

1. **Title of proposal:** Acute vasoprotective effects of nitrate-rich Beetroot juice
2. **Purpose:** Dietary nitrate supplementation, a natural means of increasing bodily stores of the vascular protective molecule nitric oxide, has well-established blood pressure lowering effects (Kapil et al; Vanhatalo et al; Webb et al) and considerable therapeutic potential for the prevention and treatment of hypertension and other cardiovascular diseases (Lundberg et al). Our long range goal is to examine the effectiveness of chronic dietary nitrate supplementation in reversing age- and disease-related stiffening of the cardiovascular system and its functional consequences. However, before we can initiate this new line of investigation and submit research proposals for extramural (NIH, AHA) funding, we need to develop an infrastructure for conducting dietary nitrate supplement research and generate preliminary data supporting the feasibility of our approach and our working hypotheses. The purpose of the proposed activities in this level I application is to establish the expertise, methodologies, and experience needed to evaluate the *acute* vasoprotective effects of Beetroot juice consumption in humans. The collaborative experience and preliminary data resulting from this proposed work, if funded, would provide the basis for a more extensive level II SSRI proposal to evaluate the vascular- and cardio-protective effects of *chronic* nitrate supplementation in a population with accelerated arterial stiffening (post-menopausal women). These results, in turn, would support a subsequent R21 application (responsive to PAS-11-280; Novel interventions for prevention and treatment of age-related conditions) to investigate the effectiveness of chronic dietary nitrate supplementation as a potential adjunctive cardiovascular de-stiffening therapy in older women and persons with type 2 diabetes (i.e. populations that exhibit attenuated cardiovascular adaptive responses to conventional therapies such as exercise training).
3. **Background and description of activities:** The first objective of this level I proposal is to formally assemble a group of investigators with the expertise needed to conduct dietary nitrate supplementation research in humans. A preliminary group of interested investigators has been identified (see below). Additional researchers/clinical personnel will be added to our investigative team as this research program develops. For example, we may need to collaborate with a Food Chemist to produce a more cost-effective placebo (i.e., Beetroot juice with nitrate extracted). Our second objective is to refine the methodological procedures for administering the nitrate supplement (dose and timing of ingestion, pre-study visit dietary control) and quantifying its absorption and conversion to nitrite/nitric oxide. This will involve collaboration with our colleagues in Nutritional Sciences (Drs. Kris-Etherton and Skulas-Ray) and the Biomarker Core Laboratory (Dr. Klein). Our third objective is to conduct a preliminary study (pending IRB approval) to investigate the acute vascular effects of Beetroot juice consumption (vs. placebo) in a sample of 8 healthy young men (19-30 yr). Primary outcome variables for this study will include plasma nitrates/nitrites and cGMP (gold standard measure of nitric oxide activity), resting blood pressure, measures of arterial stiffness (pulse wave velocities), conduit artery pulsatility and endothelial shear patterns, and lastly, forearm exercise-induced dilation of the brachial artery (Wray et al). All vascular measurements will be obtained between 2.5 and 4 hours post-nitrate/placebo consumption (time of peak nitrate absorption/nitrite conversion). We hypothesize that acute Beetroot juice consumption will reduce resting blood pressure and central (aortic) artery stiffness, and augment exercise-induced artery dilation in these subjects, and that these alterations will be

correlated with plasma nitrite/cGMP concentrations. Subject screening procedures (medical history, CBC blood draw) will be conducted in the Clinical Research Center by clinical (CTSI) staff, while experimental visits (placebo and nitrate supplementation, random order) will be conducted in Dr. Proctor's laboratory in Noll. The experience gained as a result of this preliminary study will enable the development of a follow-up investigation to determine the vascular effects of chronic Beetroot juice supplementation in post-menopausal women.

4. **Relevance to SSRI's mission:** The relevance of our proposed dietary nitrate research program for the SSRI rests primarily in the potential long range health and economic benefits of this nutraceutical. Nitrate-rich Beetroot juice supplementation increases systemic levels of nitric oxide, a potent vasodilator and vasoprotective molecule, and has established blood pressure lowering effects in both healthy adults and in persons with cardiovascular disease. Developing our ability at Penn State to investigate the acute (level I application) and chronic (level II application, NIH/AHA) vasoprotective effects of Beetroot juice consumption will enable a broader range of dietary nitrate supplement-related effects to be identified, particularly those which could benefit the vascular health of our rapidly expanding elderly and diabetic populations (i.e., through reduced heart and central artery stiffening). Findings from this line of investigation could ultimately be used to support dietary nitrate therapy for people with cardio-vascular and/or cerebro-vascular diseases, the leading two causes of death and disability in this country. The resultant increases in longevity and quality of life afforded by slower cardiovascular aging and delayed progression of disease would enable greater social engagement among aging parents and their families/peers. The economic benefits of an easily accessible and inexpensive dietary supplement would also be substantial and potentially wide-reaching (i.e., accessible to all socio-economic groups).

5. **Indicate if SSRI services will be used (GIS, PRI, SRC):** None of these services is requested for this initial (level I) proposal.

6. **Timeline:**

July 2012: Meet with collaborators; prepare/submit IRB/CRC application materials for acute Beetroot juice study.

Aug/Sept 2012: Data collection for acute Beetroot juice supplementation study.

Sept/Oct 2012: Prepare level II SSRI application.

7. **Investigator information:**

**Lead investigator:**

David Proctor

**Students:**

David Moore, Doctoral Student, Physiology Program

David Maurer, Masters Student, Kinesiology Program

Mark Bundschuh, Schreyer Honor's Student

**Collaborating investigators:**

Penny Kris-Etherton, Professor of Nutritional Sciences

Ann Skulas-Ray, Post-doctoral fellow, Nutritional Sciences

Laura Klein, Associate Professor of Biobehavioral Health

Courtney Whetzel, Research Associate, Healthy Aging Center

<b>Proposed Budget:</b>	<b><u>Totals</u></b>
<i>Beetroot juice costs (includes shipping):</i>	
Beetroot supplement for 10 subjects <sup>1</sup>	\$100.00
Beetroot placebo supplement	\$500.00
Snack (juice, granola bar)     \$2 per study visit x 2 visits x 10 subjects	\$40.00
<i>Subject remuneration costs:</i> \$75 per study visit x 2 visits x 10 subjects	\$1500.00
<i>Subject screening costs (CRC):</i>	
Vital signs/nurse check             \$ 6.00 per subject x 2 visits x 10 subjects	\$120.00
Screening blood draw (Quest) \$12.00 per subject x 1 visit x 10 subjects	\$120.00
<i>Blood draw costs:</i>	
CRC charges: \$12.50 per blood draw x 2 per visit x 2 visits x 10 subjects	\$500.00
<i>Blood assay costs:</i>	
Nitrates/nitrites                     \$185 per colorimetric kit x 2 kits	\$370.00
cGMP                                     \$270 per ELISA kit x 5 kits	\$1350.00
Ultrapure water	\$100.00
Collection/storage tubes	\$ 50.00
<i>Exercise device related costs:</i>	
Handgrip device construction, etc	\$150.00
<b>TOTAL</b>	<b>\$4900.00</b>

<sup>1</sup>Our goal is to complete all study visits/measurements on 8 subjects. We are budgeting for 10 subjects to account for possible 1) subject drop-outs, 2) missing/unusable data, 3) equipment malfunction, and 4) repeat/additional assays on stored blood samples.

**References:**

**Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Tarr J, Benjamin N, and Jones AM.** Dietary nitrate supplementation reduces the O<sub>2</sub> cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *Journal of Applied Physiology* 107: 1144-1155, 2009.

**Kapil V, Milsom AB, Okorie M, Maleki-Toyserkani S, Akram F, Rehman F, Arghandawi S, Pearl V, Benjamin N, Loukogeorgakis S, MacAllister R, Hobbs AJ, Webb AJ, and Ahluwalia A.** Inorganic Nitrate Supplementation Lowers Blood Pressure in Humans. *Hypertension* 56: 274-281, 2010.

**Kenjale AA, Ham KL, Stabler T, Robbins JL, Johnson JL, VanBruggen M, Privette G, Yim E, Kraus WE, and Allen JD.** Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. *Journal of Applied Physiology* 110: 1582-1591, 2011.

**Lundberg JO, Carlström M, Larsen FJ, and Weitzberg E.** Roles of dietary inorganic nitrate in cardiovascular health and disease. *Cardiovascular Research* 89: 525-532, 2011.

**Presley TD, Morgan AR, Bechtold E, Clodfelter W, Dove RW, Jennings JM, Kraft RA, Bruce King S, Laurienti PJ, Jack Rejeski W, Burdette JH, Kim-Shapiro DB, and Miller GD.** Acute effect of a high nitrate diet on brain perfusion in older adults. *Nitric Oxide* 24: 34-42, 2011.

**Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, Benjamin N, Winyard PG, and Jones AM.** Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 299: R1121-R1131, 2010.

**Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P, Deanfield J, Benjamin N, MacAllister R, Hobbs AJ, and Ahluwalia A.** Acute Blood Pressure Lowering, Vasoprotective, and Antiplatelet Properties of Dietary Nitrate via Bioconversion to Nitrite. *Hypertension* 51: 784-790, 2008.

**Wray DW, Witman MAH, Ives SJ, McDaniel J, Fjeldstad AS, Trinity JD, Conklin JD, Supiano MA, and Richardson RS.** Progressive handgrip exercise: evidence of nitric oxide-dependent vasodilation and blood flow regulation in humans. *American Journal of Physiology - Heart and Circulatory Physiology* 300: H1101-H1107, 2011.



Department of Nutritional Sciences  
College of Health and Human Development  
The Pennsylvania State University  
110 Chandlee Laboratory  
University Park, PA 16802-6109

814-863-0772  
Fax: 814-863-6103

June 27, 2012

Dear David,

Thank you for inviting us to collaborate on your SSRI Level I proposal "Acute vasoprotective effects of nitrate-rich Beetroot juice." We are delighted to be a part of this line of research. Your proposed use of nitrate supplementation to reduce aging related increases in vascular stiffness may identify a cost effective means of improving the health and quality of life for many aging individuals. We appreciate the importance of studying novel means of reducing the burden of cardiovascular diseases. Further, your selection of Beetroot juice supplementation for this first study is appealing in that it is a non-pharmacological approach available to the general population. As you have identified, the conduct of dietary supplementation studies are an area in which we can bring experience. We look forward to this valuable opportunity to better understand the functional vascular assessments in which your lab has expertise. It is our intent and hope that this study serves as a springboard for future collaborations on the study of nutritional interventions that may be effective for preserving arterial health.

Best regards,

A handwritten signature in black ink, appearing to be 'ASR', with a horizontal line extending to the right.

Ann Skulas-Ray and Penny Kris-Etherton



June 29, 2012

David Proctor, Ph.D.  
Professor of Kinesiology  
College of Health and Human Development  
The Pennsylvania State University

Dear David,

I am delighted to collaborate on your SSRI Level I application, "Acute vasoprotective effects of nitrate-rich Beetroot juice." I direct the Biomarker Core Laboratory where we successfully conducted nitrate/nitrite assays for your NIA grant and believe that this proposal is the perfect extension of your NIH funded research. I look forward to working with you and our colleagues on this project.

Best regards,

A handwritten signature in black ink that reads 'Laura Klein'.

Laura Cousino Klein, Ph.D.  
Associate Professor of Biobehavioral Health  
Director, Biomarker Core Laboratory



David Proctor  
Professor, Department of Kinesiology

June 29<sup>th</sup>, 2012

Dear David,

I look forward to collaborating with you and colleagues on your SSRI Level 1 proposal "Acute vasoprotective effects of nitrate-rich Beetroot juice." As the coordinator for the Biobehavioral Health Biomaker Core lab I will be interacting with our laboratory techs to bring you the best data possible from your samples. This will open up another area of assays that we look forward to providing for future collaborations as well. I have an extensive background in biomarker data and will be available for any questions and problem solving needed regarding biomarker sample collection and assaying.

Sincerely,

A handwritten signature in black ink, appearing to read "Courtney A. Whetzel". The signature is fluid and cursive, with a large initial 'C' and 'W'.

Courtney A. Whetzel, Ph.D.  
Research Associate