Is this study intended to support a significant change in the advertising for this product?	No
Does this planned use of the product in this study, taking into consideration the route of administration, the dosage level, and the subject population, significantly increase the risk (or decrease the acceptability of the risk) associated with the use of this product?	No
Rationale:	Numerous published research studies [Kapil et al., Hypertension, 2015; McDonagh et al., Nitric Oxide, 2017; London-Hoyos et al., Physiology Measurement, 2018] have used beetroot juice without adverse events (other than red/pink urine/stool) including recent publications at Iowa [Bock et al., JAP Heart Circ Physiology, 2018; Schneider et al., Nitric Oxide, 2018] which uses an identical beetroot protocol to

	this one. This dose of SuperBeets will contain 250 mg (~4.03 mmol) of nitrate and 20 mg (~0.29 mmol) of nitrite, approximately equivalent to eating a large spinach salad [Bock et al., JAP Heart Circ Physiology, 2018] in addition to 130mg of sodium, 8g of carbohydrates, 6 g of sugar and 2g of protein. SuperBeets beetroot powder concentrate is made from Non-GMO beetroot powder, non-GMO beetroot powder (fermented), natural apple flavor, malic acid, ascorbate, Stevia leaf extract. See 'SuperBeets Nutrition Label' for nutrition info or the manufacturer website (https://www.humann.com/products/superbeets-superfood/. Additionally, we submitted an IND application to the FDA. The FDA decided to waive the IND requirement, using their regulatory enforcement discretion, and the absence of safety concerns. See the miscellaneous category attachment 'FDA Response_ IND 140893 Advice-Information Request (Final) (2).pdf'

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?***
Yes

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

VI. Subjects

VI.1 ***How many adult subjects do you expect to consent or enroll for this project?***
76

VI.2 ***What is the age of the youngest adult subject?***
50.0

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***

Participants: Seventy-six (n=76) adults between the ages of 50-79 years will be recruited from the Iowa City, Iowa community using mass email containing an online screening survey, advertisements to the University of Iowa community (noon news and 'volunteer research' clinical trials website on the UIHC website https://uihc.org/clinicaltrials/studies/), or from the following studies: Aging, Vascular Disease and Cognition (PI: David J. Moser, R03 AG047306-01, IRB#200301057), Targeting Inflammation in CV Disease study (PI: Gary L. Pierce, 1R21 AG043722, IRB# 201201739), Comparison of Cerebral Blood Flow (CBF) Measured by Arterial Spin Labeling (ASL) MRI and Quantitative Water O15 PET (PI: Laura Ponto, IRB# 201801811) and Cognitive Function in Preeclampsia (COVA, PI: Gary Pierce, IRB#201808755. Individuals being contacted from the perviously mentioned studies will be contact by the research team via email (see TIVA, INI and AVD email letter) only if they have previously indicated they would like to be contacted regarding future studies. All individuals interested in the study will be asked to contact research staff by phone or email. All individuals interested in the study will be invited to complete the RedCap eligibility survey sent via email. If an individuals appears to be eligible for the study based on their RedCap Eligibility survey responses, a member of the research staff member will contact them via phone or email to complete to tell the individual more about the study, answer any questions and perform a phone screening to determine eligibility.

- After our interim analysis, the data demonstrate a trend for a decrease in our primary outcome variable (carotid artery stiffness) after 4-weeks beet juice supplementation in older adults with carotid-femoral pulse wave velocity > 7.6 m/s. About 1/3 of our participants had carotid-femoral pulse wave velocity >7.6 m/s, therefore we wish to recruit and randomize 30 more subjects to further test our findings that beet root juice has a beneficial effect on carotid artery stiffness in older adults with carotid-femoral pulse wave velocity > 7.6 m/s. The justification for recruiting 30 more subjects is because we will be screening for carotid-femoral pulse wave velocity > 7.6 m/s after consenting the subjects, so we are accounting for 1/3 of the subjects to meet eligibility criteria of carotid-femoral pulse wave velocity > 7.6 m/s and randomize 10 more subjects. We plan to screen subjects for carotid-femoral pulse wave velocity > 7.6 m/s during visit 1, prior to randomization, to determine eligibility (described further in section VII.E.6).

Pre-Consent Phone Screen:
A study team member will perform a phone screening (see attached phone screening form) to confirm their RedCap survey responses and collect some additional information regarding eligibility. If they are determined to be eligible and they are interested in participating in the study, the research staff will schedule them for the Visit 1.

Visit 1 Consenting: A member of the research staff will provide a detailed explanation of the study, review the informed consent and inform the individual of all potential risks of the study. The research staff will answer all questions asked by the potential subject before the subject signs the consent. Individuals will in

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

• Eligible subjects will be randomized to either experimental or placebo-controlled arms according to a 1:1 scheme. Inclusion/exclusion criterion specified as below:

Inclusion criteria:
• Age 50-79 years
• Cognitively healthy, having mild cognitive impairment based on Alzheimer's Disease Neuroimaging Initiative (ADNI-II) clinical criteria
• Able to undergo cardiovascular testing procedures including fasting overnight and holding selected morning medication doses.
• Ability to understand and willingness to sign a written informed consent document.
• Ability to lie comfortably for up to 90 minutes
• Women only: Post-menopausal
carotid-femoral pulse wave velocity >7.6 m/s

Exclusion criteria:
• Current or history of CVD disease (heart attack, stroke, heart failure, cardiomyopathy or peripheral artery disease, heart angioplasty/stent or bypass surgery, valve replacement, carotid endarterectomy, heart transplant.
• Medical history of stroke or other neurological disorder or systemic illness that could potentially affect cognition or brain function (outside of a diagnosis of MCI, Alzheimer's Disease) or could affect their safety or comfort while undergoing the imaging or cardiovascular studies.
• Subjects with evidence of cardiovascular disease at baseline or during the exercise test (evidence of myocardial infarction, abnormal cardiac arrhythmia, myocardial ischemia, conduction delays, >1mm ST segment depression or elevation; >3 beat ventricular tachycardia; atrial fibrillation) will be excluded from the study.
• Wilson's disease, hemochromatosis
• Individuals taking clonidine or other short-acting beta blocker
• Resting blood pressure > 200/ 110 mmHg or systolic <90 mmHg
• Medical history of chronic major psychiatric or current diagnosis of major psychiatric disease (other than dementia).
• Systemic illness or neurological disorder potentially affecting cognition or cerebral blood flow other than mild cognitive impairment
• Unable to provide informed consent due to cognitive impairment
• Currently taking medications that may affect cerebral blood flow (e.g. papaverine, indomethacin, acetazolamide, etc) or efficacy of beetroot juice (proton pump inhibitors)
• Current clinically abnormal thyroid function not adequately regulated by thyroid hormone supplementation or medication.
• Allergic to beets
• Current tobacco user or history of tobacco use within the past 3 months (cigarettes, cigars, chewing tobacco, hookah, electronic cigarettes) or living with someone who smokes/has smoked in the past 3 months.
• Current diagnosis of diabetes (Type I or insulin dependent Type II)
• Current diagnosis of COPD, cystic fibrosis, emphysema, chronic bronchitis
• History of renal failure, dialysis or kidney transplant
• Current diagnosis or history of liver disease or HIV/AIDS, or cancer requiring chemotherapy or radiation.
• Current diagnosis or history of rheumatoid arthritis, systemic lupus erythematosus, Wegener's granuomatosis
• Vulnerable populations (prisoners, etc) will not be eligible.
• Unwillingness to wash out from a vitamin or dietary supplement regime prior to enrollment and maintain throughout the duration of the study.
• Inability to comply with experimental instructions.
• Uncontrolled intercurrent illness that would limit compliance with study requirements per investigator.
• Inability to fast or hold morning medications doses until after testing is complete.
• Hormone replacement use within the past 6 months
• Currently enrolled in another study using an study medication, supplement, device or intervention.
• Initiation of a new prescription medication or change in dose/frequency in the past three months. Individuals will be become eligible after the medication/dose/frequency has been stable for 3 months.

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)***

In Johnson county and the adjacent 8 counties, the 2010 U.S. census indicates that there are 133,475 adults between the age of 50-79 and 139,165 between the ages of 18-39. We estimate that approximately 2/3 of the adults age 50-79 in Johnson and adjacent counties have clinical disease or on medications that would make them ineligible for our study. Therefore, we estimate that approximately 44,045 adults age 50-79 in Johnson and adjacent counties would be eligible for our study (9500 in Johnson County alone).

VI.15 ***Describe how you will have access to each of your study populations in sufficient number to meet your recruitment goals.***

Participants will be recruited from the University of Iowa and the Iowa City, Iowa community by email and Noon News, a newsletter available on campus at UIHC, and from the study Aging, Vascular Disease and Cognition (PI: David J. Moser, R03 AG047306-01, IRB#200301057), Targeting Inflammation in CV Disease study (PI: Gary L. Pierce, 1R21 AG043722, IRB# 201201739) and from the Comparison of Cerebral Blood Flow (CBF) Measured by Arterial Spin Labeling (ASL) MRI and Quantitative Water O15 PET (PI: Laura Ponto, IRB# 201801811, and Cognitive Function in Preeclampsia (COVA, PI: Gary Pierce, IRB#201808755. Individuals being contacted from the perviously mentioned studies will be contact by the research team via email (see TIVA, INI and AVD recruitment email)only if they have previously indicated they would like to be contacted regarding future studies. This study will also be advertised on the UIHC website under "volunteer research" on the clinical trials webpage https://uihc.org/clinicaltrials/studies).

VI.16 ***Do you plan to recruit/enroll non-English speaking people?***

No

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

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

- No

VI.20 ***Will subjects provide any information about their relatives?***

No

VI.23 ***Will anyone (other than the subject) provide you with information about the subject (e.g. proxy interviews)?***

No

VI.26 ***Is this project about pregnant women?***

No

VI.27 ***Will this project involve fetuses?***

No

VI.28 ***Does this project involve adult subjects who may be incompetent or have limited decision-making capacity on initial enrollment into the study?***

No

VI.32 ***Does this project involve subjects whose capacity to consent may change over the course of the study?***

No

VI.37 ***Does this project involve prisoners as subjects?***

No

VII.A. Project Description (A)

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

- CRU
- UIHC - C202/204 GH
- Other UI campus site - E141 Field House, E101A Field House, 528 Field House

VII.A.2 ***Is this project also being conducted by other researchers at their own sites (e.g. a multi-site collaborative project)?***

No

VII.B. Project Description (B)

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

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

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

- ☑

Phase I trials – include initial studies to determine the metabolism and pharmacologic actions of drugs in humans, the side effects associated with increasing doses, and to gain early evidence of effectiveness; may include healthy participants and/or patients (ClinicalTrials.gov & FDA)

- ☐

Phase II trials – include controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks(ClinicalTrials.gov & FDA)

- ☐

Phase III trials – include expanded controlled and uncontrolled trials after preliminary evidence suggesting effectiveness of the drug has been obtained, and are intended to gather additional information to evaluate the overall benefit-risk relationship of the drug and provide an adequate basis for physician labeling(ClinicalTrials.gov & FDA)

- ☐

Phase IV trials – studies of FDA-approved drugs to delineate additional information including the drug's risks, benefits, and optimal use(ClinicalTrials.gov & FDA)

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

VII.B.3 ***Describe the management plan, including when you would stop the subject's participation in the event the subject worsens during the washout period.***
If participants are on over the counter antioxidant, herbal supplements, vitamins, omega-3 fatty acids, they may be in the study but will be asked during the consenting process to go off the supplements/vitamins for 1 week before participating and for the duration of the study. If subjects are unwilling or medically unable to go off these supplements/vitamins for 1 week and during the course of the study, they will be ineligible to participate in the study and not consented. If they are willing to go through the 1-week washout, they can sign the consent during Visit 1 but will have the Visit #2 procedures scheduled to be completed 1 week following the washout. If the subject is unclear if they can medically go off the supplements/vitamins, they will be required to contact their personal physician to confirm they can go off any of the prescription medications listed above for the study duration.

VII.B.4 ***Describe the method (phone/in person) and frequency of contact with the subject during the washout period.***
After the subject signs the informed consent and agrees to go off the aforementioned supplements/vitamins for 1 week, they will be called by the study coordinator on the phone after 1 week of the washout and invited back to the CRU for Visit #2.

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

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 Trials and Research Website where volunteers are able to learn about different clinical trials being performed at the University (https://uihc.org/clinicaltrials/).
- E-mail -
- Advertisements -
- Other - Subjects from "Aging, Vascular Disease and Cognition" study, Targeting Inflammation in CV Disease (TIVA) study, the Comparison of CBF Measured by ASL MRI and Quantitative Water O15 PET and Cognitive Function in Preeclampsia study may be contacted if they have indicated they wish to be contacted regarding future studies. Individuals being contacted from the perviously mentioned studies will be contact by the research team via email (see TIVA, INI and AVD email letter).

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 in the ICTS Clinical Research Unit (CRU) or in the Iowa Translational Vascular Physiology lab (C202/204 GH) or 528 Field House.

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:***
Yes, after viewing a mass email, Noon News advertisement or email to previous study participants, the potential subject will complete the online screening survey. Research staff will discuss the project over the phone with individuals and collect a brief health history in order to determine eligibility over the phone. This will take place in the research office in 520FH. During the screening call, the research team member will answer any questions and describe the study to the individual as needed. If the individual 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 1).

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

Name	Consent Process Involvement
Gary Pierce, PHD, MS	Yes
Matthew Armstrong, PHD	No
Eric Axelson, BSE	No
Thorarinn Bjarnason, MD	No
Kyle Bos, BA	Yes
Donald Brown, MD	No
Josh Cochran, BS	No
Kristen Davis, MS	No
Jackson Ernst, High School	Yes
Colin Gimblet, MS	Yes
Lauren Hopkins, PHD	No
Karin Hoth, PHD	No
Diana Jalal, MD	No
Tim Koscik, BS	No
Vincent Magnotta, PHD	No
David Moser, PhD, PHR	No
Michael Muellerleile, MD	No
Virginia Nuckols, MS	Yes
Laura Ponto, PHD	No

Mark Santillan, MD	No
Steve Slevinski, BS	No
Meaghan Smith, High School	No
Emma Somers, BS	No
Ryan Staub, High School	No
Amy Marie Stroud, MSN	Yes
Samalya Thenuwara, High School	No

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

- Consent Document

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

No

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

Yes

VII.D.20 ***List any screening questions you will directly ask the potential subject to determine eligibility.***

Email advertisement with eligibility survey:
Participants will be invited to complete a survey to determine their eligibility. Individuals will receive this survey via a mass email advertisement containing a link to the survey or will be invited to fill out the survey by a member of the research staff if they respond via telephone or email that they are interested in the study. Below are questions that are asked on the online screening form. Please see the PDF (name: INCA_Online PreScreen Survey or link: https://redcap.icts.uiowa.edu/redcap/surveys/?s=EW4JL3LC9P)

Please complete the survey below.
Please complete the survey below to determine your eligibility for our study. You do not have to answer questions you do not wish to answer during this screening survey. However, failure to answer some of the screening questions may exclude you from participating in the study. Thank you in advance for your interest!

The purpose of this study is to determine the effects of acute (i.e. single dose) and chronic (i.e. 4 weeks) beetroot juice consumption on blood vessel function in middle-aged and older adults between the ages of 50-79 years. Beetroot juice contains dietary nitrate and nitrite which have been shown to increases nitric oxide bioavailability that is normally reduced with aging. Nitric oxide is an important molecule regulating blood vessel function. This study is being created and maintained by members of the University of Iowa Translational Vascular Physiology Research Lab, Department of Human Physiology, University of Iowa.

Seventy-six people will take part in this study at the University of Iowa. Participation in any study is voluntary. Submitting your name does not obligate you to respond to any requests for study volunteers. If you would like to determine your eligibility, please read the following summary of information we are requesting.

If you agree to fill out this brief survey to determine eligibility for the INCA study, we will ask you to provide information pertaining to your medical history including cardiovascular diagnoses, history of lung disorders, kidney and liver disorders, autoimmune disorders, history of neurologic disorders, previous smoking habits, height, weight current medications and willingness to participate in a research study.

If you do not wish to participate in the study, do not provide your information.

We will keep information you provide confidential. Only the Pierce Research Team has access to the data in the survey. Your information is kept in a password-protected database. The information you provide for us will only be used for the purpose of contacting you for a possible future research study. Your contact information will not be sold or distributed to any outside party or institution. If you are deemed ineligible your information will be destroyed.

If you have any questions about the INCA study itself, contact the Pierce lab at 319-356-2710. If you have questions about the rights of research subjects, please contact the Human Subjects Office, 105 Hardin Library for the Health Sciences, 600 Newton Rd, The University of Iowa, Iowa City, IA, 52242-1098, 319-335-6564, or by email at irb@uiowa.edu. To offer input about your experiences as a research subject or to speak to someone outside the research staff, call the Human Subjects Office at the number above.

Thank you very much for your consideration.

Sincerely,

Gary Pierce, PhD
Iowa Translational Vascular Physiology Research Lab
Department of Human Physiology
University of Iowa
(319)-335-9488

1. After reading the above text, would you like to be screened for eligibility for the INCA study? Yes/ No
2. Where did you first hear about the study? Flyer, Newspaper ad, friend, Noon News, UIowa Email, Other
3. Are you between the ages of 50-79 years? Yes/no
4. Do you currently have or have a history of cardiovascular disease such as heart attack, stroke, congestive heart failure, heart angioplasty/stent, bypass surgery or cardiomyopathy? Yes/no
5. Do you currently have or have a history of valvular disease, peripheral artery disease, diabetes type I or type II, bleeding disorders, connective tissue disease? Yes/no
6. Do you currently have or have a history of chronic obstructive pulmonary disorder (COPD) such as lung emphysema or chronic bronchitis, organ transplant, kidney disease, liver disease, gastrointestinal or stomach disorders such as Crohns, inflammatory bowel disease? Yes/no
7. Do you currently have or have a history of serious neurological disease or disorders, treatment of major psychiatric conditions within the past year, brain aneurysm or clip? Yes/no
8. Do you currently have or have a history of rheumatoid arthritis, systemic lupus, erythematous, Wegener's granulomatosis or any other autoimmune disease? Yes/no
9. Have you ever been diagnosed with a medical illness such as cancer (that required chemotherapy or radiation), abdominal aortic aneurysm, hepatitis B or C, or HIV/AIDs? Yes/no
10. Have you used any tobacco products (i.e., cigarettes, cigars, chewing tobacco, Hookah) within the past 3 months? Yes/no
11. Are you currently taking prescription medications for: Psychiatric illnesses or neurological disorders, dementia, seizures, pulmonary disorders such as COPD, cystic fibrosis, emphysema, chronic bronchitis, kidney disease, inflammatory disorders, acetazolamide, indomethacin, ginkgo, clonidine?
12. Are you currently taking any prescription vitamins, supplements, minerals that you are medically unable to go off of? yes/no
14. Have you started any new medications or changed current medications dosage in the past three months? Yes/no
15. Are you postmenopausal? Yes, no; NA If yes, have you been on any hormone replacement therapy within the past 6 months? Yes/no
16. What is your height, weight
17. Are you willing to: Fast overnight, take a study supplement for 4 weeks, to hold morning blood pressure or vasoactive medications on the morning of testing, washout of any nonprescription vitamin, minerals, supplements for the duration of the testing, have your blood drawn, record food intake for 1 day, eat foods low in nitrate one day prior to experimental visits. Yes/No
18. Do you have any metal in your body including metal plates or screws in your head, face or neck, pacemaker(s), brain aneurysm clip or become claustrophobic easily? Yes/No

Thank you for your time and interest in our study. You will be contacted by a research team member at the contact information provided if your responses indicate you may be eligible for the study. Please indicate below if you agree to be contacted by a research staff member if you are deemed to potentially be eligible for the study. Thanks again and have a nice day!
- The Iowa Translational Vascular Physiology Lab
20. May a member of the research staff contact you via telephone if you may be eligible for the study based on your survey responses? Yes/ No
Information to be collected to for the pre-phone consent phone call if their survey responses deem they may be eligible:
First name
Last name
Email address
Phone number
Preferred mode of contact: email/phone
Preferred time of day to contact

Below are questions that are asked on the pre-consent phone screening form (see attachment Pre-consent Phone Screening_7.24.18) 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. Date of birth will be collected to confirm in EPIC they are not enrolled into another research study that would make them ineligible for the present study.

"Thank you for your interest in our research study. Based on the online screening survey, you are eligible to participate in our study. However, we will need to complete a second screening survey to ensure your eligibility.

Before proceeding with the survey, let me tell you a little bit more about our study to hopefully answer any questions you may have. Our study is aiming to investigate the effects of beetroot juice on blood vessel function and cerebral blood flow. If you are eligible and willing to participate, your participation would require 3 visits ranging in total time from 3-5 hours, to UIHC over the course of 5-8 weeks. Some visits will require a blood draw. Our goal is to study the effects of beet root juice on blood vessel function and your brain blood flow. To measure this, you will consume a dose of beet root juice in our lab and we will measure your blood vessel function after this single dose and again after you have consumed beet root juice or the placebo for four weeks.

You will be randomly assigned to one of two groups: one group will receive beetroot juice and the other group will receive a nitrate-depleted beverage. All pre- and post-intervention testing will be the same between the two groups. You will be compensated for each visit you complete. Compensation for the entire study is $260 if you complete all three visits.

1. Do you have any questions?
2. Does this sound like something you'd be interested in? Yes No
If no, thank them for their interest in the study and let them know they are not eligible for the study.
If yes, proceed to following questions:
3. We would like to collect some additional information regarding your current and past health history to confirm our eligibility for this study. This survey will collect information regarding your height, weight and information regarding your medical history including cardiovascular diagnoses, history of lung disorders, kidney and liver disorders, autoimmune disorders, history of neurological disorders, previous smoking habits and willingness to participate in a research study. You do not have to answer questions you do not wish to answer during screening. However, failure to answer some of the screening questions may exclude you from participating in the study." Would you like to complete this phone screening to determine your eligibility? Yes No

If no, thank them for their interest in the study and let them know they are not eligible for the study.
If yes, proceed to following questions:

Contact information:
First Name
Last Name
Email Address
Phone Number
Preferred mode of contact?
Preferred time of day to contact?

Demographics: What is your age? Birthdate?
Height
Weight
BMI
1. Are you between the ages 50-79 years?
2. Are you currently participating in another clinical research study at the University of Iowa or elsewhere? (Yes/no)If yes, what study?
3. Allergies: Are you allergic to beets? (Yes/No);
4. Do you have an allergy to latex or any other medications? (Yes/No)

Medications:
What prescription medications are you currently taking? ? Include the frequency (e.g. qd, prn), dosage and reason for taking

Have any of the medications you are on changed in dose or frequency within the past 3 months?
YES NO If yes, please describe:

Are you planning to start any new medications in the next 4-6 weeks?
YES NO If yes, please describe:

Are you on any over-the-counter medications, supplements, vitamins, minerals, etc.? Include the frequency (e.g. qd, prn), dosage and reason for taking

Medical History:
Have you smoked or used any type of tobacco within the past 3 months? YES NO

Now I'm going to read you a list of disease and conditions. I will group several diseases and conditions together at once. If you currently have or have a history of one of the diseases or conditions I mention, don't say anything when I name the disease or condition. Wait until I get through the whole list and then you can say, 'Yes'. I'm not suppose to know which diseases you have, just if you have any of them, so wait until I finish the list before saying 'yes' or 'no.'

Do you currently have or have a history of any of the following conditions or diseases?
Cancer (now or in the past) that required chemotherapy or radiation
Lung Disease
Kidney disease
Diabetes type I or type II
Hemochromatosis or Wilson's disease
Blood clots

Do you currently have or have a history of any of the following conditions or diseases?
Heart attack, angioplasty, stent, bypass surgery
Heart valve disease
Heart valve replacement
Congestive Heart Failure
Peripheral Artery Disease
Chest pain or tightness or pressure upon physical exertion or emotional stress

Do you currently have or have a history of any of the following conditions or diseases?
Fibromyalgia/lupus
Rheumatoid arthritis
Immunodeficiency or systemic autoimmune disease
Gastrointestinal or stomach disorders
Vasculitis

Do you currently have or have a history of any of the following conditions or diseases?
Bleeding disorders or conditions the microcirculation
Connective tissue disease
Organ (heart, lung, kidney, liver) transplant
Orthopedic problems that prevents you from completing a bike test?

Do you currently have or have a history of any of the following conditions or diseases?
Treatment of psychiatric disorders within the past year?
Seizures or epilepsy?
Neurological disorders such as Parkinson's disease or Multiple Sclerosis?

Questions for women only: Yes No NA
Are you post menopausal? If yes, when did you reach menopause?
Have you taken hormone replacement therapy within the past 6 months?

Do you currently or plan to: Yes/No
1. Currently using an investigational or study medical device or drug?
2. Plan to enroll in an investigational or research study (other than this one) using a study medical device or drug in the next 6 weeks?

Current Health History: Yes No
Are you willing to fast overnight for 8 hrs?
Are you willing to take a study supplement?
Are you willing to have an IV place?
Are you willing to have your blood drawn?
Are you willing to record your food intake for 1 day?
Are you willing to eat foods low in nitrate one day prior to all experimental visits?

Are you willing to hold AM blood pressure or vasoactive medications on the morning of testing?
Are you willing to washout of any non-prescription vitamins, minerals and supplements for the duration of testing?

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

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

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

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

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

VII.D.26 ***List and describe screening***
- Health history survey
- WMS-III Memory and MMSE
- Maximal exercise VO2max test with 12-lead EKG
- Thyroid TSH and/or reflex T4 to determine thyroid status
-carotid-femoral pulse wave velocity

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

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

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

- ***Describe each study population separately including control population***
- ***Include when recruitment and consent materials are used***
- ***Use 3rd person active voice "The Principal Investigator will identify subjects. For example, the principal investigator will identify potential subjects, the study coordinator will discuss the study with subjects over the telephone and schedule the first study visit, etc..."***

- ***Describe the steps that will be taken by the research team to minimize the possibility of coercion or undue influence during the consent process***

The subjects will consist of middle-aged/older adults (50-79 yrs) who will undergo randomization to either beetroot juice or nitrate depleted beet root juice. The PI and research staff will recruit subjects from the community via flyers, emails to the UI community, "Noon news" and UIHC website advertisements and from the "Atherosclerotic Vascular Disease and Amyloid Dysregulation" Study (PI: Dr. David Moser, IRB#201104739) and Targeting Inflammation in CV Disease (TIVA) study, (PI: Dr. Gary Pierce, IRB# 201201739), and from the Comparison of Cerebral Blood Flow (CBF) Measured by Arterial Spin Labeling (ASL) MRI and Quantitative Water O15 PET (PI: Laura Ponto, IRB# 201801811), Cognitive Function in Preeclampsia (COVA, PI: Gary Pierce, IRB#201808755). Individuals being contacted from the perviously mentioned studies will be contact by the research team via email (see TIVA, INI and AVD email letter)only if they have indicated they wish to be contacted regarding future research opportunities. The purpose of the email will be to inform them of this new study and invite them to complete the online survey to determine eligibility.

For potential subjects who view the email advertisement will be invited to complete a brief Redcap survey via a linked contained in the survey to determine eligibility. If subjects are believed to be eligible according to the online survey, a study team member will contact the potential subject regarding the study via telephone or email. A study team member will discuss the study with the potential subject and answer any questions about the study. A study team member will also perform a phone screening (see attached for INCA Phone Screen) to determine eligibility. If the potential subject is eligible, they will be scheduled for their Visit 1, which will include a detailed explanation of the study and review of the informed consent document.

For potential subjects who view study flyer or advertisements or the UIHC Clinical Trials and Research Website advertisement, they will be invited to contact a study team member by phone or email. The potential subject will be sent an email contain a link to the Redcap survey to determine eligibility.

Potential subjects who are believed to be eligible based on their Redcap responses will be contacted by a study team member by phone to complete a pre-consent phone screening (see attached for INCA Phone Screen) to determine eligibility. If they are believed to be eligible and they are interested in the study, the research staff will schedule them for their Visit 1, which will include a detailed explanation of the study and review of the informed consent document.

During the potential subject's Visit 1, the study coordinator or research staff will answer all questions asked by the potential subject and the subject will be informed of all potential risks before the subject signs the consent document. Subjects will in no way be coerced to sign the consent form and will be informed that it is their choice whether to volunteer for this study. Even after subjects sign the consent, they are free to withdraw from the study at any time for any reason.

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)?***

- No

VII.E. Project Description (E)

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

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

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

VII.E.1.c ***Describe the circumstances under which subjects will be told what study arm they have been assigned.***
Subjects will be told which experimental beverage they were assigned to at the completion of the study.

VII.E.2 ***Describe randomization scheme/assignment including ratio such as 1:1, 2:1 etc.***

Subjects will be randomized following a 1:1 randomization scheme to consume either beetroot juice (experimental beverage) or nitrate-depleted beetroot juice (control beverage).

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)***

NIH Toolbox cognitive battery
Demographics and Health History questionnaire
1-day dietary record
Cognitive Reserve Survey (CRIq)
Letter/Pattern comparison
Stroop Color and Word test
Trail Making Test A & B
Pregnancy history survey
Menopause history survey

Note: demographics and health history questionnaire and the pregnancy history survey will be administered online through Redcap.

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***

Seventy-six (n=76) middle-aged and older adults will be recruited (see power calculations for rationale). Participants will complete baseline vascular, cognitive and neuroimaging testing. Participants will be enrolled and randomized to 4 weeks supplementation of beetroot juice (experimental beverage) or nitrate-depleted beetroot juice (control beverage) in a randomized (1:1), double-blind, placebo controlled study design. Testing will be done pre supplementation and post supplementation of either beetroot juice or nitrate depleted control beverages (vascular and neuroimaging). The order of visits are as follows:

Visit 1 (3.5 hours): Screening and consent
Prior to the study visit we would ask the participant to do the following prior to arrival:
• Fast for at least four hours.
• Refrain from caffeine and smoking for at least 4 hours prior to visit.
• Refrain from alcoholic beverages for at least 24 hours prior to visit.
- • Refrain from moderate or vigorous exercise for at least 24 hours

• Explanation of study; reading and signing of informed consent
• Subjects will complete cognitive testing
• The participant will complete the NIH Cognitive Battery Toolbox on an iPad.
• Blood testing: TSH and T4
• Subjects will complete maximal cardiopulmonary exercise test with 12-lead EKG and cardiopulmonary gas analysis for determining VO2max in the research Exercise Physiology Lab in E141 Field House (see methods for more details).
• Subjects will complete measurement of carotid-femoral pulse wave velocity which takes about 15 minutes (see methods below). Subjects with carotid-femoral pulse wave velocity > 7.6 m/s will be eligible for randomization.
• Subjects on over the counter antioxidants, herbal supplements, vitamins, omega-3 fatty acids, will be asked to go off the supplements/vitamins for 1 week before participating and for the duration of the study.

If subjects are unwilling or medically unable to go off these supplements/vitamins for 1 weeks and during the course of the study, they will be ineligible to continue, but they will have already been screened out before signing the consent form. If a subject is eligible and consents but requires a 1-week washout of vitamins/supplements, they will be invited back to the lab for Visit 2 after completing the 1-week washout.
• Subjects will be given a list of foods/beverages to avoid, consume in low quantities and 'okay' to consume prior to experimental visits. Subjects will be recommended to eat from this list 24-hours prior to each experimental visit (V2, V3).
• Subjects will also be taught to fill out a 1-day food diary for each experimental visit to measure dietary intake of vitamins, minerals and nitrate/nitrite. Subjects will be asked to avoid using mouthwash the day before visit 2 and for the duration of the study.
Vascular measurement:

Visit 2 (5 hours) Experimental Vascular Visit
• Participants will arrive at the CRU or 528 Field House following an overnight 8-hour fast. Subjects on vasoactive medications will be asked to hold medications on the morning of vascular testing but will be instructed to bring the medications with them to be taken at the completion of the visit.
• Participants will return the 1-day food diary
• An IV catheter will be placed in the arm to draw blood at baseline to measure plasma nitrate/nitrite, glycocalyx components, lipids, glucose and markers of oxidative stress.
• Glycocalyx Screening: A small camera will be placed under the participant's tongue to look at the relationship between vascular function and the thickness of a protective layer (called glycocalyx) coating the inside of their blood vessels. The tip of a small handheld camera, about the size of a tongue depressor, fitted with a sterile cover for their protection, will be gently placed under the tongue and the smallest vessels under the tongue will be recorded. The tip of the camera will record about 12 short (about 2 seconds each) movies of the vessels under the tongue.
• PI/research staff collects baseline vascular testing: Measures of radial, brachial, carotid and femoral waveforms will be collected with non-invasive tonometry to calculate carotid-femoral pulse wave velocity. Carotid ultrasound images will be collected to calculate carotid compliance/beta-stiffness index and pressure wave reflection, expressed as reflection coefficient, computed from characteristic impedance (Zc).
• PI/research staff collects baseline cerebrovascular testing: internal carotid artery flow will be measured non-invasively using Doppler ultrasound. Heart rate, beat-by-beat blood pressure and respiration rate are recorded.
• Participants will be randomized to consume beetroot juice or nitrate-depleted control beverage. Subject consumes beverage. Dietary nitrate is absorbed in the intestine and converted to nitrite in the salivary glands of the mouth, peaking in the blood around 1.5 hours post-consumption. Therefore, post-acute consumption measurements will be repeated 1.5 hours after the experimental beverage is consumed.
• During the time in which the beverage is being absorbed, participants will complete cognitive testing to measure memory, processing speed and other domains of cognition, pregnancy and menopause history surveys (if applicable)
• PI/research staff will repeat bloodwork measuring plasma nitrate/nitrite, markers of oxidative stress. Vascular, cardiac, and cerebrovascular testing will be repeated approximately one and a half hours after experimental beverage is consumed.
• PI/research staff will repeat blood work measuring plasma nitrate/nitrite approximately 3 hours after beverage consumption.
• Subject receives meal/snack from CRU bionutrition.

Visit 3 (3 hours) Post supplementation vascular and cognitive testing visit
• Participants will report to the lab approximately 4 weeks after their Visit 2.
• Participants will complete cognitive testing to measure memory, processing speed and other domains of cognition.
• Blood will be drawn via butterfly needle to measure plasma nitrate/nitrite, lipids, glucose and measures of oxidative stress.
• PI/research staff collects baseline vascular testing: Measures of radial, brachial, carotid and femoral waveforms will be collected with non-invasive tonometry to calculate carotid-femoral pulse wave velocity. Carotid ultrasound images will be collected to calculate carotid compliance/beta-stiffness index and pressure wave reflection, expressed as reflection coefficient, computed from characteristic impedance (Zc).
• PI/research staff collects baseline cerebrovascular testing: internal carotid artery flow will be measured non-invasively using Doppler ultrasound. Heart rate, beat-by-beat blood pressure and respiration rate are recorded.

Experimental Methods for Visit 1:
Maximal 12-lead EKG Exercise Test: Subjects will undergo a maximal exercise test with 12-lead EKG and cardiopulmonary gas analysis for determining VO2max. Tests will be completed in the Exercise Physiology testing lab in E141 Field House. All tests will be supervised by research staff trained in maximal exercise testing procedures. A licensed physician will be present during the exercise test. Subjects with evidence of cardiovascular disease at baseline or during the exercise test (evidence of myocardial infarction, abnormal cardiac arrhythmia, myocardial ischemia, conduction delays, >2mm ST segment depression or > 1 mm elevation; >3 beat ventricular tachycardia; atrial fibrillation) will be excluded from the study. Cognitive reserve surveys (WMS III & MMSE) will be conducted.

Experimental Methods for Visit 2 and Visit 3:
Blood draw: A blood draw will be performed by a trained staff nurse or phlebotomist to draw blood for measurement of glucose, lipids, inflammation, oxidative stress, glycocalyx components and plasma nitrate/nitrite.

Arterial stiffness, hemodynamics, and cardiac imaging:
• Carotid artery compliance/stiffness. Carotid artery compliance and Beta-stiffness index will be determined noninvasively by high-resolution ultrasonography (Ge Logiq 7) of the 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 (e.g., 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.
• 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 to calculate the transit time (t) between the foot of the carotid and the respective peripheral (femoral, brachial, radial) waveforms. The carotid-femoral transit distance (CFTD) is estimated between the 2 anatomical sites as the difference between the suprasternal notch (SSN) to carotid (SSN-C) and femoral (SSN-F) sites. Thus, the CFTD is calculated as CFTD= (SSN-F)-(SSN-C) and PWV calculated as CFTD/t. This approach accounts for parallel transmission of the pulse wave up the brachiocephalic and carotid arteries, and simultaneously along the aortic arch using the SSN as a fiducial point where parallel transmission begins (e.g., bifurcation site of aortic arch and brachiocephalic artery).
• Carotid artery pulsatility and resistance index: Carotid artery pulsatility and resistance will be measured using carotid Doppler ultrasound. Velocity flow will be recorded for 60 seconds to determine the peak systolic and end diastolic velocity flows. Baseline diastolic diameters will be measured from the carotid compliance image. Mean blood flow velocity will be calculated as the average of all peak systolic and end diastolic blood flow velocity waveform. Carotid flow pulsatility index will be calculated as [(peak systolic-end diastolic blood flow velocity/ mean blood flow velocity)]. Resistance index will be calculated as [(peak systolic – end diastolic/ peak systolic blood flow velocity)].
• Carotid characteristic impedance: Carotid Zc will be measured using the NIHem Workstation (Cardiovascular Engineering, Inc). Briefly, brachial artery blood pressure will be obtained using an automated cuff that deflates at 2 mmHg/sec. Arterial tonometry of the carotid artery will be performed using a noninvasive transducer and then 2D echocardiographic images of the carotid artery outflow tract will be obtained followed. Tonometry waveforms are signal-averaged using the EKG R-wave as a fiducial point. Average systolic and diastolic cuff blood pressures are used to calibrate peak and trough of the signal-average brachial waveform. Diastolic and integrated mean brachial pressures are then used to calibrate the carotid waveforms. Carotid artery outflow tract diameter will be measured by finding the early systolic diameter at a point just proximal to the carotid bulb. The carotid outflow tract pulsed doppler velocity waveform is then multiple by outflow tract area to compute carotid volume flow. Carotid Zc is calculated in the time domain by dividing the increase in carotid pressure during early systolic by the corresponding increase in 95% peak volume flow in the carotid output tract expressed in dynes x sec/cm5. The pressure reflection coefficient (R) in the carotid artery will be computed from the ratio of forward to backward pressure wave amplitude derived from carotid Zc.
• Carotid artery endothelial function: Internal carotid artery blood flow velocity (ICAv) and vessel diameter will be measured non-invasively using Doppler signal. Arterial diameter and blood flow velocity will be collected concurrently to measure peak blood flow velocity at baseline and during the hypercapnic protocol described above.

• Aortic characteristic impedance (Zc): Aortic Zc will be measured as described by Mitchell et al. using the NIHem Workstation (Cardiovascular Engineering, Inc.). (32, 33, 34) Briefly, auscultatory brachial artery blood pressure will be obtained using an automated cuff that deflates at 2 mmHg/sec. Arterial tonometry of the carotid artery will be performed with a custom transducer and then 2D echocardiographic images of the LV outflow tract will be obtained from a parasternal long axis view followed by the pulsed Doppler of the LV outflow tract from an apical 5-chamber view. Tonometry waveforms are signal-averaged using the ECG R-wave as a fiducial point. Average systolic and diastolic cuff blood pressures are used to calibrate peak and trough of the signal-averaged brachial waveform. Diastolic and integrated mean brachial pressures are then used to calibrate the carotid waveforms. LV outflow tract diameter will be measured by finding the largest early systolic diameter at a point just proximal to the aortic leaflets. The LV outflow tract Pulsed Doppler velocity waveform is then multiplied by outflow tract area to compute aortic volume flow. Aortic characteristic impedance is calculated in the time domain by dividing the increase in carotid pressure during early systole by the corresponding increase in 95% peak volume flow (Q95) in LV outflow tract expressed in dynes x sec/cm5.

Cognitive function testing. The NIH toolbox cognition battery will be administered. Participants will complete a series of cognitive tests (Flanker Inhibitory Control and Attention; Picture Vocabulary; Dimensional change card-sort test; List sorting; Picture Sequence; Oral Reading recognition; Pattern comparison processing speed test) using a tablet guided by a member of the research team. A member of the research team will administer the Trails A & B test, Letter/Pattern Comparison, and the stroop task using pen and paper (attached). All records (i.e., collected electronically on tablet and on paper) will be de-identified. These are assessments of the participant's ability to pay attention, learn and remember information, and solve problems. These assessments will be completed within 40-45 minutes.
-Flanker task: measures attention and inhibitory control. The test requires the participant to focus on a given stimulus while inhibiting attention to stimuli (arrows) on either side of the object. Sometimes the middle stimulus is pointing in the same direction as the “flankers” (congruent) and sometimes in the opposite direction (incongruent). Twenty trials are conducted.
-Picture vocabulary: The subject is presented with an audio recording of a word and four photographic images on the iPad screen, and is asked to select the picture that most closely matches the meaning of the word.
-Dimensional change card sorting test: Two target pictures are presented that vary along two features (e.g., shape and color). Participants are asked to match a series of bivalent test pictures (e.g., yellow balls and blue trucks) to the target pictures, first according to one feature(e.g., color) and then, after a number of trials, according to the other feature (e.g., shape)
-List sorting: Pictures of different foods and animals are displayed with accompanying audio recording and written text (e.g., “elephant”), and the participant is asked to say the items back in size order from smallest to largest, first within a single dimension (either animals or foods, called 1-List) and then on two dimensions (foods, then animals, called 2-List)
-Picture Sequence: The participants are asked to recall the sequence of pictures demonstrated over two learning trials
-Oral reading recognition: the participant is asked to read and pronounce letters and words as accurately as possible.
-Pattern comparison: participants to discern, as quickly as possible, whether two side-by-side pictures are the same or not. The items are presented one pair at a time on the iPad screen, and the participant is given 85 seconds to respond to as many items as possible.

Surveys: Cognitive reserve status, pregnancy history survey, menopause history survey

Blood draw: A blood draw will be performed by a trained staff nurse or phlebotomist to draw blood for measurement plasma nitrate/nitrite.

Experimental Beverage: Plasma levels of nitrate and nitrite will be augmented using a commercially available beetroot powder concentrate (Super Beets, HumanN, https://www.humann.com/) blended in water to create a beverage. A nitrate-depleted control (Beet Essence, Green Foods, Inc., http://www.greenfoods.com/store/) beverage containing similar caloric, sodium and antioxidant properties (only devoid of nitrate/nitrite) will be used as the control beverage. SuperBeets concentrate will be used for the experimental beverage: 20g of active beetroot powder (Superbeet, HumanN) will be mixed in 120-180mL of water. Each dose will contain 500 mg (~8.06 mmol) of nitrate and 40 mg (~0.58 mmol) of nitrite. The nitrate-depleted control beverage will be made identical to above only Beet Essence, a nitrate-depleted powder, will be substituted.

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

VII.E.8 ***Describe - any procedures need to be included in the consent:***
If a subject does not return for a scheduled visit, every reasonable effort will be made to contact the subject. However, we will limit our efforts to more than 3 phone calls and 3 emails with no response from the participant. Except in the case of withdrawal of consent by the subject, any data acquired will be used in the analyses where applicable.

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

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

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

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

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

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

VII.E.17 ***Describe:***
Parking passes for UIHC ramps

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

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

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

- Compensation scheme:
  Visit 1: $55
  Visit 2: $75
  Visit 3: $30
  + $100 for 4 weeks supplementation
  Total: $260

  If a subject is completes carotid-femoral pulse wave velocity testing during visit 1 and is determined ineligible because of a carotid-femoral pulse wave velocity < 7.6 m/s, they will still be compensated $30 for their time.

  If a 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.

VIII. Risks

VIII.1 ***What are the risks to subjects including***
- emotional or psychological
- financial
- legal or social
- physical?
Physical risks
Blood sample: A total of 5 tablespoons of blood will be obtained from a vein during the course of the study during the vascular visits. Potential risks and discomforts associated with obtaining blood samples include slight bruising, pain, a temporary feeling of faintness, and rarely, infection at site of blood draw. All blood draws will be performed by a research nurse or research staff trained in drawing blood.

No known risks of the Gylcocalyx screening.

Cognitive testing/surveys: There are no known risks to participating in the questionnaire assessments, or

cognitive tests. Subjects may feel uncomfortable answering survey questions. They may skip any questions they do not want to or feel uncomfortable answering. Subjects may feel boredom, fatigue, or frustration during cognitive tests. Subjects will be encouraged to take breaks as necessary between cognitive tasks. Subjects may request to skip or discontinue cognitive tests at any time.

Fasting for 4 or 8 hours: The most common risk when fasting is dehydration, therefore subjects 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.

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

carotid artery compliance/stiffness echocardiography. There are no known risks associated with the use of carotid echocardiography. ECG electrodes may cause minor irritation to the skin.

Carotid-cerebral Pulse Wave Velocity (ccPWV): There are no known risks associated with Carotid-cerebral Pulse Wave Velocity (ccPWV)

12-lead ECG Maximal Exercise Stress Test: Potential risks of a maximal exercise stress test are fatigue, dry mouth, heart palpitations, muscle strain, shortness of breath and chest pain. There is a very small risk of heart attack (0.04%) and death (0.01%) for middle-aged and older healthy adults. The risk is significantly lower for young healthy adults.

Confidentiality and financial risks: Subjects may be at risk of breach of their confidentiality. All research team members have undergone confidentiality training to minimize this risk. Measures in place to protect subject confidentiality are noted in the 'What About Confidentiality' section later in this document. Other than the cost of transportation, no financial risk is anticipated.

Risks of beetroot juice beverage to be used in the study
Possible side effects or adverse reactions to beetroot juice (experimental or control beverage). Beetroot juice is a supplement and not a pharmaceutical substance. Beetroot juice is a safe, well-tolerated supplement available over the counter at major grocery or vitamin stores. The amount of nitrate being administered per day in this study corresponds to what is found in a diet high in green leafy vegetables (~about 300g of spinach or lettuce). The only known risk of beet root juice is the risk of pink or red urine/stool.

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.)***

To address the potential risk of boredom, fatigue or frustration during the behavioral tasks, we will provide participants with opportunities for breaks. Participants will be encouraged to ask questions as needed to minimize potential frustration.

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.

If answers to various health questionnaires lead to a concern for the participant's safety or the safety of others, the mentor (Gary Pierce) will be notified immediately and will contact the study physician to perform a risk assessment. The participant will be kept informed of the need for additional evaluation and will be encouraged to ask questions.

The investigators will check response to all question about suicide before the subject leaves the study site. If there are concerning answers to any of these questions, the investigator will page the psychiatry resident

on call for further advice. If the psychiatrist recommends immediate evaluation, the investigator will accompany the subject to the psychiatry clinic or emergency room as directed.

To address the risk of flying metal objects, we require that all people involved with the study remove all metal from their clothing and pockets. No metal objects will be brought into the magnet room while participants are inside the room. In addition, the door to the room remains closed throughout the study so that no one can accidentally bring a metal object into the room.

ECG Cycle Ergometer Exercise Stress Test with VO2 Monitoring: The test will be conducted by research staff trained in stress testing procedures and monitoring 12 lead ECG, blood pressure, and any symptoms in all subjects. A licensed cardiologist will be present to monitor subjects. American Heart Association/American College of Sports Medicine guidelines for contraindications to begin the test, and indications to stop the test will be followed. After the ECG stress/VO2 test, the subjects will be monitored for 15 minutes ensuring they are symptom free and ECG, blood pressure, and heart rate have returned to baseline before being released. The Exercise Physiology Testing lab in E141 Fieldhouse is equipped with a Quinton ECG 12-lead ECG stress test system, cycle ergometer, Parvomedics metabolic cart, AED, fully stocked crash cart with emergency supplies and drugs (same used in UIHC), telephone and easy first floor access to S. Grand Avenue.

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

How will information/data be collected and stored for this study?

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 PIs 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 the folder and locked in storage cabinet in the study coordinator's office. The office is locked when personnel are not in the office. Signed informed consent documents and will be kept in a separate binder in a different locked file cabinet in the study coordinator's office.

Electronic records (computer files, electronic databases, etc.) - Data will be entered using subject ID code into the ICTS REDCap web-based database application that is password protected. No personal identifiable data will be entered. Only research staff on the IRB approved study will be allowed access this database. The ICTS REDCap staff are responsible for maintaining security of the data. Some data using subject ID code will also be entered into a Microsoft Excel and SPSS datasheets that will be kept in a shared server for CLAS that is password protected. Only research staff on the IRB approved study will have access to the folder the study on the server. This server is maintained by Bryan Ringen, IT Support Consultant, College of Liberal Arts and Sciences.
Name - Bryan Ringen
Title - IT Support Consultant, College of Liberal Arts and Sciences
University Job Classification - IT Support

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

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

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

The Data Safety Monitoring plan will consist of an annual review of the protocol by Dr. Mark Santillan, MD, PhD, Department of Obstetrics and Gynecology. The PI will provide an annual report to Dr. Santillan summarizing the following:
- Data on progress of the protocol including subject recruitment, attrition, and minority involvement.

- Reasons for attrition or other recruiting issues.
- Data on compliance in reporting of any adverse events
- Data on protocol compliance and any amendments to the protocol
- Summarize outcome data and provide to Dr. Santillan for review of the efficacy of the treatment intervention on primary outcomes.
- 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.

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, PhD, OB/GYN at UIHC

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)?***
Subjects will not directly benefit from this study.

IX.2 ***What are the potential benefits to society in terms of knowledge to be gained as a result of this project?***
Inorganic nitrate, such as beet root juice, could be used to reduce or attenuate vascular aging in middle-aged and older adults.

X. Privacy & Confidentiality

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

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

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

X.4 ***How will information/data be collected and stored for this study (check all that apply):***
- Biologic samples (blood draws, check swabs, saliva samples, tissue samples, etc.) - Basic blood chemistries will be sent to the UIHC pathology lab for analyses. Remaining biological specimens such as blood, 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. 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
    - Title - Associate Professor
    - University Job Classification - Faculty/Staff
- Electronic records (computer files, electronic databases, etc.) - Data will be entered using subject ID code into the ICTS REDCap web-based database application that is password protected. No personal identifiable data will be entered. Only research staff on the IRB approved study will be allowed access this database. The ICTS REDCap staff are responsible for maintaining security of the data. Some data using subject ID code will also be entered into a Microsoft Excel and SPSS datasheets that will be kept in a shared server for CLAS that is password protected. Only research staff on the IRB approved study will have access to the folder the study on the server. This server is maintained by Bryan Ringen, IT Support Consultant, College of Liberal Arts and Sciences.
    - Name - Bryan Ringen
    - Title - IT Support Consultant

- University Job Classification - Faculty/Staff
- Paper/hard copy records (hard copy surveys, questionnaires, case report forms, pictures, etc.) - 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 PIs 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 the folder and locked in storage cabinet in the study coordinator's office. The office is locked when the she is not in the office. Signed informed consent documents and will be kept in a separate binder in a different locked file cabinet in the study coordinator's office.

X.5 ***Do the confidentiality protections indicated above allow only members of the research team to access the data/specimens?***
No

X.6 ***Describe***
No, mean data in the form of a progress report will be sent to a funding agency (ie. American Heart Association, NIH, etc) if/when funding is secured. Summary (mean) data will not include any personal identifiable information of the study subjects.
Deidentified blood samples will be sent to Jennifer Pollock, PhD, at the University of Alabama Birmingham to assess plasma nitrate and nitrite levels. The samples will not include any personal identifiable information of the study subjects.

X.7 ***Does your study meet the NIH criteria for a Certificate of Confidentiality or will you be applying for Certificate of Confidentiality?***
No

XI. Data Analysis

XI.2 ***Provide the rationale or power analysis to support the number of subjects proposed to complete this study.***
Power analyses were calculated based on the primary aim:
For aim 1: The hypothesis for is that acute BRJ supplementation will improve (i.e., reduce) common carotid artery beta-stiffness index in older adults to the levels of middle-aged adults. Using new pilot data from our lab for mean differences in carotid beta-stiffness index values in middle-aged compared with older adults, an effect size of 1.40 was calculated. Therefore, we estimate a sample of 24 older adults middle-aged and older adults will be needed. With an estimated 15% attrition rate the total number to be recruited is 28.

For Aim 4: The hypothesis is that chronic (4-week) supplementation of beetroot juice will result in improvements in 1) carotid artery stiffness, pressure wave reflection and blood flow pulsatility beyond a single acute dose, and 2) that chronic beetroot juice supplementation for 4 weeks will improve select cognitive domains such as processing speed, working memory and executive function. Using mean differences in carotid beta;-stiffness values between middle-aged and older adults from our lab, using the effect size of 1.40, we estimate an additional sample of 12 people per group (total= 24) will be needed. With an estimated 15% dropout rate for the chronic studys, a total sample of 14 per group (total n= 28) additional participants is required for the chronic study (initial approved 18 + 28 additional participants= total 46 total.

After our interim analysis, the data demonstrate a trend for a decrease in our primary outcome variable (carotid artery stiffness) after 4-weeks beet juice supplementation in older adults with carotid-femoral pulse wave velocity > 7.6 m/s. About 1/3 of our participants had carotid-femoral pulse wave velocity >7.6 m/s, therefore we wish to recruit and randomize 30 more subjects to further test our findings that beet root juice has a beneficial effect on carotid artery stiffness in older adults with carotid-femoral pulse wave velocity > 7.6 m/s. The justification for recruiting 30 more subjects is because we will be screening for carotid-femoral pulse wave velocity > 7.6 m/s after consenting the subjects, so we are accounting for 1/3 of the subjects to meet eligibility criteria of carotid-femoral pulse wave velocity > 7.6 m/s and randomize 10 more subjects. We plan to screen subjects for carotid-femoral pulse wave velocity > 7.6 m/s during visit 1, prior to randomization, to determine eligibility (described further in section VII.E.6).

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 blood samples 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.