Published in final edited form as:

Appl Physiol Nutr Metab. 2015 February ; 40(2): 122–128. doi:10.1139/apnm-2014-0228.

Acute dietary nitrate supplementation does not augment submaximal forearm exercise hyperemia in healthy young men

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Abstract

Despite the popularity of dietary nitrate supplementation and the growing evidence base of its potential ergogenic and vascular health benefits, there is no direct information about its effects on exercising limb blood flow in humans. We hypothesized that acute dietary nitrate supplementation from beetroot juice would augment the increases in forearm blood flow, as well as the progressive dilation of the brachial artery, during graded handgrip exercise in healthy young men. In a randomized, double-blind, placebo-controlled crossover study, 12 young (22 ± 2 years) healthy men consumed a beetroot juice (140 mL Beet-It Sport, James White Juice Company) that provided 12.9 mmol (0.8 g) of nitrate or placebo (nitrate-depleted Beet-It Sport) on 2 study visits. At 3 h postconsumption, brachial artery diameter, flow, and blood velocity were measured (Doppler ultrasound) at rest and during 6 exercise intensities. Nitrate supplementation raised plasma nitrate (19.5-fold) and nitrite (1.6-fold) concentrations, and lowered resting arterial pulse wave velocity (PWV) versus placebo (all $p < 0.05$) indicating absorption, conversion, and a biological effect of this supplement. The supplement-associated lowering of PWV was also negatively correlated with plasma nitrite ($r = -0.72$, $p = 0.0127$). Despite these systemic effects, nitrate supplementation had no effect on brachial artery diameter, flow, or shear rates at rest (all $p \geq 0.28$) or during any exercise workload (all $p \geq 0.18$). These findings suggest that acute dietary nitrate supplementation favorably modifies arterial PWV, but does not augment blood flow or brachial artery vasodilation during non-fatiguing forearm exercise in healthy young men.

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Keywords

inorganic nitrate; vascular function; pulse wave velocity

1. INTRODUCTION

Nitric oxide (NO) plays a key contributing role in the modulation of blood vessel tone at rest and under conditions of increased metabolic demand, such as exercise (Heinonen et al. 2011; Joyner and Casey 2009; Joyner and Tschakovsky 2003). As a result, there has been a growing interest in the use of dietary interventions that increase bioavailable NO as both cardiovascular health-promoting (Kapil et al. 2010; Lundberg et al. 2011; Machha and Schechter 2011) and ergogenic aids (Bailey et al. 2009; Bescos et al. 2012; Jones et al. 2011; Lansley et al. 2011; Vanhatalo et al. 2011). In particular, dietary supplementation with inorganic salts or high-nitrate containing foods (e.g., beetroot juice) has recently grown in popularity for its potential blood pressure-lowering and aerobic exercise performance-enhancing effects. With regard to the latter function, many of the studies performed in humans have largely focused on the influence of dietary nitrate on exercising muscle metabolism, such as a putative ability to reduce the oxygen cost of muscle contraction (Bailey et al. 2010; Bailey et al. 2009; Bentley et al. 2014; Bescos et al. 2012; Jones et al. 2011; Larsen et al. 2011). However, evidence from a rodent model suggests that dietary nitrate supplementation may also improve the perfusion of active skeletal muscles during exercise (Ferguson et al. 2013b). Ferguson et al. (2013b) have recently demonstrated that consumption of nitrate-rich beetroot juice augments hindlimb skeletal muscle blood flow and vascular conductance during treadmill running in rats. In humans, 2 studies have investigated the effects of intra-arterially infused doses of sodium nitrite on forearm exercise hyperemia, but these studies involve supra-physiological doses of nitrite. Therefore it is unclear whether increasing plasma nitrite within the physiological range by dietary nitrate supplementation can impact vascular function during exercise. To our knowledge, no studies have investigated the influence of dietary nitrate supplementation on exercise hyperemia or local vascular responses to exercise in humans.

The purpose of the present study was to determine whether an acute dose of nitrate-rich beetroot juice augments forearm exercise hyperemia, as well as brachial artery dilation during graded handgrip exercise in healthy young adults. We hypothesized that this nitrate-rich supplement would augment the increases in forearm blood flow, as well as the progressive dilation of the brachial artery, during graded handgrip exercise based on the potent vasodilator effects of intra-arterially infused nitrite in the human forearm (Cosby et al. 2003; Dejam et al. 2007) and the effects of beetroot juice consumption on blood flow to the exercising rodent hindlimb (Ferguson et al. 2013b).

2. MATERIALS and METHODS**2.1 Participants**

Young (age, 22 ± 2 years; range 19 – 24 years) healthy men were recruited from the local university community. Interested volunteers provided written informed consent prior to

enrollment. On the same day, medical screening was performed to determine eligibility, followed by familiarization with the study procedures. Participants were selected if they were low- to moderately active (i.e., < 3 days/week of exercise), free of overt chronic disease as assessed by clinician reviewed medical history questionnaire and venous blood chemistry (hematologic, liver, and kidney function), and met the following criteria: (i) resting blood pressure between 110/60 and 140/90 mmHg, (ii) body mass index between 18.5 and 30 kg/m², (iii) fasting plasma glucose < 100 mg/dL, (iv) fasting plasma low-density lipoprotein < 130 and/or high-density lipoprotein > 40 mg/dL, (v) non smoker, (vi) not taking any cardiovascular medications, and (vii) had not donated blood or blood products in the past 3 months. Characteristics of the 12 participants that completed this study are shown in Table 1. All procedures were approved by the Office of Research Protections at Pennsylvania State University in agreement with the guidelines set forth by the Declaration of Helsinki.

2.2 Study design

After the baseline medical screening visit, participants attended 2 study visits that were at least 5 days apart, during which they consumed 140 ml (two 70-ml bottles) of either a concentrated beetroot juice supplement (BR_{nitrate}; Beet-It Sport, James White Drinks Ltd., Ipswich, UK) or the equivalent nitrate-depleted placebo (BR_{placebo}). The order of these 2 study visits (nitrate visit vs. placebo visit) was randomized by clinical support staff, with primary study investigators blinded to the treatment order during testing and data analysis. Each 70 ml Beet-It Sport beverage has an average nitrate content of 6.45 mmol (0.4 g), equivalent to approximately 400 ml of beetroot juice. Therefore the expected dose of inorganic nitrate administered to each subject during their BR_{nitrate} visit was 12.9 mmol (0.8 g).

Participants followed their usual diet (i.e., they did not follow a nitrate-restricted diet), but were instructed to refrain from alcohol and dietary supplements (48 hours) and to arrive at each study visit after an overnight fast (water only). They were asked to abstain from caffeine consumption (12 hours) and from aerobic or resistance training (24 hours) prior to each study visit. Importantly, participants were instructed not to brush their teeth or to use mouthwash the morning of each study visit to prevent killing anaerobic nitrate-reducing bacteria on the dorsal surface of the tongue, which are essential for the conversion of nitrate-to-nitrite following beetroot juice ingestion (Gladwin et al. 2005; Webb et al. 2008). Both study visits took place at approximately the same time of day starting between 0700 and 1000 hours.

Upon arrival at the Clinical Research Center a venous blood sample was collected for determination of baseline plasma nitrate and nitrite concentration. Participants then consumed two 70-mL bottles of BR_{nitrate} or BR_{placebo}. Two hours and 30 minutes after consuming the nitrate supplement or placebo, a second venous blood sample was taken to determine the post-consumption change in plasma nitrate and nitrite because peak plasma nitrite concentration in healthy younger men is reported to occur 2 h postconsumption and remain elevated above baseline until 8 h postconsumption (Wylie et al. 2013). Following the

second venous blood sample, participants were escorted to the Vascular Aging and Exercise laboratory for vascular testing.

2.3 Pulse wave velocity (PWV)

Brachial-to-ankle PWV (PWV_{BA}) was determined by a vascular profiling machine (VP2000, Colin Corp., Komaki, Japan) after 15 minutes of supine rest, which was approximately 3 h post $BR_{nitrate}$ or $BR_{placebo}$ consumption. Briefly, PWV_{BA} was calculated by dividing the estimated distance between the brachial and posterior tibial artery (determined from the participants' height by the vascular profiling machine) by the pulse transit time between the 2 sites. Reported PWV values are the mean of the left and right measurements.

2.4 Graded handgrip exercise

Handgrip exercise was performed with each subject's left hand using a custom-built pulley based device. Subjects lied supine with their elbow extended to facilitate Doppler Ultrasound imaging of their brachial artery. Following 1 minute of resting brachial artery hemodynamic measurements, subjects performed rhythmic contractions of their forearm muscles at a controlled rate (30 contractions per minute, 1-s contraction: 1-s relaxation) and range of motion (10-cm excursion of the pulley wire with each contraction). Each participant completed six 3-min bouts of exercise with progressively increasing weight (200, 400, 600, 800, 1000, and 1200 g), with 1 min of quiet rest between bouts. The graded handgrip exercise protocol began approximately 3 h after consumption of the $BR_{nitrate}$ or $BR_{placebo}$.

2.5 Vascular responses to exercise

Diameter and blood flow velocity of the brachial artery were measured using Doppler ultrasound (HDI 5000, Philips; Bothell, Wash., USA). For blood flow velocity, the artery was insonated at a constant angle of 60° with the sample volume adjusted to cover the width of the artery. Velocity measurements were sampled in real time (400 Hz) using a data acquisition system (Powerlab, AD Instruments; Castle Hill, Australia). Mean blood velocity was calculated from the final 30 s of the third minute during each bout. High-resolution diameter measurements (6 MHz probe) were taken from 15-s recordings performed during baseline rest and immediately following the measurement of flow velocity during each exercise bout. Images were recorded directly to computer using a video capture device and software (Dazzle Video Creator, Pinnacle, Mountain View, Calif., USA). Brachial artery diameter was determined as the average across the cardiac cycle using edge-detection software (Brachial Analyzer Software, Medical Imaging Applications; Coralville, Iowa). Forearm blood flow at each work rate was calculated by multiplying the cross-sectional area of the brachial artery with mean blood velocity using the equation $\pi (\text{diameter}/2)^2 \times \text{velocity} \times 60$. The dilatory response of the brachial artery (% increase above resting baseline) to graded handgrip exercise was examined as a function of workload and estimated shear rate. All vascular analyses were conducted by a single investigator who was blinded to the participant and supplement order.

2.6 Plasma [nitrate/nitrite] analysis

Venous blood samples were drawn into EDTA tubes (10mL K₂ EDTA tubes, BD Vacutainer, Franklin Lakes, N.J., USA) and immediately centrifuged at 3200 r/m (1590 g) and 4°C for 10 min. Plasma was then extracted and stored in -80°C freezer for later analysis of nitrate and nitrite concentration. The ENO-20 analyzer (EICOM, San Diego, Calif., USA) with a sensitivity of 0.1 pmol for nitrate and nitrite was used to measure nitrate and nitrite concentration in the plasma samples. Briefly, plasma was mixed with an equal volume of 100% methanol and centrifuged at 13 000 r/m (12000 g) for 10 minutes. Samples were then loaded into a 96-well plate. Nitrate and nitrite were then separated via column chromatography and individually reacted with a Griess reagent, synthesizing a diazo compound. The absorbance of this red diazo compound was then read at a wavelength of 540nm using a visible light detector.

2.7 Statistical analysis

Data were analyzed using IBM SPAA Statistics for Windows (version 20.0.; IBM Corp., Armonk, N.Y., USA). Comparisons of resting supine blood pressures and arterial PWV between the BR_{nitrate} and BR_{placebo} visits were performed using paired *t* tests. Paired *t* test was also performed to compare plasma nitrate and nitrite concentrations between the BR_{nitrate} and BR_{placebo} for both baseline and 2.5 h postconsumption. Two-factor ANOVA with repeated measures was used to assess the effects of nitrate supplementation on vascular responses during graded handgrip exercise. The association between PWV and plasma nitrite concentration was determined by simple linear regression. All data are presented as means ± SE. A value of *p* < 0.05 was considered statistically significant.

3. RESULTS

Subject characteristics are provided in Table 1. Ingestion of the beetroot juice supplement and its nitrate-depleted placebo was well tolerated by all participants with no side effects reported.

3.1 Concentrated beetroot juice ingestion raised plasma nitrate and nitrite (Figure 1)

Prior to beverage ingestion, resting baseline plasma nitrate (*p* = 0.79) and nitrite (*p* = 0.92) concentrations were not different between visits. Two hours and 30 minutes following ingestion of the BR_{placebo}, plasma nitrate (*p* = 0.09) and nitrite (*p* = 0.40) concentrations were not elevated above baseline. However, plasma nitrate and nitrite concentrations were significantly elevated following ingestion of the BR_{nitrate} (nitrate 19.5-fold, nitrite 1.6-fold; both *p* < 0.03).

3.2 Acute nitrate supplementation and resting arterial hemodynamics

Resting supine systolic (120 mmHg vs. 119 mmHg, *p* = 0.24) and diastolic (63 mmHg vs. 63 mmHg, *p* = 0.30) blood pressures, which were measured approximately 3 h after beverage consumption were not different between BR_{nitrate} or BR_{placebo} visits. However, simultaneously measured PWV was significantly lower 3 h after consuming the BR_{nitrate} beverage (1065 ± 32 cm/s) compared with the BR_{placebo} (1106 ± 28 cm/s; *p* = 0.03, Figure 2.a). Moreover, a significant negative correlation (*r* = -0.72, *p* = 0.01) was observed between

the difference in postconsumption plasma nitrite concentrations and the corresponding difference in PWV measured between the 2 days (Figure 2.b).

3.3 Acute nitrate supplementation does not alter forearm exercise hyperemia (Figure 3)

Resting brachial artery blood flow measured immediately prior to graded handgrip exercise was similar between BR_{placebo} and BR_{nitrate} visits ($p = 0.55$). As expected, there was an observed increase in blood flow across exercise intensities ($p = 0.001$). However, exercising forearm blood flow between BR_{placebo} and BR_{nitrate} visits was similar at each exercise intensity (all $p > 0.05$).

3.4 Nitrate supplementation does not alter the brachial artery dilatory response to exercise (Figure 4)

Resting brachial artery diameter measured immediately prior to graded handgrip exercise was similar between BR_{placebo} and BR_{nitrate} visits ($p = 0.67$). Brachial artery diameter and calculated shear rate increased in an intensity-dependent manner following treatment with both the BR_{placebo} and BR_{nitrate} ($p < 0.0001$), with no significant difference in these responses between treatments ($p = 0.70$).

4. DISCUSSION

The primary objective of the current study was to determine whether acute dietary nitrate supplementation augments forearm blood flow and conduit vasodilator responses during graded handgrip exercise. Dietary nitrate supplementation in the form of a single dose of highly concentrated beetroot juice raised plasma nitrate (19.5-fold) and nitrite (1.6-fold) concentrations, and lowered resting PWV versus placebo (all $p < 0.05$). These differences indicate that there was absorption, conversion, and a systemic biological effect of the ingested nitrate, respectively, in these young healthy subjects. However, in contrast with our hypothesis, this supplement had no obvious effects on forearm blood flow or brachial artery dilatory responses of healthy young men to the exercise protocol we used.

4.1 Nitrate supplementation and forearm exercise hyperemia

The lack of any observable effects of acute nitrate supplementation on handgrip exercise blood flow in the present study were unexpected, especially considering the relatively high dose of nitrate we administered (BR_{nitrate} visit) and the significant role of nitric oxide in forearm exercise hyperemia in healthy younger adults as previously demonstrated using nitric oxide synthase (NOS) inhibition with L-N^G monomethyl arginine (L-NMMA) (Schrage et al. 2004; Wray et al. 2011). The dose of ingested nitrate (800 mg) and the increase in plasma nitrate (19.5-fold) were well within the range of prior studies demonstrating improved exercise performance and beneficial muscle metabolic effects in healthy adults (Bescos et al. 2012; Vanhatalo et al. 2011; Wylie et al. 2013). However, the conversion of nitrate to nitrite in the present study (1.6-fold increase in nitrite) appears low in relation to most of these prior reports (2.5 to 4-fold increase). The absolute increase in venous nitrite concentration after our subjects consumed the BR_{nitrate} supplement averaged 210 nmol/L (360 nmol/L at baseline, 570 nmol/L at 2 hrs post consumption). This increase in venous nitrite concentration is just below the venous nitrite concentration difference (220

nmol/L) associated with the threshold for increased forearm blood flow induced by intra-arterial infusion of (sodium) nitrite in healthy adults (Dejam et al. 2007). Collectively, these findings raise the possibility that the single dose of dietary nitrate we administered, and any subsequent intravascular conversion to nitric oxide, may have been insufficient to impact exercise-induced hyperemia in the forearm of these subjects.

Exercise intensity is another important factor to consider when interpreting the vascular effects of nitrate supplementation. Recent evidence in humans (Casey and Joyner 2011) and rodent models (Copp et al. 2013) implicate an intensity-dependent role for NO on muscle blood flow responses to exercise (i.e., greater impact of NO synthase blockade at higher intensities), including the rhythmic handgrip exercise model we employed (Wray et al. 2011). Our subjects completed 6 steady-state work rates that resulted in a modest ~4-fold increase in forearm blood flow above baseline (Figure 3), but we did not have them exercise at high (i.e., fatiguing) intensities, conditions which would likely have promoted increased activation of the nitrate-nitrite-NO signaling pathway. Thus, it is possible that contraction intensities during our handgrip exercise protocol were not high enough to tax local (microvascular) oxygen supply in relation to metabolic demand, at least in subjects with no vascular or metabolic limitations and average grip strength (Table 1). No direct information regarding the influence of dietary nitrate supplementation on exercising limb blood flow in humans is available for comparison; however, the effects of dietary nitrate supplementation on active limb muscle oxygenation (near-infrared spectroscopy-based measurements) and metabolic perturbation (i.e., fall in pH, rates of phosphocreatine degradation and inorganic phosphate accumulation, accumulation of lactate) are more evident during high versus low intensity exercise, particularly under conditions of hypoxia (Bailey et al. 2009; Masschelein et al. 2012; Vanhatalo et al. 2011). Recent observations of enhanced local oxygenation following dietary nitrate supplementation in exercising calf muscles of peripheral arterial disease patients (Kenjale et al. 2011) and in low (but not high) oxidative hindlimb muscles of exercising rodents (Ferguson et al. 2013b), is further evidence that the vascular effects of dietary nitrates are more evident when there is a greater mismatch between local muscle oxygen supply versus demand. It remains to be determined whether nitrate supplementation augments conduit or microcirculatory responses in exercising limb muscles during higher intensity, intermittent, and/or blood flow restricted (isometric) contractions i.e., non-steady state conditions that more closely mimic activities of daily life.

4.2 Nitrate supplementation and brachial artery vasodilation

Wray et al recently reported that nitric oxide plays a highly significant role in the brachial artery dilator response to graded handgrip exercise in young healthy adults (Wray et al. 2011), which they observed as a 70% reduction in this response during local endothelial NOS inhibition with L-NMMA. The use of a similar handgrip exercise protocol (6 × 3 min stages with 1 min recoveries) resulted in a shear stress response (Figure 4C) comparable with that of Wray et al. (2009) in young men, and afforded us the opportunity to determine if acute nitrate supplementation impacts this graded, nitric oxide-dependent dilatory response. Our results suggest that a single, moderately high dose (800 mg) of inorganic nitrate does not affect exercise-induced, endothelium-mediated increases in brachial artery diameter in healthy young men.

All previous studies examining the effects of acute dietary nitrate supplementation on endothelial vasodilator function in humans have utilized the brachial artery dilator response following a 5-min period of forearm cuff ischemia (the Flow-Mediated Dilation (FMD) test), the noninvasive standard measure of endothelial function (Thijssen et al. 2011). Upon release of the occlusion cuff, there is a brief, uncontrolled increase in blood flow through the brachial artery. While the size of the FMD response (peak % dilation of the brachial artery above baseline) is prognostic for the occurrence of cardiovascular events, this response depends on the magnitude and duration of the vascular shear stimulus, a variable that to our knowledge has not been reported in any dietary nitrate supplementation studies to date. Failure to quantify the actual shear stimulus for vessel dilation makes it difficult to compare brachial FMD responses between groups and interpret the response to interventions. This omission could contribute to the variable findings in the literature with some studies reporting substantial increases in brachial FMD following nitrate supplementation (Heiss et al. 2012) and others very small (Bondonno et al. 2012) or no changes (Bahra et al. 2012; Kapil et al. 2010; Kenjale et al. 2011; Webb et al. 2008). In the present study, we chose to utilize the graded handgrip exercise model to evaluate this question as it provides sustained, wide-ranging endothelial shear rates through the brachial artery and produces intensity-dependent vasodilation, which was recently shown to be nitric oxide-mediated in younger healthy adults (Wray et al. 2011). The lack of any observable BR_{nitrate} versus BR_{placebo} differences in the estimated shear rate within the brachial artery, or in the conventional assessment of percentage of brachial artery dilation, suggests that an acute dose (800 mg) of dietary nitrate does not alter brachial artery dilator function in healthy young men. It is important to note, as indicated above, that dietary interventions to augment the conversion of nitrite to NO might not result in improved hyperemic or vasodilatory responses during exercise at lower (mild to moderate) intensities, particularly in subjects with no apparent endothelial dysfunction.

The lack of any nitrate supplementation associated alterations in resting brachial artery blood flow or vessel diameter, or brachial artery dilation in response to exercise, are surprising given the reduction we observed in PWV. The reasons for this are unclear as alterations in resting PWV are often observed when systemic (nitric oxide-mediated) vasomotor tone is acutely altered (Fok et al. 2012). However, Bahra et al. (2012) also observed a reduction of PWV in the absence of any changes in brachial FMD after nitrate supplementation (potassium nitrate salt ingestion) in a group of healthy men and women aged 18–45 years. Interestingly, the reduction of PWV that we observed in the present study was correlated with changes in plasma nitrite concentration (Fig. 2B), suggesting that there was a hemodynamic effect associated with the consumption of this concentrated beetroot juice supplement.

4.3 Experimental considerations

Strengths of the current investigation include the use of a double-blind, randomized cross-over study design in a carefully screened sample of healthy younger men. This was also 1 of only 2 studies examining vascular outcomes of dietary nitrate supplementation that used a “true placebo” (i.e., nitrate-depleted beetroot juice; e.g., Gilchrist et al. 2013) and one of very few acute supplementation studies that did not restrict subjects’ diets of nitrate

containing foods prior to supplementation (i.e., nitrate-free run in diet; e.g., Coles and Clifton 2012). The use of a true placebo that differs only in nitrate content, along with standardized pre-visit instructions to avoid caffeine, exercise, and oral anti-bacterial hygiene (teeth brushing, mouthwash) is arguably the most rigorous means of testing the effects of dietary nitrate supplementation *per se* on vascular function.

There are several limitations of the present study that should be considered alongside these strengths. First, the present results are specific to graded submaximal forearm exercise and cannot be extrapolated to large muscle (leg or whole body) exercise. Second, we measured plasma nitrite concentration at baseline and 2.5 h postconsumption of beetroot juice, but not immediately before or during the graded handgrip exercise protocol. Therefore, it is unclear whether the current exercise protocol was sufficient to reduce nitrite to nitric oxide within the exercising limb. Finally, we assessed maximal isometric grip strength (MVC, Jamar device), but not the peak dynamic power output of our subjects' forearm muscles using our testing device (pulley-based handgrip device). Thus we were unable to quantify either the contraction intensity or the "relative" exercise intensity associated with each work level of our graded forearm exercise protocol. This is an important consideration given the intensity-dependent role of nitric oxide for muscle blood flow responses to exercise (Ferguson et al. 2013a; Wray et al. 2011) and the ergogenic/metabolic effects of dietary nitrate supplementation (Bailey et al. 2009; Bescos et al. 2012; Jones et al. 2011; Lansley et al. 2011; Vanhatalo et al. 2011). To address this issue, we closely compared brachial artery blood flow, shear rate, and dilator responses between our subjects and the handgrip exercise study of Wray et al (2011), which examined the nitric oxide dependency of these responses in healthy young adults using a protocol with a similar number of exercise stages (n=6) and work/rest durations (3 min/1 min). Comparisons between these 2 studies revealed a similar range of hyperemic (up to ~4 fold above rest), shear rate (300–400 s⁻¹) and conduit dilator (6–10%) responses that include workloads extending to a range that Wray et al demonstrated (via L-NMMA infusion) to be nitric oxide-dependent (Wray et al 2011). Collectively, this suggests that the range of workloads we examined were sufficiently intense to detect the local vascular effects of dietary nitrate supplementation in the population we studied.

As indicated above, the conversion of nitrate to nitrite on the BR_{nitrate} visit was less than we expected and is a potential explanation for the null effects on forearm exercise blood flow. However, the 1.6-fold increase in plasma nitrite concentration we observed is comparable in magnitude and time course with previous dietary nitrate supplementation studies reporting significant effects on resting hemodynamic outcomes (Bahra et al. 2012; Kapil et al. 2010; Webb et al. 2008). Given this, and our use of an ingested nitrate dose (800 mg) recently shown to elicit maximal blood pressure-lowering effects in younger men (Wylie et al. 2013), it was surprising to find no evidence of a nitrate treatment effect on resting blood pressure in the present study. However, establishing the effects of dietary nitrate supplementation on resting blood pressure was not the focus of our study. Consequently, we only performed these measures once (3 h postconsumption) at each study visit, as opposed to performing serial measurements before and after consumption on both days. It is possible that day to day variability in resting blood pressure may have limited our ability to detect differences in resting blood pressure on the placebo versus nitrate supplement visit. Reported reductions in

resting systolic and/or diastolic blood pressure following acute ingestion of nitrate supplements such as beetroot juice are relatively small in absolute terms (-4.4 mmHg and -1.1 mmHg, respectively; Siervo et al. 2013), particularly in healthy subjects with low resting blood pressures. It is also important to note that there are several published studies, including those in healthy younger adults, that have not observed a nitrate supplementation treatment effect on resting blood pressure (Cermak et al. 2012; Heiss et al. 2012; Larsen et al. 2006; Larsen et al. 2010; Miller et al. 2012).

4.4 Conclusions

To the best of our knowledge, the present study is the first to show a correlation between the changes in plasma nitrite concentration and PWV with dietary nitrate supplement and to evaluate *directly* the effects of dietary nitrate-supplementation on human exercise hyperemia. The results indicate that a single 800 mg dose of inorganic nitrate, delivered in the form of concentrated beetroot juice, lowers PWV, but does not augment dilation of the brachial artery or blood flow to the forearm muscles during graded, nonfatiguing handgrip exercise in healthy young men. Further investigation into the effects of dietary nitrate supplementation on resting and exercise induced vascular responses, particularly in populations with reduced nitric oxide bioavailability and under conditions of greater muscle metabolic demand, is warranted. While these findings are limited to healthy young men, and cannot be extrapolated to large muscle, dynamic exercise, this study provides important, methodological information for future investigations of the effects of dietary nitrate-supplementation on exercise induced vascular responses.

Acknowledgements

Funding was provided from Social Sciences Research Institute at Penn State University (to D.N.P.), and HL058091 (to D.K.S.). In addition, the project described was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1 TR000127. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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a)

b)

Fig. 1.

Plasma nitrate (a) and nitrite (b) concentrations at baseline (white bars) and 2.5 h after (hashed bars) ingestion of nitrate-rich (BR_{nitrate}) or nitrate-depleted (BR_{placebo}) beetroot juice. μM, μmol/L. Data are presented as means ± SE. *, Significant difference from baseline of same treatment, $p < 0.05$.

a)

b)

Fig. 2.

Brachial-to-ankle pulse wave velocity (PWV_{BA}) measured at 3 h after ingestion of nitrate-rich (BR_{nitrate}; solid bar) or nitrate-depleted (BR_{placebo}; white bar) beetroot juice (a), and correlation of PWV_{BA} difference and plasma nitrite concentration difference between postconsumption of BR_{nitrate} and BR_{placebo} supplementation (b). μM, μmol/L. Data are presented as means ± SE. *, Significant difference from baseline of same treatment, $p < 0.05$.

Fig. 3. Brachial artery blood flow during graded forearm exercise performed 3.5 h after ingestion of nitrate-rich (BR_{nitrate}; filled circles) or nitrate-depleted (BR_{placebo}; unfilled circles) beetroot juice. Data are presented as means $\pm$ SE for each workload.

a)

b)

c)

Fig. 4. Brachial artery diameter (a), percent change in diameter (b), and shear rate (c) during graded forearm exercise performed 3.5 h after ingestion of nitrate-rich (BR_{nitrate} ; filled circles) or nitrate-depleted (BR_{placebo} ; unfilled circles) beetroot juice. Data are presented as means $\pm$ SE for each workload.

Table 1

Subject characteristics.

	Young men
Subjects, n	12
Height, cm	179±5
Weight, kg	79±8
Age, years	22±2
BMI, kg/m ²	25±2
Glucose, mg/dL	91±5
Cholesterol, mg/dL	145±20
HDL, mg/dL	52±7
LDL, mg/dL	78±16
Triglycerides, mg/dL	77±27
Systolic blood pressure, mmHg	120±7
Diastolic blood pressure, mmHg	64±7
HR, bpm	58±11
Handgrip MVC, kg	50±7

Note: Values are means± SE. BMI, body mass index; HDL, high-density lipoprotein; HR, heart rate; LDL, low-density lipoprotein; MVC, maximal voluntary contraction.