Cover Page

Study Title: Creatine Use and Muscle Stretching in Peripheral Artery Disease

NCT Number: NCT04471792

Date of Study Protocol: 03-09-2022

Study Protocol to Follow

PROTOCOL TITLE:

Creatine supplementation and muscle stretching in peripheral artery disease patients

PRINCIPAL INVESTIGATOR:

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VERSION NUMBER/DATE:

Version 6 (3/9/2022)

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1	11/7/19	Added use of Magnetic Resonance Imaging	Yes
2	3/16/20	Research device (section 2.1), renal disease (section 10.2 (exclusion); page 2&4 of consent), conflict of interest (consent form) and recruitment language (section 13.0).	Yes
3	5/01/20	Ability to consent patients at VSA	No
4	7/1/20	Changed Dr. Delp to PI; Increased subjects to 50; clarified inclusion criteria for healthy volunteers; changed age range 40-95; Added demographics, vitals, SF36, WIQ, and iPAQ to visit 2; added 5 day study visit window; limited MRIs to 4-6 healthy and 6-8 PAD in each supplement group; added diary for splint stretching and supplement use tracking; added REDCap for data management.	Yes
5	8/25/21	Added SF-36, WIQ, and IPAQ to visit 3.	No
6	3/9/21	Added a Fitbit to visit 1 & 3 to monitor step count (section 6.2). Added 8 foot up and go test, 30 second chair rise test, and chair sit and reach to visit 2 & 3 (section 6.2). Added heel raises for 1 minute with NIRS to visit 2 & 3 (section 6.2). These changes are also reflected in the informed consent document (pages 5-7). Changed estimated visit length in the consent form from 2-3 hours to 2-3.5 hours to reflect addition of new tests.	Yes

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1.0 Study Summary

Study Title	Creatine supplementation and muscle stretching in peripheral artery disease patients
Study Design	Randomized, single blind, cross sectional
Primary Objective	To utilize near-infrared spectroscopy and magnetic-resonance imaging to investigate if stretch training, when combined with creatine loading improves the cardiovascular performance in PAD patients
Secondary Objective(s)	To characterize near-infrared spectroscopy derived tissue oxygenation responses at rest and during a walking test (i.e., six-minute walk test).
Research Intervention(s)/ Investigational Agent(s)	Creatine Monohydrate
IND/IDE #	IND 145110
Study Population	Individuals aged 40 - 95
Sample Size	Up to 50
Study Duration for individual participants	About 5-6 weeks
Study Specific Abbreviations/ Definitions	6MWT: six-minute walk test NIRS: Near-infrared spectroscopy PAD: Peripheral Artery Disease

2.0 Objectives*

2.1 1. To utilize near-infrared spectroscopy to investigate if our research device for stretch training, and creatine loading impact cardiovascular performance in aged and PAD patients.
2. To characterize near-infrared spectroscopy derived tissue oxygenation responses at rest and during a walking test (i.e., six-minute walk test). We will also assess parameters with Magnetic Resonance Imaging.

2.2 It is hypothesized that the stretching protocol will improve both NIRS derived tissue oxygenation and magnetic resonance derived leg blood flow measures and that creatine supplementation will further improve phosphorus metabolite muscle performance. All patients will undergo either 4 weeks of stretch training with- or-without creatine supplementation according to previously defined creatine guidelines (Sanchez-Gonzalez et al 2011).

3.0 Background*

3.1 Lower extremity peripheral artery disease (PAD) has been estimated to impact nearly 8.5 million U.S. adults above the age of 40, significantly increasing the rate of morbidity and mortality with concomitant decreases in quality of life. These patients are often given medical therapy (e.g., statins, antiplatelet, anticoagulants) and are also recommended to begin structured exercise programs. However, limb ischemia is common in these patients during physical activity which may limit exercise tolerance. Specifically, (Bauer, Brass, & Hiatt, 2004; 2011) have shown that a major limitation in PAD patients is an impaired muscle metabolism. These data are important in that they show alterations in blood flow and metabolic machinery likely impact exercise tolerance. As such, development of tolerable countermeasures to improve limb blood flow and muscle energetics may increase adherence to exercise therapy and improve health outcomes in PAD patients. Our lab has previously shown that daily stretching, 30-minutes of dorsiflexion, significantly improved soleus muscle function and muscle blood flow during exercise in a rat model of aging (Hotta et al., 2018). In a follow up study, we have also shown that this model improves vascular function in human PAD patients (Hotta et al; in review). As noted above, muscle energetics are delayed in PAD patients, so improving the rest-to-exercise transition, with creatine supplementation, and muscle blood flow may help PAD patients sustain exercise longer.

3.2 Our lab has previously shown in animal models and human that muscle stretching improves leg blood flow Hotta et al. (2018, 2019). We are now testing to see if an added supplement can further improve these findings.

4.0 Study Endpoints*

4.1 Improvement in muscle oxygenation, shown via near-infrared spectroscopy, and blood flow/phosphorus metabolites, via magnetic resonance imaging.

4.2 Improvement in walking distance, shown via 6MWT.

5.0 Study Intervention/Investigational Agent

5.1 Creatine Monohydrate (Cr) ($C_4H_{11}N_3O_3$) is a dietary supplement commonly found in red meat and seafood (3). Cr is given in doses ranging from 2grams/day up to 30 grams/day for up to 5 years safely (1). Previous work suggests that an initial 5 day loading period (10g/day) followed by a maintenance phase (5 g/day) be followed to obtain maximal response (3). The objectives of the current pilot study are to investigate if Cr supplementation plus muscle stretching improves 6MWT distances in patients with peripheral artery disease (see (1) for review). The route of administration for the Cr is by mouth. With over 1,000 studies using Cr, the safety and efficacy of Cr are very consistent with the main side effect being weight gain (2, 3). The use of Cr even extends to ischemic heart disease, aging, and neurodegenerative disease. Currently, there are no studies suggesting adverse effects that would contraindicate use in patients with peripheral artery disease

5.2 Drug/Device Handling: The researchers will purchase the creatine monohydrate from GNC supplements. The creatine containers will be stored in the laboratory and remain unopened until the patient takes them home. This supplement has been given full approval by the FDA (See IND above).

1. *Hotta K, Behnke BJ, Arjmandi B, Ghosh P, Chen B, Brooks R, Maraj JJ, Elam ML, Maher P, Kurien D, Churchill A, Sepulveda JL, Kabolowsky MB, Christou DD, and Muller-Delp JM. Daily muscle stretching enhances blood flow, endothelial function, capillarity, vascular volume and connectivity in aged skeletal muscle. J Physiol 596: 1903-1917, 2018.*

2. *Hotta, K., Batchelor, W. B., Graven, J., Dahya, V., Noel, T. E., Ghai, A., . . . Muller-Delp, J. (2019). Daily Passive Muscle Stretching Improves Flow-Mediated Dilation of Popliteal Artery and 6-minute Walk Test in Elderly Patients with Stable Symptomatic Peripheral Artery Disease. Cardiovasc Revasc Med, 20(8), 642-648. doi:10.1016/j.carrev.2019.05.003*

3. *Hultman E, Soderlund K, Timmons JA, Cederblad G, and Greenhaff PL. Muscle creatine loading in men. J Appl Physiol (1985) 81: 232-237, 1996.*

4. *Kreider RB, Kalman DS, Antonio J, Ziegenfuss TN, Wildman R, Collins R, Candow DG, Kleiner SM, Almada AL, and Lopez HL. International Society of Sports Nutrition position stand: safety and efficacy of creatine supplementation in exercise, sport, and medicine. J Int Soc Sports Nutr 14: 18, 2017.*

5. *Shao A, and Hathcock JN. Risk assessment for creatine monohydrate. Regul Toxicol Pharmacol 45: 242-251, 2006.*

5.3 If the drug is investigational (has an IND) or the device has an IDE or a claim of abbreviated IDE (non-significant risk device), include the following information:

- Judy Muller-Delp.
- 21 CFR 11: Data collection/security parameters are outlined in the data management section of this protocol.
- 21 CFR 54: FDA COI forms will be completed as required.
- 21 CFR 210: All creatine monohydrate and placebo supplementation will comply with good manufacturing practice regulations.
- 21 CFR 211: GNC and Allergy Research Group both comply with the good manufacturing regulations.
- 21 CFR 312: We have submitted an IND and have approval for the use of creatine monohydrate in this investigation.

	Applicable to:		
FDA Regulation	***IND Studies***	***IDE studies***	***Abbreviated IDE studies***
21 CFR 11	***X***	***X***	
21 CFR 54	***X***	***X***	
21 CFR 210	***X***		
21 CFR 211	***X***		
21 CFR 312	***X***		
21 CFR 812		***X***	***X***
21 CFR 820		***X***	

6.0 Procedures Involved*

6.1 All patients (PAD or healthy volunteers) will undergo 4 weeks of splint stretching (30min/day; 5days/week – procedures attached with this submission) and be randomized into groups with or without creatine supplementation (10g/day for the first 5 days and 5g/day for the remainder). Placebo supplementation (Fiber powder; cellulose) will be given during the opposite arm of the study and according to section 6.2 (below).

6.2 Each potential patient/participant will be screened to determine potential eligibility by the research team. If the individual is eligible, see inclusion/exclusion criteria, they will be asked to consider joining the study. Individuals meeting the initial criteria and who agree to participate in the study will sign informed consent and complete any medical history forms. All participants must abstain from alcohol for 24 hours and caffeine/food for ~ 4 h prior to testing sessions. The current investigation is a randomized single-blind study design.

- C1. Health and exercise questionnaires: Subjects' medical history and exercise habits will be

assessed by self-reported health and exercise questionnaires: demographics and health history (Visit 1), physical activity readiness (Visit 1), health survey-SF36 (Visit 2 and 3), international physical activity questionnaire (I-PAQ, Visit 2 and 3), walking impairment questionnaire (WIQ, Visit 2 and 3).

- C2. Fitbit: Subjects will be given a Fitbit Charge 2 (Fitbit, San Francisco, California) to wear for a 1 week period starting at Visit 1 and Visit 3 to measure daily step count before and after the intervention. At this time, we anticipate 4-8 subjects with PAD using a Fitbit during the course of the study. We will recruit 2-4 PAD patients to serve as time controls and not partake in the intervention.
- C3. 'Anthropometric measurements and vitals will consist of subject height measured on a stadiometer and weight measured on a calibrated scale, heart rate (resting) and baseline blood pressure after 10-min of quite supine rest. Next, we will measure circumference of the waist and lower leg and range of motion of the knee and ankle. All of these measurements will be done at Visit 2. *Resting heart rate and blood pressure will be repeated at Visit 3.*
- C4. `Muscle oxygenation characteristics' of the lower leg (i.e., gastrocnemius and soleus) will be measured via near-infrared spectroscopy (NIRS) (Moxy monitoring systems, Hutchinson, Minnesota) at Visit 2 and 3. NIRS is a completely noninvasive measure that uses infrared light waves emitted from a skin surface probe. NIRS measurements will be made continuously during the entirety of the experimental protocol. Protocol one will utilize a blood pressure cuff around the knee joint and inflated to ~250mmHg to occlude flow for 5-minutes. At minute 5 the cuff is instantaneously released and rest will continue for ~5-minutes. The NIRS will be take off for C3.A and then put back on in place to complete C4 (see below).
- C5.A 'Muscle blood flow and metabolic kinetics" (i.e., gastrocnemius and soleus) will be measured in the 3T MRI system (Visit 2 and Visit 3; limited subjects). All patients and controls will be placed in the MRI feet first and undergo each trial by placing a blood pressure cuff just proximal to the knee joint and inflating it to ~250mmHg to occlude flow for 5-minutes. At minute 5 the cuff is instantaneously released and rest will continue for ~5-minutes. After a brief rest period, the patient's foot and ankle will be positioned in the splint device to produce dorsiflexion and stretch of the calf muscles and scanning will resume. Due to associated MRI costs, only a subset of patients and controls will be using the MRI. At this time, we anticipate ~25 total subjects will undergo pre/post MRI: 4-6 healthy/placebo; 4-6 healthy/creatine; 6-8 PAD/placebo; 6-8 PAD/creatine. Subjects who undergo MRI will be selected in

the order they present and are eligible to receive an MRI (based on MRI exclusion criteria)

C6. 'Cardiovascular assessment' will be measured via the "6-minute walk test (6MWT)" (Visit 2 and Visit 3) a sub-maximal clinically relevant test to assess efficacy of the 4-week intervention. All patients/participants will undergo a 6MWT pre/post intervention. Briefly, a standardized model will be followed according to American Heart Association guidelines. The 6MWT will be performed on a level surface by a research team member. Patients will be instructed to cover the greatest distance they can in 6-minutes at a pace of their choosing. Chairs will be placed periodically along the hallway in case a patient, or participant, needs to rest until they can begin again. The 6MWT data collection form and corresponding rating scales are provided with this submission.

- C7. "Physical function" will be measured via the 8 foot up and go, 30 second chair rise, and chair sit and reach tests. The 8 foot up and go requires the subject to start in a seated position, stand up, walk 8 feet, then return to the seated position. The score for this assessment will be the number of seconds needed to complete this task. The subject will perform this assessment twice, and the best score of the two trials will be used. Next, the subject will perform the 30-second chair stand test. In this assessment, the number of full stands from a seated position will be counted in the 30-second time period. The chair sit and reach will require the subject to reach his/her fingertips towards a straightened leg from a seated position. This assessment will be performed three times on each leg. The score will be the number of centimeters between the fingertips and toes for each leg.
- C8. "Muscle oxygenation characteristics" of the lower leg (i.e., gastrocnemius and soleus) will also be evaluated via NIRS with a heel raise test. The subject will complete heel raises for a 1 minute period while we continuously monitor muscle oxygenation during exercise and recovery.
- C9. 'Creatine supplementation and placebo control' will consist of an initial loading dose (see consent) of 10g/day for 5 days and then a daily 5g/day for 4 weeks. All creatine and placebo powders will be given in containers marked with a date for consumption and serving spoon and instruction guide. All creatine will be purchased from a local nutrition store (General Nutrition Center, GNC). All cellulose will be purchased from an online supplement store (Allergy Research Group, ARG). Subject instructions for taking the supplement will be provided to them and are attached to this submission.
- Subjects' visits (after screening visit) are planned for baseline (Visit 2) and Day 28 (Visit 3). However, due to scheduling

conflicts that are likely to arise, if a subject cannot come back on Day 28, there will be a 5-day study visit window (+/-) to allow for scheduling but not acquire protocol deviations. Stretching and supplementation will continue to occur during that time. Any study visit outside of this extended visit window will be documented as a deviation. Finally, any subject/patient that doesn't adhere to a "no alcohol for 24h or food/caffeine 4h" prior to testing will be rescheduled for another later date.

6.3 Describe:

Specific risks:

Healthy control subjects and peripheral artery disease patients: uncomfortable muscle stretch and blood pressure cuff. Fitbit may cause skin irritation.
Placebo: uncomfortable muscle stretch, blood pressure response to walking. Bloating, gas, diarrhea from fiber pill.
Creatine: uncomfortable muscle stretch, blood pressure response to walking. Bloating, gas, diarrhea, muscle cramping during creatine supplementation. We are unaware of any further risks with creatine supplementation.

We will reduce risk of patients with a standardized protocol across all subjects. For example, we will maintain weekly contact with the patients both inside the laboratory and while at home with phone calls and messages. In addition, the cardiologist's knowledge of the patient will provide us with the information to include or exclude the patient. During the most vigorous task (six-minute walk test) we will place chairs along the hallway in case the patient gets too tired to continue and can rest until they are recovered.

To reduce the risk of skin irritation associated with the Fitbit, all subjects will be instructed to keep the device clean, dry, and to not wear the device too tightly. If skin irritation occurs and does not resolve within 3 days, subjects will be instructed to discontinue use of the device. If any soreness, tingling, numbness, burning, or stiffness in the hands occurs in the hands or wrists, subjects will be instructed to discontinue use of the device immediately. To reduce risk of the above, subjects will be given the following instructions:

1. Do not attempt to replace the battery, open the enclosure or disassemble your Fitbit product.
2. Do not use your Fitbit product if the display is cracked.
3. Do not expose your Fitbit product to extremely high or low temperatures.
4. Do not use your Fitbit product in a sauna or steam room.

5. Do not use abrasive cleaners to clean your Fitbit product.
6. Remove your Fitbit product immediately if it feels warm or hot.
7. Charge the battery with the charging cable provided. Do not use other cables.
8. Do not wear your Fitbit product while charging it.
9. Do not charge your Fitbit product while it is wet.

Creatine monohydrate will be used in the current investigation. The purpose of using creatine is to create a favorable metabolic environment to improve patients' ability to walk.

- The use of creatine monohydrate in this investigation has been approved by the FDA.

The source records that will be used to collect data about subjects are attached to the IRB submission and will be kept in a locked cabinet in a locked office.

6.4 All data will be collected by trained members of the study team (NIRS, MRI, blood pressure, heart rate, anthropometry, Fitbit, and cardiovascular (i.e., six-minute walk test) variables.

6.5 N/A

6.6 N/A

7.0 Data and Specimen Banking*

7.1 N/A

8.0 Sharing of Results with Subjects*

8.1 Deidentified data will be provided for the 6MWT and the tissue oxygenation if the patient asks. These results will not include their name or any way to identify the patient.

9.0 Study Timelines*

9.1 All subjects will be asked to come to 3 visits (Visit 1: initial screen, paperwork and surveys; Visit 2: pre-testing and surveys; Visit 3: post-testing). Day one is approximated to take 30-45 minutes. Testing days two and three are expected to take up to ~2.5 hours. The whole study will take 5-6 weeks to complete.

9.2 We estimate 6 – 12 months for subject enrollment.

9.3 We anticipate complete primary analysis by summer 2021

10.0 Inclusion and Exclusion Criteria*

10.1 The inclusion criterion for the PAD study group is a resting ankle-brachial index (ABI) of 0.90 or less in either leg or clinical diagnosis by a medical doctor. All patients must have a stable condition for at least 3 months. Subjects in the healthy volunteer group do not need to meet the PAD inclusion criteria.

- Subjects must be ages 40 - 95
- PAD patients will be screened/cleared to participate by their primary care physician/cardiologist and then by our research team. Healthy volunteers do not require physician clearance to participate.
- All subjects must have the ability to speak and read English to be included in this study.

10.2 Subjects (PAD and healthy controls) are excluded according to whether they meet one or more of the following criteria: • habitual exercise or cardiovascular rehabilitation program during the past 3 months • below or above-knee amputation, critical limb ischemia (ulceration or gangrene) • leg pain at rest • major surgery or lower extremity revascularization during the previous 3 months • major medical illness treatment during the prior 12 months • central neurological disease • limited ankle or knee joint range of motion • requirement of oxygen with activity or exercise • Any New York Heart Association level of heart failure • atrial fibrillation • wheelchair confinement, or inability to walk • cognitive disorder • vasculitis problem including Takayasu's arteritis, Berger's disease, collagen disease or Raynaud's disease • Subjects whose physician feels that participation in this research project would not be appropriate for him/her will be excluded. • overt cardiovascular disease • metabolic disease • renal disease

Exclusion specific to the MRI: If patients have any history of pacemakers or pacer wires, open heart surgery, artificial heart valves, aneurysm clips, cochlear implants, braces or extensive dental work, implanted electrical or mechanical devices, tissue expanders, foreign metallic objects from explosives, shrapnel or metalwork fragments, or artificial limbs. Subjects will also be excluded if they are pregnant, claustrophobic, have tremors or cannot lie still for 1-2 hours.

If subjects only have exclusion criteria listed in the MRI section, they can be included in the study, but will not participate in the MRI portion at Visit 2 and Visit 3.

- *This investigation will not include:*
- *Adults unable to consent*

- *Individuals who are not yet adults (infants, children, teenagers)*
- *Pregnant women*
- *Prisoners*

11.0 Vulnerable Populations*

11.1 N/A

12.0 Local Number of Subjects

12.1 The goal of this study is to enroll 20 individuals with PAD and 20 healthy volunteers. To account for screenfails and withdrawals, we will enroll up to 50 subjects in this study.

12.2 N/A

13.0 Recruitment Methods

13.1 Subjects/patients will be identified based on required demographics (i.e. age) via word of mouth, email, flyers and visits to local clinics (e.g., cardiologists). We will also recruit by going to support groups, senior centers, or retirement homes in the area. These are not specific and we always contact the property to set up a meeting and get approval to be on their grounds to talk with residents.

We have also partnered with Vascular Surgery Associates in Tallahassee, FL (VSA) to recruit PAD patients. Prior to any contact with VSA patients, applicable study team members will also sign related documents that are provided by the VSA legal department. VSA employees will assess patient records based on our set inclusion/exclusion criteria. The VSA will then contact potential subjects that match our inclusion/exclusion criteria. If they are interested in being in the study, we will speak to these individuals to explain the study and enroll them if possible.

13.2 Source of the subjects, Tallahassee, FL and surrounding areas.

13.3 All PAD patients will be identified by their primary care physician/cardiologist and our health history questionnaires. Each PAD patient must complete the consent form (with exclusion criteria) and obtain their physician approval or will not be included in the current investigation. Healthy controls will be identified by recruitment shown in 13.1 and consented to participate.

13.4 Materials to recruit patients and controls have been uploaded.

13.5 Each patient and control that completes all the testing will get $100.00 via cash or gift card.

14.0 Withdrawal of Subjects*

14.1 Failure to comply with creatine/placebo use or stretching protocol or inability to attend study visits.

- Meets any of the exclusion criteria

14.2 The subject will be informed via telephone, email or in person that they have been removed from the study. We will also remind them at multiple time points that this study is completely voluntary and they may stop at any time.

14.3 If the patient removes themselves from the investigation all data collected up to the point of withdrawal will be kept. The reason for subject withdrawal will be recorded.

15.0 Risks to Subjects*

15.1 Specific risks:
Healthy control and peripheral artery disease patients: uncomfortable muscle stretch and blood pressure cuff.
Placebo: uncomfortable muscle stretch, blood pressure response to walking. Bloating, gas, diarrhea from fiber pill.
Creatine: uncomfortable muscle stretch, blood pressure response to walking. Bloating, gas, diarrhea, muscle cramping during creatine supplementation. We are unaware of any further risks with creatine supplementation.

- *MRI:* According to the FDA, there is currently no evidence that MRI with approved scanners of up to 4 Tesla signal strength are associated with adverse effects. Notwithstanding, there are several major potential risks. The first of these is discomfort that subjects may experience from being in a confined and sometimes noisy environment. Second, strong magnetic fields can have a deleterious effect on electronic, magnetic and metal devices that subjects may carry on them or have been implanted in their bodies. Third, if unprotected, hearing loss can occur with protracted exposure to the noise generated during MRI imaging. In addition, subjects may temporarily experience dizziness and/or nausea if they move rapidly through the magnetic field. There is also the potential for radio frequency (RF)-induced local tissue heating and for mild electrical stimulation due to induced currents as magnetic gradients switch rapidly. Known as peripheral nerve stimulation (PNS), this is typically not harmful but may cause discomfort. Subjects may also experience physical discomfort from lying on their backs for 1 – 1.5 hours in the scanner.
- *To mitigate these risks the following steps will be taken:*
- When running certain scan types 3T MRI scanners can generate sound on the order of *126 – 131 dB (Foster et al., 2000; Hattori et al., 2007).*

The FDA has recommended that safe audible levels are no more than 99 dB(A) (US Food and Drug Administration, 2014). Therefore, anyone in the magnet room while it is in operation is required to correctly wear earplugs. Subjects will be encouraged to move slowly when entering and exiting the magnetic field. The scanner bed is designed to move slowly in order to minimize the possibility of dizziness. RF-induced local heating is unlikely due to software requirements to enter the subject's height and weight accurately to determine specific absorption rates of RF energy. Moreover, padding will be extensively used to ensure that the subject is not in contact with parts of the scanner where RF energy emanates. Subjects are warned of the possibility of mild electrical stimulation and are monitored throughout the session by means of audio intercom. *Exclusions will be made for the following: cardiac pacemaker, metal fragments in eyes/skin/body (shrapnel), history of being a metal worker/welder, history of eye surgery/eyes washed out because of metal, aortic aneurysm clips, prosthesis, by-pass surgery/coronary artery clips, hearing aid, heart valve replacement, subjects with an I.U.D. (birth control device), a shunt (ventricular or spinal), electrodes, metal plates/pins/screws/wires, or neuro/bio-stimulators (TENS unit), vision problems uncorrectable with lenses, claustrophobia, inability to lie still on one's back for approximately 90 minutes, prior neurosurgery, older tattoos with metal dyes, unwillingness to remove nose, ear, tongue or face jewelry, and women who are pregnant or currently lactating.*

While in the scanner voice contact will be maintained at all times and subjects will be encouraged to report discomfort. Furthermore, well trained staff are on hand to watch for signs of distress, anxiety or fatigue and terminate the study should the subject exhibit signs of distress.

Subjects will be provided with an emergency squeeze ball to use if they would like to terminate the scan and talk to subject personnel. Subjects can terminate their scanning session at any time if they wish.

We will make every effort to train subjects properly prior to the MRI scan. For example, a mock scanner can be used to assess subject's ability to tolerate confined spaces and lie on their backs. They can also be played noises typical of the scanner environment. Subjects will be asked questions about their fear of confined spaces during the screening stage to reduce the risk of recruiting claustrophobic subjects. However, should subjects become claustrophobic during study or for any reason be unable to remain in the scanner, the scan

will be terminated and the participant expeditiously removed from the scanner.

Risks of assessments: The main risks are that the interviews and questionnaires are time consuming. These assessments, however, rarely pose psychological risks for the study participants.

Mitigation of risk of assessment: While most subjects will find the interviewing to be a positive experience, some may become anxious or tearful because of the personal nature of the questions. Interviewers will exercise caution and sensitivity and terminate the interview as necessary in order to protect the emotional well-being of the subject.

Risk of breach of confidentiality: *Breaches in confidentiality could have an negative impact, but protections are in place to minimize the risk of this happening.*

Mitigating the risks of breach of confidentiality: To minimize the possible risk of breach of confidentiality, each study participant will be assigned, upon enrollment in the study, a study ID code that will be used as an identifier for the subsequent research-related activity. All information collected, including self-reported questionnaires and imaging data are identified solely with the study ID without any personal identifiers. Only strictly anonymized data will be used for statistical analysis. Contact information for the subjects will be collected in order to reach them for appointment reminders and to be able to communicate with them weekly. This information will be kept on a separate form accessible only to the research team members who will be contacting the subjects. Access to the subject ID list will be limited to specific members of the research team. Research paper files will be kept in a locked office and only made available to qualified personnel for research purposes. All data will be entered into REDCap database and stored in a HIPAA compliant environment. No verbal or written information concerning a subject will be released to anyone without the express written authorization of the subject.

15.2 N/A

15.3 N/A

15.4 N/A

16.0 Potential Benefits to Subjects*

16.1 Benefits
Healthy control: None.

Placebo: increasing functional capacity and cardiovascular health outcomes.
Creatine: increasing muscle blood flow, increasing functional physical capacity and cardiovascular health outcomes.

Static muscle stretching has been shown to improve muscle blood flow. We believe that combining creatine supplementation with muscle stretching will enhance the efficacy of the treatment protocol, further enhancing cardiovascular function.

17.0 Data Management* and Confidentiality

17.1 Statistical analysis will include repeated measures ANOVA with Bonferroni post hoc analysis and paired samples t-test when applicable. Due to the nature of the current investigation (pilot study) we will be running a power analysis once the data set is collected.

17.2 During this study, and after consent is obtained, medical history questionnaires will be filled out prior to the start of the study. Experimental measurements will be obtained for statistical analyses and for research purposes only, and data will be kept in strict confidence. No information will be given to anyone without written permission from the subject. Data will be entered into REDCap and stored in a HIPAA compliant environment. Confidentiality will be ensured by use of identification codes. All data, whether generated in the laboratory or in post-processing will be identified with an identification code unique to the subject. The master identification code list will be kept under lock and key access limited to the P.I. and necessary members of the study team. Any presentation or publication of the data will be done in cumulative fashion without identifiers.

17.3 Our protocols include standard operating procedures for calibration of instrumentation and all data collection.

17.4 Describe how data or specimens will be handled study-wide:

- *All data collected on paper will be identified only by subject ID. Paper forms will be entered and/or uploaded into REDCap database which only the research team will have access to. Subject contact information will be necessary, but this information will be stored separately with limited access to the study team members who must contact the subjects for AE tracking and appointment reminders.*

- Individual participant paper files will be stored in a locked cabinet in a locked office only accessible to the research team. Any data extracted from the REDCap database will be secured with password protection. Electronic communication between investigators will only involve anonymous information.

  All data forms and study specific information will be kept in a locked file cabinet and in a password protected capable computer database with access limited to only the P.I. and key personal of the research team. Confidentiality will be protected by utilizing a code number as the only identifier for each subject.

  The PI or study staff will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance. A check list will be used to verify compliance. The frequency of data review for this study differs according to the type of data and can be summarized as follows:

Data Type: Subject accrual (including compliance with protocol enrollment criteria); Frequency: Weekly; Reviewer: PI
Data Type: Status of all enrolled subjects, as of date of reporting; Frequency: Weekly; Reviewer: PI
Data Type: Adverse Events* and rates (including out-of-range lab values); Frequency: Weekly; Reviewer: PI
Data Type: Serious adverse events**; Frequency: Per occurrence; Reviewer: PI *An adverse event (AE) is any untoward medical occurrence in a subject during participation in the study or with use of the experimental agent being studied.

Upon completion of the study, the data will completely de-identified so that it may be kept indefinitely. Once publications have been approved we will transfer all paper data into the FSU online system that is password protected.

All study records must be kept in compliance with FDA regulations.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects*

- We will not use data to monitor safety of subjects/patients. We are doing this by talking with the patients and subjects weekly.

19.0 Provisions to Protect the Privacy Interests of Subjects

19.1 Only the PI and the research team will have full access to patient records. All data will be deidentified when post processing. *This*

investigation is not to be kept in secret. All patients may talk about the study and study components to whomever they choose.

19.2 We will be sure to inform the patients that this study is totally voluntary, and they are not required to answer anything they feel is uncomfortable or they do not want to answer.

19.3 We will let the patients know their data will be coded and locked away with a similar level of security as a doctor's office. Besides the PI and research team, no other lab help will have sensitive information (i.e., health history) or any information to link the data back to the subject.

20.0 Compensation for Research-Related Injury

20.1 N/A

20.2 N/A

21.0 Economic Burden to Subjects

21.1 N/A

22.0 Consent Process

22.1 Indicate whether you will you be obtaining consent, and if so describe:

- *Consent process will take place in the College of Medicine and/or at Vascular Surgery Associates.*
- *Patients must first have doctor's approval prior to second visit and consent.*
- *Every week we will speak with patients and make sure they are comfortable and without any distress.*
    - *We will be following the HRP-090 consent process.*

Non-English Speaking Subjects

- *Non-English patients will be excluded from the current study due to inability to translate.*

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception)

- *N/A we will obtain consent.*

Subjects who are not yet adults (infants, children, teenagers)

- *N/A*

Adults Unable to Consent *N/A*

23.0 Process to Document Consent in Writing

23.1 We are using the common consent form for patients.

23.2 We will obtain written consent and then doctor's approval of PAD patients. All healthy volunteers will not be required to get physician approval but will also document consent in writing.

24.0 Setting

24.1 Subjects with PAD will be identified and recruited from Vascular Surgery Associates Clinic. Screening visit for healthy volunteers will take place at FSU College of Medicine. Study procedures (for all subjects) for Visit 2 and 3 will be conducted at FSU College of Medicine facilities.

- *For research conducted outside of the organization and its affiliates describe: N/A*

25.0 Resources Available

25.1 Describe the resources available to conduct the research: For example, as appropriate:

- *Given that the percent of individuals over 60 years of age with PAD is 12-20% we believe this allows us with a sufficient pool of individuals to recruit in the Tallahassee area.*
- *80% of Matthew Martenson, 10% of Dr. Caldwell and 15-20% of Dr. Muller-Delp's time will be committed to this study.*
- *Our space for human research is approximately 1000 sq feet. We have access to patient rooms in the MRI facility where we are conducting part of the current study. This room is outfitted with chairs and an examination table we will use for resting measurements. We are currently using these facilities on another human study with splints and muscle stretching. Down from the MRI room we have access to a hallway where we will conduct the 6MWT.*
- *We are located within the College of Medicine and will have an AED close by. In addition, we will have a College of Medicine physician (Dr. Lawrence D. Kaelin) that will have oversight of the study. He will not directly interact with patients; however, he is aware of the protocols being followed.*
- *All individuals will be trained to understand data collection and methods prior to first patients with mock run throughs to ensure that all research runs accordingly and will be the primary individual taking measurements.*

26.0 Multi-Site Research* -- N/A