THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF KINESIOLOGY

EFFECT OF DIETARY NITRATE SUPPLEMENTATION ON NITRIC OXIDE-MEDIATED VASCULAR RESPONSES

MARK AUSTIN BUNDSCHUH
SPRING 2013

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Science
with honors in Kinesiology

Reviewed and approved* by the following:

David N. Proctor
Professor of Kinesiology and Physiology
Thesis Supervisor

Steriani Elavsky
Assistant Professor of Kinesiology
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Objective: To assess the effect of dietary nitrate supplementation on nitric oxide (NO)-mediated vascular responses in the brachial artery. **Methods:** Five healthy, young men (22 ± 2 yrs) consumed a standard dose (140 mL, equivalent to the nitrate content of 5 servings of spinach) of nitrate-rich beetroot juice (active) or a similar quantity of nitrate-free beetroot juice (placebo) on two separate days, approximately one week apart. Three hours following beetroot juice consumption, Doppler ultrasound was used measure brachial artery diameter and mean blood velocity in response to a discontinuous protocol of six stages of handgrip exercise (200 g, 400 g, 600 g, 800 g, 1000 g, 1200 g) separated by 1 minute rest periods. Participants were paced via a metronome to contract 30 times per minute. **Results:** Resting supine blood pressures taken ~ 3 hours following beetroot juice consumption were similar ($p > 0.05$) during the nitrate (mean = 120/65 mmHg) and placebo (mean = 118/64 mmHg) visits. Nitrate supplementation did not alter the overall dilator response of the brachial artery to graded handgrip exercise (rest to peak; 6.9% placebo vs. 5.2% nitrate, $p > 0.50$), but it did delay this response, possibly due to the elevated baseline artery diameter observed during the nitrate visit (4.15 vs. 3.85 mm, $p = 0.12$). Nitrate supplementation also modified the rise in arm blood flow across increasing handgrip work rates (treatment by work rate interaction; $p = 0.19$). **Conclusions:** Acute dietary nitrate supplementation causes vasodilation of forearm resistance vessels, but does not appear to impact the shear-induced, nitric-oxide mediated dilation of the brachial artery in healthy, young men. The exercise intensity-dependent influence of dietary nitrate supplementation on forearm hyperemia has not previously been reported,

and adds further insight into the physiological conditions under which this dietary supplement may have its largest vascular effects (i.e., when tissue PO_2 and pH are low).

Key Words: brachial artery, dietary nitrate, enterosalivary circulation, handgrip exercise, nitric oxide

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Over the past two and a half years, I have received training, guidance, and encouragement from a great number of individuals that contributed to the success of my Schreyer Honors College thesis.

I have always considered myself incredibly fortunate to have Dr. Proctor as my honors supervisor. His extensive knowledge and patience provided me continual encouragement to do my best work. Moreover, his insight has helped me define my career interest by discovering the connections between research and medicine. This research experience has been an invaluable part of my undergraduate education.

Thank you to our research team, Dave Moore, Dave Maurer, and Danielle Kim for the hours invested in my research training and assistance with data collection/analysis. Thank you to Kate Sauder for sound advice on study design and statistics, and to Cyndi Flanagan and Tracey Banks of the Clinical Research Center for help with data collection. Thanks also to Dr. Elavsky for being a knowledgeable and kind honors advisor.

I would also like to thank Dr. Korzick and Dr. Butler of Penn State's Summer Translational Cardiovascular Sciences program (AHA 12UFEL10340001), which provided experience and financial support for my research study. Additional funding was granted by Penn State's Social Science Research Institute Level 1 Seed Grant.

Finally, I would like to thank my friends for making my time at Penn State unforgettable, and my grandparents, mom, dad, and brother for their love and support.

Chapter 1

INTRODUCTION

Cardiovascular Disease (CVD) accounts for about one in three deaths in the United States.[10,21] Therefore, interventions designed to reduce the rates of major CVD risk factors such as hypertension could potentially save thousands of lives each year. Indeed, studies have demonstrated that diets rich in fruits and vegetables, such as the DASH (Dietary Approaches to Stop Hypertension) diet can reduce the risk of major CVD events such as coronary heart disease, stroke, and heart failure.[8,19,20] Identifying the micronutrients responsible for the antihypertensive/cardioprotective effects of such diets is an area of active investigation; growing epidemiological and mechanistic evidence points to inorganic nitrates (rather than antioxidants)[11,18,22] as the key bioactive substance responsible for these cardio/vasoprotective effects.

Following dietary (inorganic) nitrate supplementation via beetroot juice[12,29] and capsule[13], both plasma nitrate (~ 1 to 3 hours) and nitrite (~ 2 to 4 hours) concentrations have been shown to significantly increase. The in vivo conversion of dietary nitrate to nitric oxide occurs via a two-step reduction. About 25% of circulating nitrate is absorbed into the enterosalivary circulation.[26] Nitrate then collects in the salivary glands and is reduced to nitrite by nitrate reductases of facultative bacteria. This mechanism was elucidated through nitrate supplementation studies which demonstrated significant increases in plasma nitrate, but not plasma nitrite concentrations when volunteers used antibacterial mouthwash concurrently, disrupting the enterosalivary circulation.[9,23,29] Nitrite is then swallowed and reduced to nitric oxide (NO) in the acidic environment of the stomach. However, large amounts of swallowed nitrite enter the systemic circulation, and can be converted to NO by several circulating and/or tissue nitrite reductases such as deoxygenated hemoglobin[5], xanthine oxidoreductase (XOR)[31], vitamin C[4], and even NO synthase[28].

A large body of literature has shown significant reductions in systolic[2,3,12,13,15,16,27,29] and diastolic blood pressure[12,27,29,2,13,16] following nitrate consumption. Further, Kapil *et al.* 2010 reported elevations in plasma cyclic guanosine monophosphate (cGMP) concentrations (i.e., the most sensitive indicator of nitric oxide bioactivity). Consistent with this enhanced production of NO, several studies have demonstrated improved dilator responses via exogenous nitrite and nitrate supplementation. Arterial infusions of nitrite in the human brachial artery have produced augmented forearm blood flow.[5,6] Moreover, by inhibiting nitric oxide synthase (NOS) with N-Methylarginine (L-NMMA), Cosby *et al.* 2010 demonstrated that much of this dilation is NOS independent. To further address the hypothesis that exogenous nitrate and nitrite can enhance endothelial function, subjects underwent flow-mediated vasodilation (FMD) testing after an acute, oral dose of nitrate. These studies showed no functional evidence of improved endothelium-dependent FMD responses in young, healthy subjects.[1,29] However, these tests of endothelial function only examine brachial dilation to a single FMD stimulus.

Graded handgrip exercise testing elicits a progressive dilation of the brachial artery, and a hyperemic response that has been recently shown to be endothelium-dependent and nitric oxide-mediated in healthy younger adults.[30] This graded handgrip test also provides a more sustained and wide ranging shear stimulus than the conventional (single, transient stimulus) FMD technique. For these reasons, we speculated that the graded handgrip exercise test would provide a more robust assessment of the potential nitric oxide-mediated effects of dietary nitrate supplementation than the FMD test protocol that has been used to date. Accordingly, the primary purpose of this thesis study was to test, in healthy, young men, the hypothesis that ingestion of nitrate-rich beetroot juice would augment the shear mediated dilator response of the brachial artery to graded handgrip exercise.

Chapter 2

METHODS

Subjects

Five young men (22 ± 2 yrs) completed the study. All subjects were normally active (neither sedentary nor performing regular aerobic exercise training ≥ 3 days/week), nonsmokers, and normotensive (resting blood pressure < 140/90 mm Hg) with clinically normal blood lipids (total cholesterol < 240 mg/dL, low-density lipoprotein cholesterol < 130 mg/dL, high-density lipoprotein cholesterol > 40 mg/dL). Subjects were free of overt chronic diseases as evaluated by medical history questionnaire. Also, no subjects were taking medications having significant cardiovascular effects. All subjects gave their written informed consent after being explained all experimental procedures and possible risks and benefits associated with the study. This study was approved by the Office for Research Protections and the Institutional Review Board at The Pennsylvania State University. Subject characteristics are presented in Table 1.

Recruitment and familiarization

Young men (19 to 35 yrs) from the Penn State campus were recruited via word of mouth and posted flyers for this study which took place over the period of January to March 2013. Subjects completed an initial screening visit to make sure they met inclusion/exclusion criteria and to familiarize them with the graded forearm exercise protocol and hemodynamic measurements. During this visit, subjects also performed maximal (isometric) handgrip testing; peak forearm muscle strength was defined as the highest of three consecutive maximal exertions with their non-dominant hand.

Experimental study visits

Pre-visit instructions

Subjects participated in two experimental study visits (placebo visit or nitrate supplement visits), at least five days apart to allow full washout of the supplement. Prior to each of these visits, subjects were instructed to abstain from consuming alcohol and caffeine for at least 48 hours and from exercise for at least 24 hours. Subjects were also asked to fast for at least 12 hours prior to these visits. Both visits started at approximately the same time of day (beetroot juice consumption typically 9am) with the resting hemodynamic measurements beginning at approximately 11:30am and the handgrip exercise protocol beginning at approximately 12:30 pm.

Beetroot juice consumption and blood draws

Upon arrival in the morning at the Clinical Research Center in Noll Laboratory, subjects had a baseline seated resting blood pressure measurement taken and a baseline venous blood sample withdrawn. Following these baseline procedures, subjects ingested one of two beetroot juice drinks. Subjects consumed 140 mL of either (1) the Beet-it Sport Shot (James White Drinks, Ipswich, UK; 0.8 g, 13 mmol of nitrate) or (2) the Beet-it Sport Shot Placebo (contains all of the same components, but is nitrate-free). Subjects were permitted a small granola bar and water during consumption of the beetroot juice.

To standardize each subject’s activity while waiting for digestion, absorption, and conversion (to nitrite and nitric oxide) of the consumed drink, subjects were required to remain in Noll Laboratory for 2.5 hours. While waiting, subjects were allowed to study, check their e-mail, and perform other sedentary tasks. Seated blood pressure was remeasured taken 1 and 2 hours after beetroot juice consumption. After 2.5 hours, a second venous blood draw was taken. Subjects were then escorted to the Vascular Aging and Exercise Laboratory, a quiet, temperature controlled room (22-25°C), for the remaining study procedures.

Resting blood pressures

Upon arrival to the Vascular Aging and Exercise Laboratory, subjects were instructed to lie supine for 15 minutes to achieve a resting steady-state condition. Subjects then underwent three consecutive bouts of left and right brachial artery blood pressure measurements (Colin VP-2000, Medical Instruments Corporation). Pulse wave velocity testing and a two-minute cognitive function test (serial subtraction) were also conducted, but these measurements will not be presented in this thesis.

Graded handgrip protocol

Following resting measures, subjects performed graded exercise using a custom-built handgrip device that restricted their range of motion (10 cm). Following a brief resting stage, subjects completed six stages of exercise (200 g, 400 g, 600 g, 800 g, 1000 g, 1200 g). Each stage of exercise was performed for approximately 3 minutes in order to achieve a hemodynamic steady-state condition. One minute breaks separated each stage of exercise to allow for recovery and limit sympathetic activation. Subjects were paced via metronome to contract their forearm muscles 30 times per minute. Brachial artery blood velocity was assessed from approximately 0 until 3 minutes at each stage of exercise. Brachial artery diameter was then measured from approximately 3 until 3:15 minutes of each stage of exercise.

Vascular ultrasound imaging

Diameter and blood velocity of the left brachial artery were measured 2 to 5 cm proximal to the antecubital fossa using Doppler ultrasound (HDI 5000; Philips, Bothell, WA). Blood velocity was measured using a 6 MHz linear array transducer at an insonation angle of 60° and the sample volume adjusted to cover the width of the brachial artery. Blood velocity signals were collected online at a sampling frequency of 400 Hz and saved via a Powerlab system (AD

Instruments, Castle Hill, Australia). Diameters were obtained from two-dimensional images using the same 6 MHz linear array transducer with an insonation angle between 60° and 90°. Two-dimensional ultrasound images were recorded with a Dazzle Video Capture Card and stored offline using Studio 14 (Pinnacle Systems).

Calculations

Calculations were derived from the average values during hemodynamic steady-state conditions. Mean blood velocity of the brachial artery was calculated during rest (for one minute) and during each stage of exercise (using the time period from 2:30 to 3 minutes). A single investigator who was blinded to the treatment order calculated the mean diameter of the brachial artery using automated edge-detection software (Brachial Analyzer Software, Medical Imaging Applications; Iowa City, IA) during rest (for the last 15 seconds) and during each stage of exercise (using the time period from 3 to 3:15 minutes). Diameters were averaged across the cardiac cycle. Brachial artery mean blood velocity and mean diameter were used to calculate brachial artery blood flow and shear rate according to the following formulas:

$$\text{BA Blood Flow} = V_{mean}\,\pi\,(\text{BA diameter}_{mean}/2)^2 * 60$$

$$\text{BA Shear Rate} = 8V_{mean}/\text{BA diameter}_{mean}$$

Statistical analysis

The data were analyzed using IBM SPSS software (version 20). Blood pressures and brachial artery vasodilation (Figures 1 and 3) were assessed using a paired sample t-test to test for significant differences in means between the placebo and nitrate treatments. For brachial artery diameter and blood flow (Figures 2 and 4), two-way repeated measures ANOVA was used to determine significance of treatment effect, stage of exercise effect, and treatment by stage of exercise effect. When a significant treatment by stage of exercise interaction effect was found, Bonferroni tests were used for post hoc comparisons between treatments at each exercise stage. In accordance with the Bonferroni correction, the statistical significance for these tests was adjusted to $p < 0.007$ (= 0.05/7, accounting for rest and 6 exercise stages). Finally, Pearson's correlation coefficients was used to determine the significance of the association between blood-derived and hemodynamic variables of the brachial artery. Excluding Bonferroni tests, statistical significance was set at $p < 0.05$. All data are expressed as a mean ± SE unless otherwise noted.

Chapter 3

RESULTS

Subject feedback

All subjects tolerated the procedures of this investigation very well, including the consumption of the beetroot juice supplements, and there were no unexpected or adverse events. One of the five subjects disliked the taste of the Beetroot juice, but was able to consume the entire dose on both visits without any complications. No subjects reported *beeturia* following beetroot juice consumption, a harmless side effect most often reported during chronic (>3 days) nitrate supplementation studies.[2,3,12,24,27,29]

Resting blood pressures (Figure 1)

Resting supine blood pressure measurements were taken 3 hours following consumption of the beetroot juice on both study visits. Diastolic pressures on the placebo and nitrate study visits were nearly identical (64.6 ± 2.7 and 64.1 ± 3.0 mmHg, respectively; $p = 0.784$). There was a modest, but non-significant difference in systolic blood pressure between the placebo (120.4 ± 2.0 mmHg) and nitrate (117.8 ± 3.4 mmHg) visits ($p = 0.225$).

Pre-exercise (resting) brachial artery responses (Figure 2)

Blood flow through the brachial artery prior to the start of the forearm exercise test tended to be higher on the nitrate compared to placebo visit (51.7 ± 16.8 vs. 29.2 ± 3.6 ml/min, $p = 0.169$; Figure 2). Differences in resting brachial artery diameter (3.86 ± 0.13 and 4.14 ± 0.05

mm, respectively; $p = 0.115$) and mean blood velocity (4.21 ± 0.56 and 6.32 ± 1.94 cm/s, respectively; $p = 0.262$) were also not statistically significant between placebo and nitrate visits.

Exercise hyperemia responses (Figure 2)

Brachial artery blood flow increased linearly with increasing forearm exercise work rate on both study visits. However, ANOVA revealed a significant treatment (placebo vs. nitrate visit) by exercise stage interaction ($p = 0.019$), indicating a modified exercise hyperemic response during the nitrate visit. Blood flow at the final exercise stage (i.e., 1200 g) was 37% higher ($p = 0.030$) during the nitrate visit, but did not reach statistical significance (adjusted p-value of 0.007) in accordance with the Bonferroni correction.

Brachial artery dilator responses to exercise (Figures 3, 4, 5, and 6)

The peak vasodilator response of the brachial artery (i.e., peak change from resting diameter or peak percent change from resting diameter) observed during the final exercise stage did not differ (both $p > 0.50$) between the nitrate (0.214 mm, 6.94% above rest) and placebo (0.268 mm, 5.17% above rest) study visits (Figure 3). There was, however, a significant treatment (placebo vs. nitrate visit) by exercise stage interaction for brachial diameter ($p = 0.037$; Figure 4). Close examination of Figure 4 reveals a different trend of brachial artery dilation during the two study visits, such that the linear brachial artery diameter increase seen during the placebo visit was delayed during the nitrate visit. There were no significant differences between shear rates across all work rates (Figure 5). To account for possible differences in shear stimulus at a given work rate, the dilator response was also plotted against the shear stimulus (Figure 6). Comparisons of slopes during the final three work rates indicated no differences in response ($p = 0.541$).

Correlates of peak artery dilation (Figure 7)

Fasting plasma low-density lipoprotein (LDL) concentrations were measured during each subject's screening visit. There was a significant inverse association between LDL concentration and the peak brachial artery dilator response normalized to shear rate measured during the placebo visit ($r = -0.929$, $p = 0.022$).

Chapter 4

TABLES AND FIGURES

TABLE 1. Subject Characteristics

Values are Means ± STD

Subjects (n)	5
Age (yrs)	22 ± 2
Weight (kg)	83.2 ± 7.5
Height (cm)	183.7 ± 3.8
Body Mass Index (kg/m2)	24.7 ± 2.2
Glucose (mg/dL)	93 ± 3
Total Cholesterol (mg/dL)	148 ± 10
High-density Lipoprotein (mg/dL)	47 ± 5
Low-density Lipoprotein (mg/dL)	83 ± 14
Triglycerides (mg/dL)	93 ± 32
Systolic Blood Pressure (mmHg)	122 ± 7
Diastolic Blood Pressure (mmHg)	77 ± 5
Heart Rate (bpm)	63 ± 5
Maximal Voluntary Contraction (kg)	54 ± 8

FIGURE 1. Resting systolic blood pressure in response to placebo and nitrate treatment

Values are mean ± SE. Nonsignficant finding.

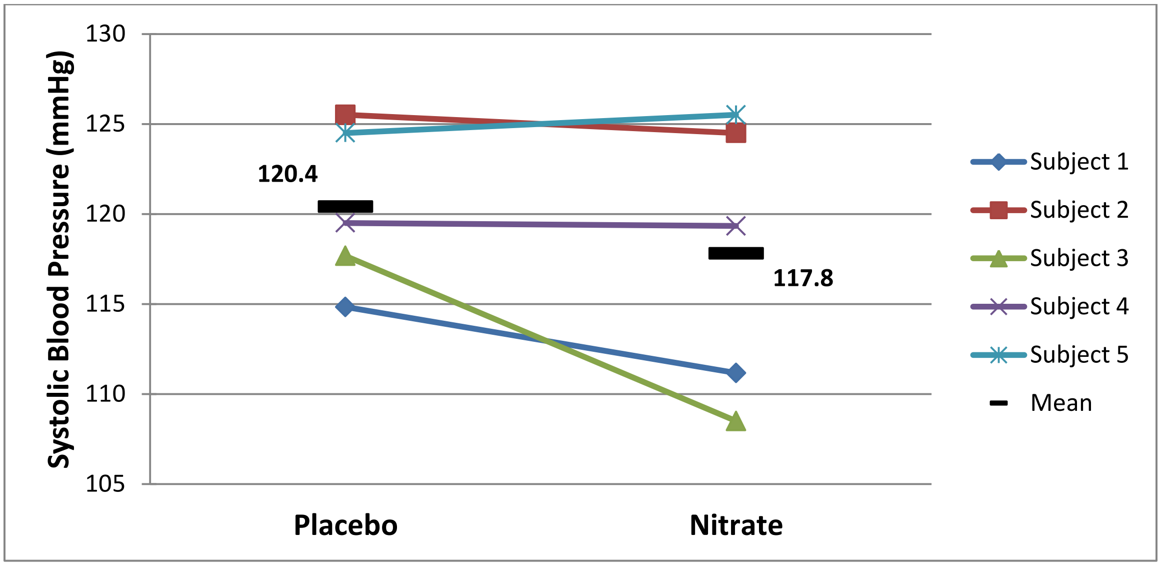


FIGURE 2. Brachial artery blood flow response to graded handgrip exercise during placebo and nitrate treatment

Values are mean ± SE. * Significant difference between Placebo and Nitrate responses ($p < 0.05$).

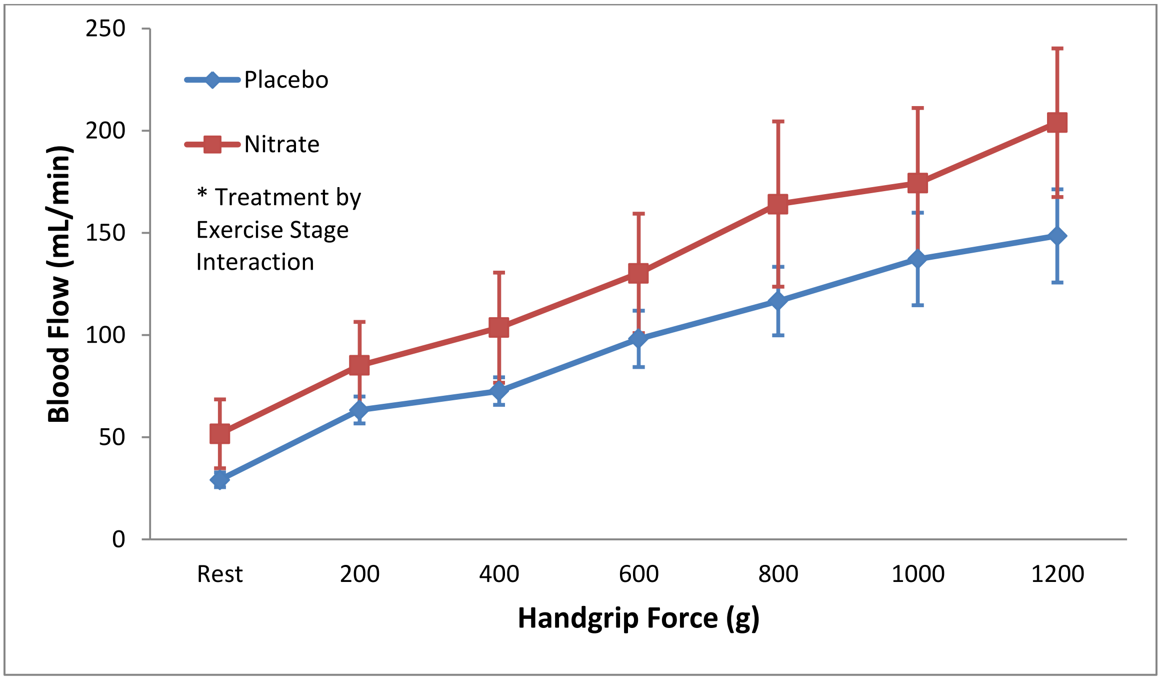
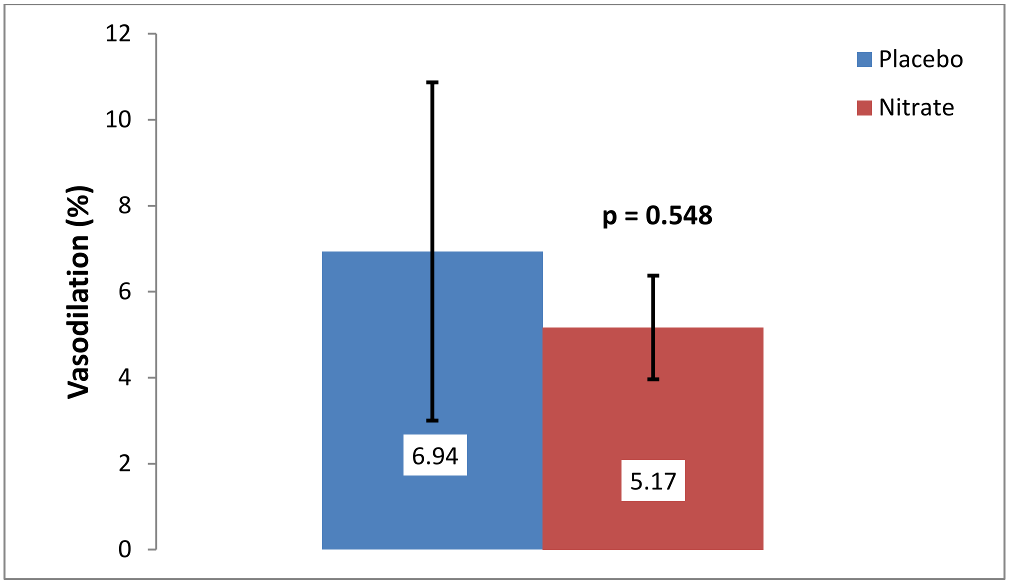


FIGURE 3. Percent vasodilation **(A)** and change in diameter **(B)** from rest to peak of the brachial artery in response to placebo and nitrate treatment

Values are mean ± SE.

(A)



(B)

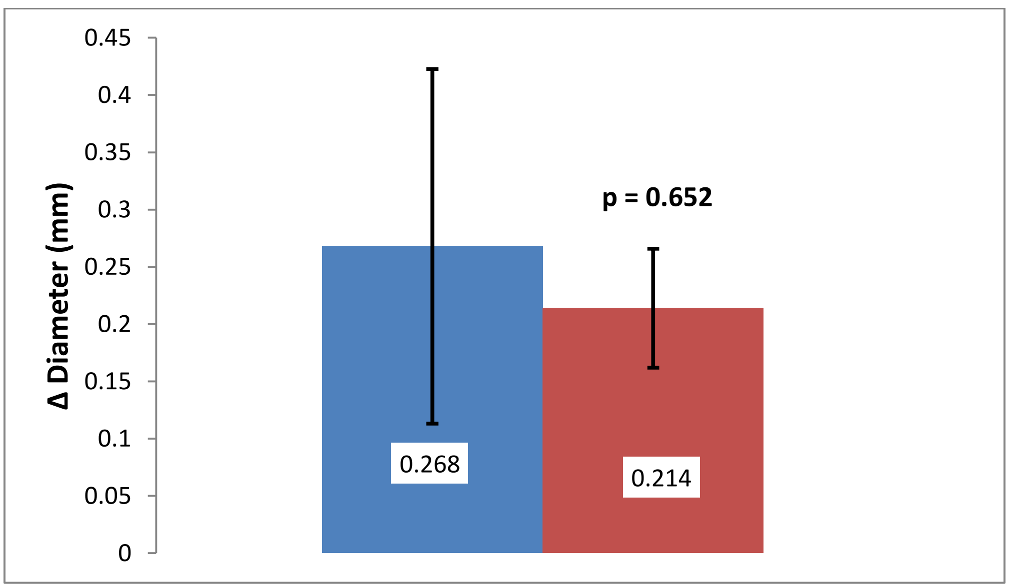


FIGURE 4. Brachial artery diameter response to graded handgrip exercise during placebo and nitrate treatment

Values are mean ± SE. * Significant difference between Placebo and Nitrate responses ($p < 0.05$).

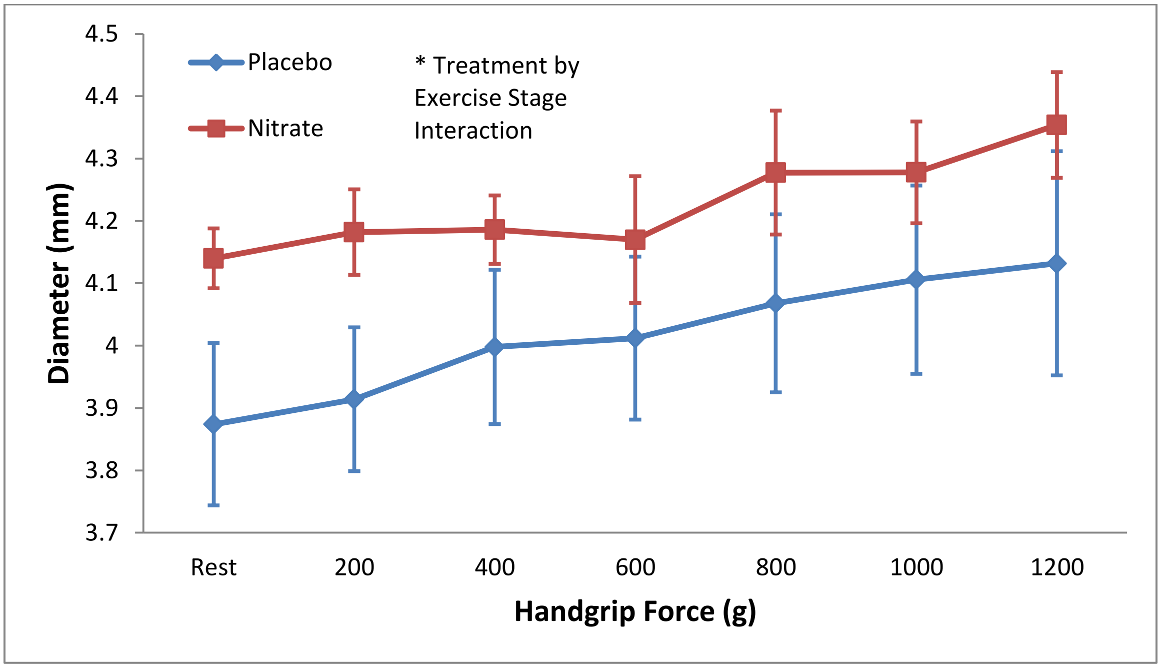


FIGURE 5. Brachial artery shear rate response to graded handgrip exercise during placebo and nitrate treatment

Values are mean ± SE. There were no statistical differences in shear rate vs. handgrip force between treatments ($p > 0.894$).

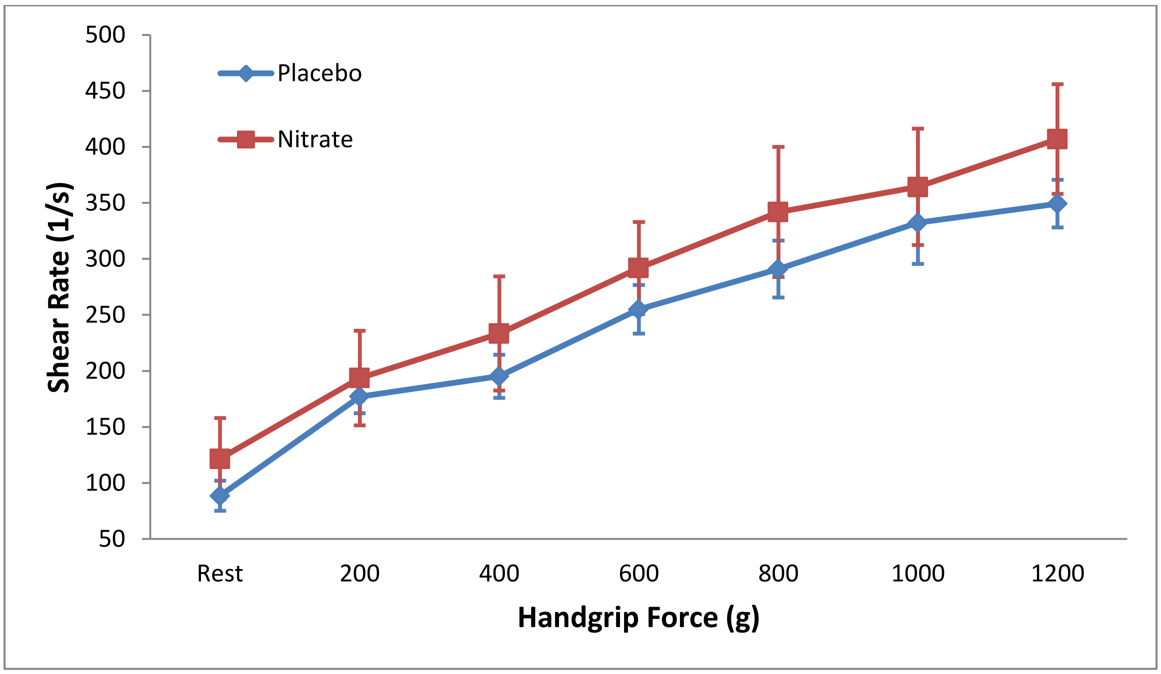


FIGURE 6. Relation between changes in shear rate and brachial artery diameter during graded handgrip exercise under placebo and nitrate treatment

Values are mean. Comparisons of slopes at final three work rates indicated no significant differences between placebo and nitrate treatment (p = 0.541).

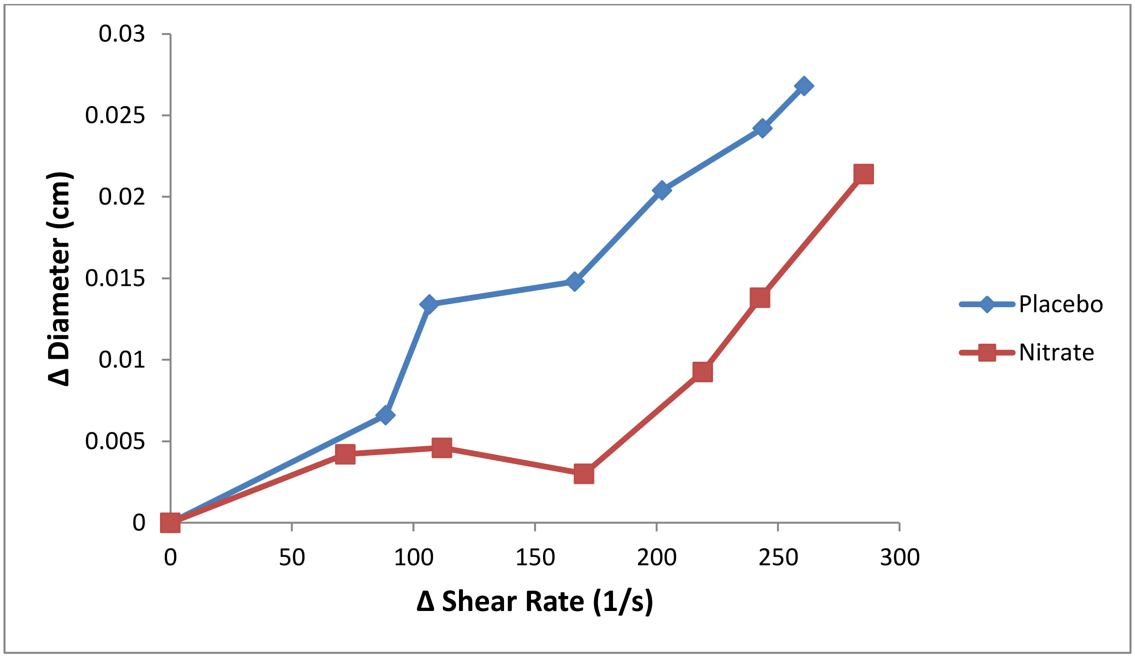
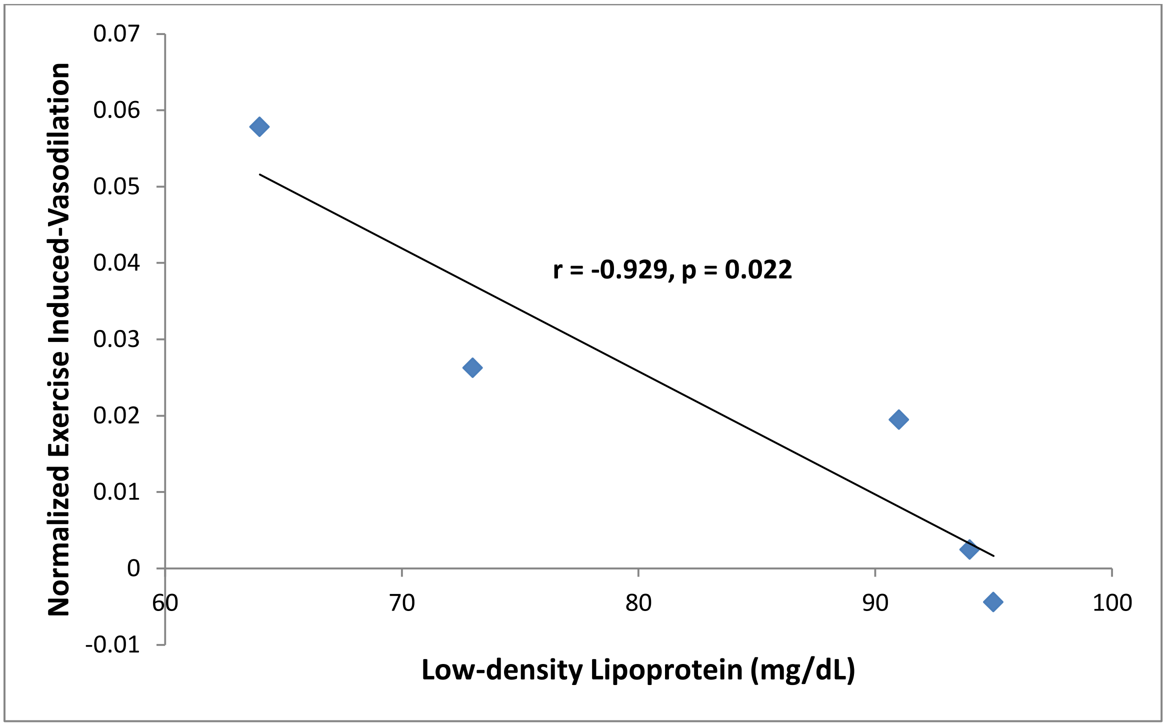


FIGURE 7. Association between plasma LDL concentration and exercise-induced brachial artery vasodilation normalized to shear rate during placebo treatment



Chapter 5

DISCUSSION

The present study utilized a randomized, double-blind, placebo-controlled design to examine the effects of acute dietary nitrate supplementation (~3 hr following consumption of 140 ml of nitrate rich beetroot juice) on nitric oxide-mediated vascular responses in healthy, young men. Preliminary findings (n = 5) are as follows: First, there were no detectable differences in resting systolic or diastolic blood pressure between the two study visits. Second, resting brachial artery diameter, blood velocity, and blood flow each appeared to be higher on the nitrate (compared to placebo) study visit. Third, nitrate supplementation did not alter the overall dilator response of the brachial artery to graded handgrip exercise (rest to peak), but it did appear to delay this response. A fourth finding was that nitrate supplementation modified the hyperemic response to graded handgrip exercise. Collectively, these results reinforce the fact that nitrite is a potent vasodilator of the forearm circulation in humans and provide further insight into the physiological conditions under which this dietary supplement may exhibit its largest vascular effects i.e., during higher intensity exercise when tissue PO_2 and pH are reduced.

Effects of dietary nitrate supplementation on resting blood pressure

Multiple studies have reported a progressive decline in resting systolic blood pressure (and in some cases diastolic pressure) in healthy volunteers following consumption of nitrate containing juice or nitrite containing capsules, with peak reductions in blood pressure coinciding with peak plasma nitrite concentrations approximately 3 to 4 hours post consumption.[13,29] Resting blood pressures taken approximately 3 hours following beetroot juice consumption in the present study averaged 120/65 and 118/64 mmHg for the placebo and nitrate visits, respectively. That there were no differences in diastolic pressure during the nitrate (vs. placebo) visit is not unexpected given that a number of nitrate supplementation studies have reported no changes in this variable.[3, 15] The non-significant difference in systolic pressure across these two visits is more surprising, but could reflect (1) the relatively low baseline blood pressures of our subjects and/or (2) our failure to track blood pressure changes *within* each visit and under consistent conditions (i.e., all measurements in the supine position and using the same blood pressure measurement technique). Between-visit comparisons of serial (rather than a single time point) of blood pressure in additional subjects will be necessary to establish whether this particular nitrate supplement product (Beet-It Sport Shot) elicits reductions in resting blood pressure in younger, normotensive volunteers.

Effects of dietary nitrate supplementation on resting forearm hemodynamics

Brachial artery blood flow 3 hours following beetroot juice consumption was, on average, 77% higher on the nitrate visit compared to the placebo visit. This difference did not achieve statistical significance ($p = 0.169$), but an elevation in resting forearm blood flow is a consistent finding based on previous studies involving sodium nitrite infusions at physiological doses into the brachial artery of healthy adults (i.e., 175-200% increase).[5,6] The elevated blood flow through the resting brachial artery resulted from a 50% higher mean blood velocity ($p = 0.262$) and a larger diameter (+7%; $p = 0.115$). The higher blood velocity through the brachial artery on the nitrate visit (at a similar or slightly lower mean arterial blood pressure) likely reflects the dilation of downstream (forearm muscle) resistance vessels in response to an increase in circulating nitrites.[14,17,25] The 7% larger resting artery diameter, in turn, likely reflects an increased endothelial shear stimulus (i.e., placebo shear rate = 88 s^{-1}, nitrate shear rate = 122 s^{-1}; Figure 7), apparently the first such measurements obtained during a study involving dietary nitrate supplementation. Additional subjects tested in a similar fashion will be necessary to strengthen the validity of these directional hemodynamic changes. However, when viewed in relation to the intra-brachial nitrite infusion results described above[5,6], there is considerable evidence that nitrates cause vasodilation of forearm resistance vessels in humans.

Effects of dietary nitrate supplementation on nitric oxide-mediated dilator responses

Two recent studies involving healthy younger volunteers reported no improvement in brachial artery flow mediated dilation (FMD) after ingestion of beetroot juice[29] or potassium nitrate[1]. Such results support the general view that dietary nitrate supplementation does not enhance endothelial vasodilator function in healthy, young adults, despite its ability to acutely (within 3 hours) raise plasma cGMP concentrations in such subjects.[13] As an alternative, we used a more sustained and wide ranging shear stimulus from a graded handgrip exercise model[30] to evaluate this question. We found that nitrate supplementation did not enhance/alter the overall dilator response of the brachial artery to this graded handgrip test (rest-to-peak response: 6.9% placebo vs. 5.2% nitrate, $p > 0.50$), but, interestingly, it did delay this response (Figure 4). We speculate that the absence of any dilation across the first 3 work rates on the nitrate visit simply reflects the “pre-dilated” state of the artery under this condition. Subsequent examination of the delta brachial artery diameter vs. delta shear rate relation across all work rates (Figure 5) revealed no obvious difference in slope between the placebo and nitrate visits. This dose-response assessment, which is more robust than a single FMD stimulus-response[30] accounts for the larger baseline diameter seen during the nitrate visit and provides evidence that dietary nitrates do not augment nitric oxide-mediated dilator responses in healthy, young men.

Effects of dietary nitrate supplementation on exercise hyperemia

Cosby *et al.* 2003 have previously shown that infusion of sodium nitrite directly into the brachial artery (5 min) significantly augments forearm blood flow during handgrip exercise. To our knowledge, the present study is the first to assess the potential of *dietary* nitrates to impact exercise-induced vascular responses in the arm. We observed a significant treatment-by-work rate interaction ($p = 0.019$), but due to a limited sample size were unable to detect at which work rate(s) these effects were significant. Nonetheless, based on close inspection of Figure 2, and the results of Cosby *et al.* 2003, it is reasonable to speculate that the nitrate supplement may have augmented forearm exercise hyperemia relatively more at the higher (compared to lower) work rates in our subjects. That beetroot juice consumption might elicit greater hyperemic benefits during higher intensity exercise is supported (indirectly) by (1) human studies demonstrating greater ergogenic effects during higher intensity exercise[1, 2, 15, 16, 27] and (2) a recent beetroot juice supplementation study in rodents that reported preferential improvements in blood flow and vasodilation during exercise in muscles composed predominantly of type II (fast-twitch) muscles[7].

All subjects completed the same number of exercise stages (x6) at the same contraction rates on both visits. Had we asked them to continue graded exercise to the point of volitional fatigue, we might have observed an even greater disparity in blood flow between visits as well as providing insight into whether this dietary supplement actually improves forearm exercise capacity. Further studies should address this “ergogenic” question and determine if subjects’ perceived effort at any given submaximal work rate is reduced.

Experimental considerations

The data contained in this thesis and the resulting conclusions that can be drawn are somewhat limited based on the small number of subjects (n = 5) that had completed the study in the time available to meet final thesis deadlines. However, this limitation is balanced against our use of a randomized, double-blind, placebo controlled interventional study design where each subject served as his own control. This is the most rigorous experimental design that can be used to test hypotheses for this type of nutritional intervention research.

Conclusion

The findings from the present study add to the growing body of evidence for a potent vasodilatory (and generally endothelium-independent) effect of acute dietary nitrate supplementation in healthy humans. The contraction-intensity (and/or time-) dependent influence of dietary nitrate supplementation on the forearm hyperemic response to graded exercise is a novel finding, and one that may reflect an enhanced local reduction of nitrates to nitrites when muscle PO_2 and pH are substantially reduced. Collectively, these findings support continued investigation into possible therapeutic effects of dietary nitrates for patients with reduced limb muscle perfusion during exercise (e.g., heart failure, peripheral artery disease, etc).

REFERENCES

1. Bahra M, Kapil V, Pearl V, Ghosh S, and Ahluwalia A. Inorganic nitrate ingestion improves vascular compliance but does not alter flow-mediated dilatation in healthy volunteers. *Nitric Oxide* 26: 197-202, 2012.

2. Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, Wilkerson DP, Benjamin N, and Jones AM. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J Appl Physiol* 109: 135-148, 2010.

3. Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, Dimenna FJ, Wilkerson DP, Tarr J, Benjamin N, and Jones AM. Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol* 107: 1144-1155, 2009.

4. Carlsson S, Wiklund NP, Engstrand L, Weitzberg E, and Lundberg JO. Effects of pH, nitrite, and ascorbic acid on nonenzymatic nitric oxide generation and bacterial growth in urine. *Nitric Oxide* 5: 580-586, 2001.

5. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO, 3rd, and Gladwin MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med* 9: 1498-1505, 2003.

6. Dejam A, Hunter CJ, Tremonti C, Pluta RM, Hon YY, Grimes G, Partovi K, Pelletier MM, Oldfield EH, Cannon RO, 3rd, Schechter AN, and Gladwin MT. Nitrite infusion in humans and nonhuman primates: endocrine effects, pharmacokinetics, and tolerance formation. *Circulation* 116: 1821-1831, 2007.

7. Ferguson SK, Hirai DM, Copp SW, Holdsworth CT, Allen JD, Jones AM, Musch TI, and Poole DC. Impact of dietary nitrate supplementation via beetroot juice on exercising muscle vascular control in rats. *J Physiol* 591: 547-557, 2013.

8. Fung TT, Chiuve SE, McCullough ML, Rexrode KM, Logroscino G, and Hu FB. Adherence to a DASH-style diet and risk of coronary heart disease and stroke in women. *Arch Intern Med* 168: 713-720, 2008.

9. Govoni M, Jansson EA, Weitzberg E, and Lundberg JO. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric Oxide* 19: 333-337, 2008.

10. Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, Finkelstein EA, Hong Y, Johnston SC, Khera A, Lloyd-Jones DM, Nelson SA, Nichol G, Orenstein D, Wilson PW, and Woo YJ. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* 123: 933-944, 2011.

11. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, and Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* 334: 1145-1149, 1996.

12. Hobbs DA, Kaffa N, George TW, Methven L, and Lovegrove JA. Blood pressure-lowering effects of beetroot juice and novel beetroot-enriched bread products in normotensive male subjects. *Br J Nutr* 108: 2066-2074, 2012.

13. 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: role for nitrite-derived NO. *Hypertension* 56: 274-281, 2010.

14. Kelm M, Preik-Steinhoff H, Preik M, and Strauer BE. Serum nitrite sensitively reflects endothelial NO formation in human forearm vasculature: evidence for biochemical assessment of the endothelial L-arginine-NO pathway. *Cardiovasc Res* 41: 765-772, 1999.

15. Lansley KE, Winyard PG, Fulford J, Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Gilchrist M, Benjamin N, and Jones AM. Dietary nitrate supplementation reduces the O2 cost of walking and running: a placebo-controlled study. *J Appl Physiol* 110: 591-600, 2011.

16. Larsen FJ, Weitzberg E, Lundberg JO, and Ekblom B. Effects of dietary nitrate on oxygen cost during exercise. *Acta Physiol (Oxf)* 191: 59-66, 2007.

17. Lauer T, Heiss C, Balzer J, Kehmeier E, Mangold S, Leyendecker T, Rottler J, Meyer C, Merx MW, Kelm M, and Rassaf T. Age-dependent endothelial dysfunction is associated with failure to increase plasma nitrite in response to exercise. *Basic Res Cardiol* 103: 291-297, 2008.

18. Lee IM, Cook NR, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, and Buring JE. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. *Jama* 294: 56-65, 2005.

19. Levitan EB, Wolk A, and Mittleman MA. Consistency with the DASH diet and incidence of heart failure. *Arch Intern Med* 169: 851-857, 2009.

20. Levitan EB, Wolk A, and Mittleman MA. Relation of consistency with the dietary approaches to stop hypertension diet and incidence of heart failure in men aged 45 to 79 years. *Am J Cardiol* 104: 1416-1420, 2009.

21. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosamond W, Sacco R, Sorlie P, Roger VL, Thom T, Wasserthiel-Smoller S, Wong ND, and Wylie-Rosett J. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation* 121: e46-e215, 2010.

22. Lonn E, Bosch J, Yusuf S, Sheridan P, Pogue J, Arnold JM, Ross C, Arnold A, Sleight P, Probstfield J, and Dagenais GR. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. *Jama* 293: 1338-1347, 2005.

23. Petersson J, Carlstrom M, Schreiber O, Phillipson M, Christoffersson G, Jagare A, Roos S, Jansson EA, Persson AE, Lundberg JO, and Holm L. Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash. *Free Radic Biol Med* 46: 1068-1075, 2009.

24. Presley TD, Morgan AR, Bechtold E, Clodfelter W, Dove RW, Jennings JM, Kraft RA, King SB, Laurienti PJ, Rejeski WJ, 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.

25. Rassaf T, Lauer T, Heiss C, Balzer J, Mangold S, Leyendecker T, Rottler J, Drexhage C, Meyer C, and Kelm M. Nitric oxide synthase-derived plasma nitrite predicts exercise capacity. *Br J Sports Med* 41: 669-673; discussion 673, 2007.

26. Spiegelhalder B, Eisenbrand G, and Preussmann R. Influence of dietary nitrate on nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds. *Food Cosmet Toxicol* 14: 545-548, 1976.

27. 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. *Am J Physiol Regul Integr Comp Physiol* 299: R1121-1131, 2010.

28. Vanin AF, Bevers LM, Slama-Schwok A, and van Faassen EE. Nitric oxide synthase reduces nitrite to NO under anoxia. *Cell Mol Life Sci* 64: 96-103, 2007.

29. 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.

30. Wray DW, Witman MA, 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. *Am J Physiol Heart Circ Physiol* 300: H1101-1107, 2011.

31. Zhang Z, Naughton D, Winyard PG, Benjamin N, Blake DR, and Symons MC. Generation of nitric oxide by a nitrite reductase activity of xanthine oxidase: a potential pathway for nitric oxide formation in the absence of nitric oxide synthase activity. *Biochem Biophys Res Commun* 249: 767-772, 1998.

Appendix A

PENNSTATE
1855

INFORMED CONSENT FORM

ORP OFFICE USE ONLY

DO NOT REMOVE OR MODIFY

IRB#40269

Informed Consent Form for Biomedical Research
The Pennsylvania State University

Title of Project: Acute vascular effects of nitrate-rich beet juice

Principal Investigator: *David N. Proctor, Ph.D.*
105 Noll Laboratory
Pennsylvania State University
University Park, PA 16802
dnp3@psu.edu
814-863-0724

Other Investigator(s):
David Moore djm411@psu.edu
Jin-Kwang Kim jok5099@psu.edu
David Maurer dgm167@psu.edu
Mark Bundschuh mab5676@psu.edu

1. Purpose of the study:

This research study will examine the effects of a single dose of beet juice on the stiffness of the arteries and the ability of blood vessels in the arms and legs to dilate (i.e., widen). The knowledge gained from this study will be useful in evaluating the growing use of dietary nitrate supplements in the prevention and treatment of cardiovascular diseases such as high blood pressure. This study is being paid for by the Social Science Research Institute at Penn State University.

2. Procedures to be followed:

This research is being performed by David Proctor, Professor of Kinesiology, trained graduate and undergraduate students working in his laboratory, and Dr. Proctor's colleagues in the College of Health and Human Development and the Penn State College of Medicine. A member of Dr.

Proctor's research team will fully explain each procedure that applies to your participation. The study and its procedures are outlined below:

- **Visit 1 – Screening/Familiarization Visit.** *Prior to this visit, you should be fasted (no food, water only for 12 hours prior to arrival) and should not consume any alcohol or dietary supplements (48 hours prior) or participate in any exercise workouts (24 hours prior: no weight lifting or sustained whole body exercise lasting longer than 15 minutes).* Upon arrival, your height, weight, resting heart rate and blood pressure will be measured at the Clinical Research Center (CRC). Medical staff at the CRC will also give you a medical review and provide you with questionnaires to evaluate your medical history, healthy history, and physical activity/dietary habits. Also, a blood sample from a vein in your arm will be taken (approximately 15 mL or about 1 tablespoon). This blood sample will be analyzed to ensure that various markers of the function and health of your kidney, liver, and heart/blood (e.g., cholesterol, fat, and oxygen carrying proteins) are within normal limits for your age. A CRC medical staff member experienced in performing blood draws will take this blood sample. If the initial blood draw is unsuccessful it will need to be repeated, possibly from a vein in your hand, with your permission.

  You will then be escorted down the hall to the Vascular Aging and Exercise Laboratory (room 201 Noll Laboratory) where you will become familiarized with our blood vessel measurement devices and forearm exercise testing machine. Finally, you will be asked to squeeze a handgrip device as hard as you can. The highest force you develop during three such maximal efforts will determine your maximal grip strength.

- **Visit 2 – Experimental Visit.** You will be asked to arrive at the CRC in the morning, after a 12 hour overnight fast (no food, only water). *Prior to this visit, you should not consume any alcohol or dietary supplements (48 hours prior) or participate in any exercise workouts (24 hours prior: no weight lifting or sustained whole body exercise lasting longer than 15 minutes).* After a seated resting blood pressure measurement, a blood sample from a vein in your arm will be taken by a CRC staff member. This sample will be used to determine the amount of nitrate (a naturally occurring substance in your body that relaxes blood vessels) and its by-products in your blood. If the initial blood draw is unsuccessful it will need to be repeated, possibly from a vein in your hand, with your permission. Following the blood draw, you will be given a small snack (e.g., granola bar or other carbohydrate food, with water) to help reduce feelings of hunger. Shortly after, you will be asked to consume 140 mL (9.5 tablespoons) of either nitrate-rich beet juice (known as the "active" drink) or beet juice with nitrates removed (known as the "placebo" drink). You will not be able to tell which drink you are consuming. You will then be asked to wait in the CRC or Noll lab for approximately 2.5 hours to allow time for the active (or placebo) drink to be fully digested and absorbed. During these 2.5 hours, you will be able to use a personal laptop computer or other portable electronic device. We will also have a CRC staff member take your seated blood pressure one hour, and two hours, after consuming the drink. A second blood sample will then be collected in the CRC after 2.5 hours, to determine if there is an increase in nitrate or its by-products in your blood after consuming the beet juice. If the initial blood draw is unsuccessful it will need to be repeated, possibly from a vein in your hand, with your permission. After this blood sample is collected, you will be escorted to the Vascular Aging and Exercise Laboratory (room 201 Noll Laboratory) to have the following procedures:

1. Measurement of blood pressures and vessel stiffness in your arms and legs.

2. Measurements of resting blood flow through an artery in your mid-thigh.

3. Measurements of blood flow through an artery in your mid-thigh and blood pressure during a serial subtraction test

4. Measurements of the size of an artery in your arm before (rest), during, and after rhythmic exercise of your forearm muscles.

*Each of the above procedures is described in more detail below.

- **Visit 3 – Experimental Visit.** At least 4 days after visit 2, you will return to the CRC to repeat the same procedures as you did during visit 2. Procedures and timeline will be identical to those during visit 2 except that you will consume whichever drink supplement you did not receive during visit 2, i.e., the active (nitrate-containing) drink or the placebo (nitrate-removed) drink. The order of these two drink supplements will be randomly determined for each participant.

The details of the measurements and procedures conducted during visits **2** and **3** are as follows:

- **Consumption of beet juice (nitrate-rich form).** Shortly after finishing your snack, you will be asked to consume a 140 mL (9.5 tablespoons) dose of a commercially available nitrate rich ("active") beet juice supplement. Beets are naturally high in nitrates; the nitrate content in this beet juice supplement is 0.8 grams of nitrate, which is about the amount of nitrate found in 5 servings of spinach (American Heart Association recommends 5 servings of vegetables a day). Nitr**a**tes obtained from beets or other food/drink sources are broken down in the body to nitr**i**tes and eventually to nitric oxide, a substance that can dilate (widen) blood vessels and mildly reduce blood pressure.

- **Consumption of beet juice (without nitrates):** On either visit 2 or visit 3 the placebo drink you consume will contain all of the same components as the "active" drink except for the dietary nitrates.

- **Experimental visit (visits 2 and 3) blood samples.** Approximately 10 mL (two thirds of a tablespoon) of blood will be taken from a vein in your forearm (or hand vein if necessary) before and approximately 2.5 hours after you consume the beet juice supplement. A CRC medical staff member experienced in performing blood draws will take these samples.

- **Measurement of blood pressures and vessel stiffness in your arms and legs:** After your second blood draw, you will be escorted from the CRC to the Vascular Aging and Exercise Laboratory where we will ask you to lay comfortably on a hospital stretcher flat on your back. Dr. Proctor's research assistants will place blood pressure cuffs around both of your upper arms and ankles, and a small sensor over your shirt (mid-chest level). You will lie quietly on the stretcher for about 15 minutes to allow your blood pressure and heart rate to stabilize. The cuffs allow us to measure the blood pressure in your arms and legs and to determine how fast heart pulses travel through your blood vessels (an indicator of the stiffness of your arteries).

During each of these resting measurements, the cuffs will inflate to a level that temporarily stops blood flow to both hands and feet, followed by a slow deflation (just like a typical blood pressure measurement in your arm). Three to six measurements of blood pressure/artery stiffness will be collected, depending on the quality of these recordings.

- **Measurements of blood flow through an artery in your mid-thigh:** After removing the blood pressure cuffs from your wrists and ankles, you will remain on the stretcher for detailed measurements of blood flow patterns in your leg. For this measurement, you will need to wear shorts, which we can provide for you if necessary. One of our research assistants will place an ultrasound probe (device which sends/receives sound waves) over a large artery in your mid-thigh. This ultrasound device allows us to measure the size (width) of this artery and the speed/patterns of blood flow through your leg. Blood pressure will also be monitored periodically during these measurements using an arm and/or finger cuff.

- **Measurements of blood flow and blood pressure during a serial subtraction test:** After the resting measures of blood flow patterns are taken in the leg, a research assistant will instruct you to count backwards by a set number starting from a random number between 900-1000. The same ultrasound device as used above will measure the speed/patterns of blood flow through your leg during this subtraction test. Blood pressure will also be monitored continuously using a finger cuff.

- **Measurements of artery size during forearm exercise:** For the final part of this visit, you will be asked to perform repeated hand squeeze exercise (as practiced during visit 1) in which the contraction speed and/or muscle force your produce is gradually increased every 2 to 3 minutes until you cannot maintain the required work rate. The size (width) of your brachial artery (located on the inside surface of your upper arm just above your elbow) will be measured using an ultrasound sensor before, during, and immediately following this graded exercise test to determine how much this vessel in your arm can dilate (widen). Blood pressure will also be monitored during these measurements using an arm and/or finger cuff.

- **Future use of stored blood samples:** During visits 2 and 3, we will collect approximately 3 mL (or 0.2 tablespoons per blood draw) of blood above the minimum we need to complete the measurements we have planned for this study (i.e., measurement of blood nitrates and it's by products). The 3 extra mL of blood we collect provides additional sample in case an error occurs (equipment and/or human error) during our laboratory analysis procedures and we need to re-run a measurement or entire test. It is also possible that some time after we have completed your study visits, we might decide that additional blood measures not described in this consent form (for example, a newly discovered protein that influences blood vessel function) may be useful in helping us learn even more about the effects of beet juice consumption on blood vessel health. Permission is needed in order for us to use your extra stored blood samples for these additional/new measurements. **Please choose one of the following two options below:**

I **DO** give permission to use my stored blood in future studies on vascular health

____ your initials

I **DO NOT** give permission to use my stored blood in future studies on vascular health.

___ your initials

3. Discomforts and risks:

It is not possible to identify all potential risks associated with these research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known or potential risks.

- **Blood draw:** For each of the blood draws (one during visit #1, two during visit #2, and two during visit #3; total of 5 for the entire study), you will feel a small pinch or discomfort while the needle is inserted in your arm. If the initial blood draw is unsuccessful it may need to be repeated, with your permission. Blood draws can also cause mild swelling, bruising (blood under the surface of the skin), and/or continued bleeding at the insertion site; the risk of these happening will be minimized by pressing on the site until any visible bleeding stops. There is also a slight chance of infection developing at the insertion site in the hours following a blood draw. This risk will be minimized and most likely eliminated by having a trained CRC medical staff member draw the blood in the CRC using sterile supplies. Dizziness or fainting during or following a blood draw is also a possible risk. If these symptoms occur, we will have you lie down and help you raise your feet until symptoms improve. If this does occur, we may ask that you remain at the CRC until we have checked your blood pressure, and we are sure that you feel okay.

- **Consumption of beet juice:** There are no known health risks or toxicities associated with daily consumption of nitrate-rich beet juice, a commonly sold health drink/supplement in Europe. The most common side-effect of beet juice consumption is pinkish-colored urine (known as “beeturia”). This can occur after consuming either the nitrate-rich or placebo version of the drink. If this does occur, the pinkish urine usually disappears in 24 hours or less. Because nitrates dilate (widen) blood vessels, there is also a possibility that consuming this supplement will cause your blood pressure to decrease slightly (i.e., 3 to 5 millimeters). Most people will not have any sensations of this reduction in blood pressure. However, in the unlikely event that blood pressure drops enough to cause lightheadedness at any point during this study visit, we will have you remain in the building under the supervision of a CRC staff member until the lightheadedness goes away completely.

- **Measurement of blood pressures and vessel stiffness in your arms and legs:** You may feel a tightness and/or tingling sensation in your hands and feet while the upper arm and ankle cuffs are inflated. These sensations are very temporary since each cuff inflation measurement only lasts for about 1 minute.

- **Measurements of blood flow through an artery in your mid-thigh:** You may feel minor discomfort (pressure) when the research assistant is pressing the ultrasound probe against your mid-thigh to locate a good image of the underlying artery. This discomfort, if it occurs, is very mild and stops immediately after the measurement is completed (measurement takes about 5 minutes). There is also a small risk that the ultrasound gel will irritate your skin. This irritation/redness, if it occurs, should go away soon after the study is completed.

- **Measurement of artery size during forearm exercise:** Minor discomfort and skin irritation associated with ultrasound imaging (described immediately above) may also be felt in your arm before and during the forearm exercise testing. These sensations, if they occur, will go away soon after the study is completed. Forearm muscle fatigue (tiredness), particularly during the higher workloads near the end of the test, is likely. This discomfort goes away quickly after the completion of the exercise. Mild soreness in the muscles of the forearm/hand could develop sometime after the exercise test is completed (usually the day following). This soreness, if it occurs, usually goes away in about 2 days.

4. Benefits:

There are no direct benefits to you for participating in this study other than receiving information about your cardiovascular risk factors (i.e., blood pressure, blood cholesterol, etc.). However, this study may benefit society by providing information about the health effects of nitrate-rich beet juice.

5. Duration/time of the procedures and study:

You will be involved in the research study for no longer than 6 weeks, which will consist of 3 separate study visits. The first study visit will take approximately 3 hours. The following two experimental visits (visits 2 and 3) will each take approximately 4.5 hours. Thus, the total duration of all the research sessions will be approximately 12 hours.

6. Alternative procedures that could be utilized:

There are alternative dietary modifications that could be used to reduce the stiffness of blood vessels (example: salt restriction) or to test the ability of blood vessels to widen (example: temporarily blocking blood flow to the arm). There are also alternative methods of measuring these vascular responses, some non-invasive, others invasive. The measurements we have selected, however, should be sensitive enough to determine if this particular food supplement (i.e., nitrate-rich beet juice) can de-stiffen blood vessels.

7. Statement of confidentiality:

Your participation in this research is confidential. Moreover, data will be stored and secured in the Vascular Aging and Exercise Laboratory (201 Noll Laboratory) in password protected computer files. Any hard copies of data will be stored in locked filing cabinets. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

The Pennsylvania State University's Office for Research Protections, the Institutional Review Board, and the Office for Human Research Protections in the Department of Health and Human Services may review records related to this research study.

8. Right to ask questions:

Please contact Dr. David Proctor at 814-863-0724 (office phone) or 814-571-5234 (cell phone) with questions, complaints or concerns about the research. You can also call this number if you feel this study has harmed you. If you have any questions, concerns, problems about your rights as a research participant or would like to offer input, please contact The Pennsylvania State University's Office for Research Protections (ORP) at (814) 865-1775. The ORP cannot answer questions about research procedures. Questions about research procedures can be answered by the research team.

9. Payment for participation:

You will be paid a total of $150 for your participation in the entire study ($0 for visit 1, $75 each for study visits 2 and 3). If for some reason you do not complete the study, you will be paid for the visits you did complete with the exception of the screening/familiarization visit.

10. Voluntary participation:

Your decision to be in this research is voluntary. You can stop at any time. You do not have to answer any questions you do not want to answer. Refusal to take part in or withdrawing from this study will involve no penalty or loss of benefits you would receive otherwise.

Additionally, if you do not comply with the study protocol (e.g., you skip/miss an excessive number of study visits or fail to follow pre-visit instructions) we may not seek your continued participation in this study.

11. Injury Clause:

In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

12. Abnormal Test Results:

In the event that abnormal lab test results are obtained during initial screening or subsequently throughout this study, the Clinical Research Center will inform you as quickly as possible of these results and instruct you to contact your private physician for further assessment. The lab test results will be made available to your private physician at your request.

If you have read the information in this form and agree to and give your permission for your participation as a volunteer in the study entitled **"Acute vascular effects of nitrate-rich beet juice"** please sign below. You must be 18 years of age or older. You will receive a signed copy of this consent form.

____ ____________________
Participant Signature Date

____ ____________________
Person Obtaining Consent Date

ACADEMIC VITA

Mark Austin Bundschuh
mab5676@psu.edu

EDUCATION

B.S., Science, May 2013, The Pennsylvania State University, University Park, PA

HONORS AND AWARDS

- **Student Marshal**, Eberly College of Science
  Selected to lead the procession of graduates and carry the banner carrier for the Spring 2013 Commencement *(May 2013)*

- **Dean's List**, The Pennsylvania State University
  All semesters (*Fall 2009 - Fall 2012*)

- **Clemens Family Corporation Academic Scholarship**, Clemens Family Corporation
  Awarded scholarship based on academic record, volunteer work, and future potential (*March 2011 and 2012*)

- **Ambassador Travel Grant**, Schreyer Honors College
  Awarded financial support for my medical mission trip to Panama *(January 2012*)

- **The President's Freshman Award**, The Pennsylvania State University
  Awarded medal for achieving a 4.0 GPA at the end of the fall semester of Freshman year (*January 2010*)

- **Eagle Scout**, Boy Scout Troop 152
  Planned and managed the removal of invasive trees, plants, and garbage on the perimeter of a historic cemetery (*December 2008*)

WORK EXPERIENCE AND ACTIVITIES

- **Penn State University Health Services**, University Park, PA
  Clinic Volunteer Program *August 2012 – Present*
  - Conducted patient intakes which included assessing vital signs and documenting medical information
  - Trained in topics such as infection and allergy control, proper documentation, and patient confidentiality

- **Penn State Global Medical Brigades**, University Park, PA
  Member *August 2011 – May 2012*
    - Traveled to underserved communities in Panama to provide vital healthcare and education
    - Collected medical supply donations from local health centers for our medical mission trip to Panama

- **Mount Nittany Medical Center**, State College, PA
  Emergency Department Volunteer *August 2011 – May 2012*
    - Performed ancillary tasks (improved sanitation, restocked medical supplies, and transported patients)
    - Acted as a liaison between the medical staff and patients

- **Penn State Alpha Epsilon Delta,** National Health Pre-professional Honor Society
  National and Local Honors Member *August 2010 – May 2012*
  Contributed valuable service to the Penn State community through the AED Blood Cup, Penn State THON, and the AED Public Health Fair

- **Towamencin Township Municipal Pool**, Towamencin, PA
  Pool Lifeguard *Summer 2007 – 2011*
    - Supervised swimmers and maintained a safe pool environment
    - Certified in Cardiopulmonary Resuscitation and Lifesaving

- **Lehigh Valley Hospital Department of Emergency Medicine**, Allentown, PA
  Externship *May 2010*
    - Shadowed an ER physician during a 12 hour shift
    - Learned how to interpret EKG, X-ray, and CT scans

RESEARCH EXPERIENCE

- **Penn State Vascular Aging and Exercise Laboratory**
  Research Assistant *January 2011 – Present*
    - Designed, organized, and implemented a research study to determine the effects of dietary nitrate consumption via beetroot juice on forearm shear-induced vasodilation of the brachial artery
    - Served as the study coordinator where I was responsible for promoting ongoing collaboration and communication among our laboratory group, the Clinical Research Center nurses, and human subjects
    - Experienced in vascular physiology laboratory data collection and analysis techniques

- **Penn State Summer Translational Cardiovascular Sciences Institute**
  Undergraduate Research Fellow *June – August 2012*
  - Proved the feasibility and effectiveness of dietary nitrate supplementation via beet juice in a small pilot study
  - Developed a broader understanding of cardiovascular health and research through an interdisciplinary lecture series

PUPLICATIONS AND PAPERS

- **Schreyer Honors College thesis**, Department of Kinesiology
  Effect of Dietary Nitrate Supplementation on Nitric Oxide-Mediated Vascular Responses